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Community ecology of parasites: functional and network approaches

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Doctoral thesis by
Cristina Llopis Belenguer

Supervisors
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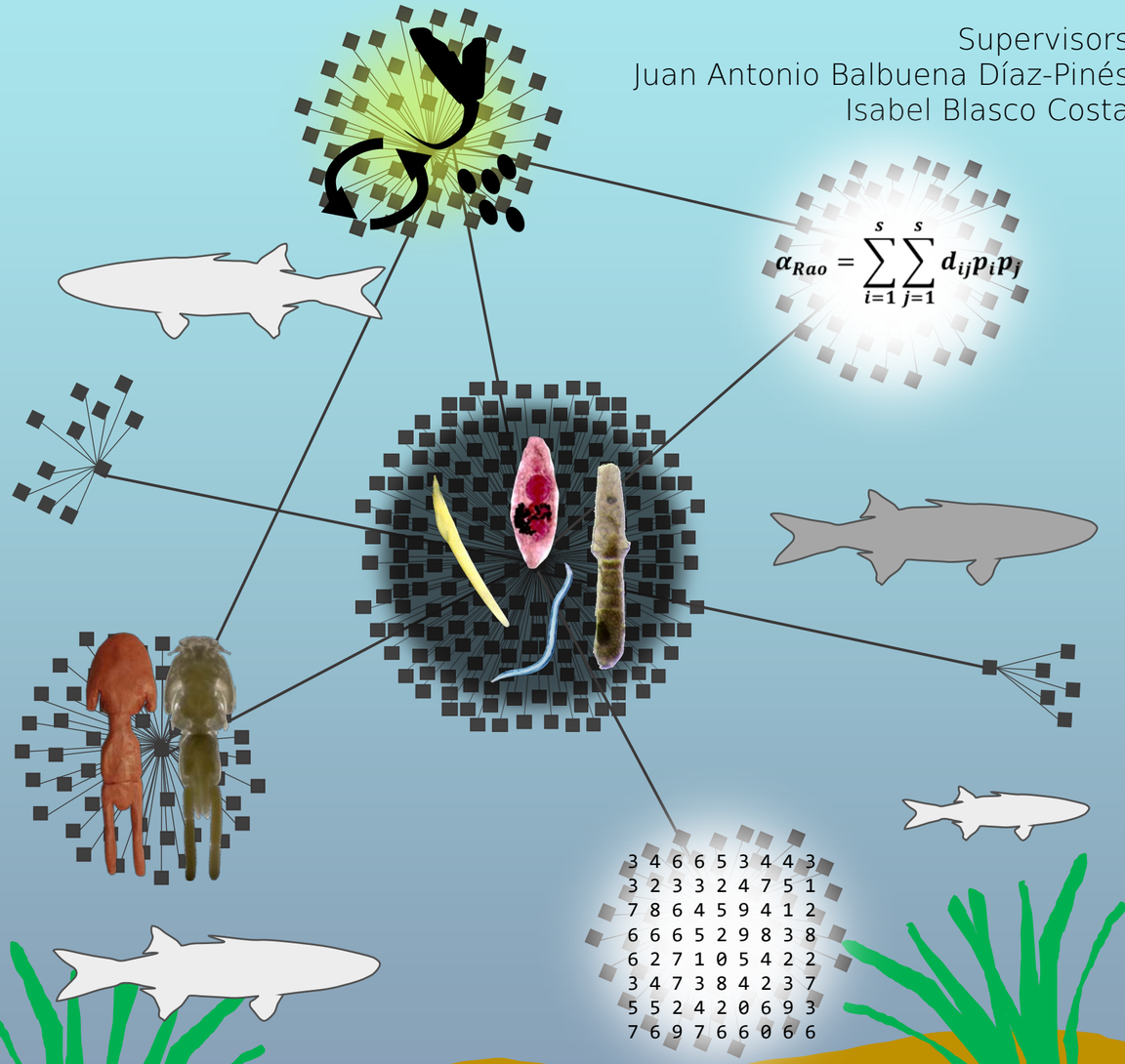

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Doctoral Program in Biodiversity and Evolutionary Biology (RD 99/2011)

Valencia, March 2020

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D. Juan Antonio Balbuena Díaz-Pinés, Profesor Titular del Departamento de Zoología de la Facultad de Ciencias Biológicas de la Universitat de València, y

Dña. Isabel Blasco Costa, Investigadora Titular del Museo de Historia Natural de Ginebra, Suiza

CERTIFICAN que Dña. Cristina Llopis Belenguer ha realizado bajo nuestra dirección, y con el mayor aprovechamiento, el trabajo de investigación recogido en esta memoria, y que lleva por título "COMMUNITY ECOLOGY OF PARASITES: FUNCTIONAL AND NETWORK APPROACHES", para

optar al grado de Doctora en Ciencias Biológicas.

Y para que así conste, en cumplimiento de la legislación vigente, expedimos el presente certificado en Valencia, a 28 de febrero de 2020.

Firmado: Juan Antonio Balbuena Díaz-Pinés

Firmado: Isabel Blasco Costa

A mi familia/ A la meua família

y a la familieta

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Resum

Resum

Introducció

Els organismes i les poblacions d'una mateixa àrea depenen els uns dels altres i el seu conjunt forma una comunitat ecològica (Krebs 2001). Conéixer els processos que regeixen la diversitat d'una comunitat ha sigut una inquietud recurrent entre els ecòlegs. La dicotomia Clements-Gleason ha estat al centre de la discussió al voltant de la composició biològica de les comunitats durant un segle (Ricklefs 2008, Liautaud et al. 2019). La teoria determinista de Clements considera les comunitats com a superorganismes, on les espècies reaccionen conjuntament als canvis, com ho fan les parts d'un organisme (Clements 1916). Segons aquestes idees, les comunitats estan clarament definides amb límits nítids (concepte unitari de la comunitat). Així, per exemple, canvis ambientals suaus poden produir grans diferències entre una comunitat i altra (Clements 1936). En canvi, la visió estocàstica de Gleason assumeix una organització individualista de les comunitats. Les espècies (concebudes com les unitats fonamentals a la natura) van substituint-se progressivament a mesura que el medi canvia i existeixen zones amples de transició d'espècies. En conseqüència, les espècies simplement conviuen perquè toleren el mateix hàbitat i si hi ha límits bruscos entre comunitats, aquests es mantenen, quasi exclusivament, per pertorbacions físiques, com ara una forta variació en la composició del sòl (Gleason 1917). Tot i que les idees de Gleason van prevaldre durant el segle XX, actualment es considera que els paradigmes de Clements i Gleason representen els extrems d'un gradient determinista-estocàstic d'organització de les comunitats ecològiques (Götzenberg et al. 2012, Liautaud et al. 2019).

Per tal de determinar les regles que regeixen la composició de la comunitat, és a dir, per establir en quin punt del gradient Clements-Gleason es troba una comunitat determinada, hem de mesurar característiques descriptives a nivell de comunitat, com és la diversitat de la comunitat (Ives 2007).

La diversitat biològica és la variabilitat de vida en una localització (Hamilton 2005). Tradicionalment, la diversitat ha estat mesurada com a Diversitat Taxonòmica (TD –de les seues sigles en anglés–, nombre i l'abundància d'espècies). Aquest enfocament ha sigut criticat ja que la TD considera que totes les espècies són igualment diferents unes respecte a altres (Chao et al. 2014). En conseqüència, si una espècie de la comunitat fora reemplaçada per una altra amb la mateixa abundància, obtindríem el mateix valor de diversitat per aquesta comunitat, independentment de la singularitat ecològica o evolutiva de la nova espècie (Pavoine et al. 2005). Per tal d'estudiar de la forma més realista possible la diversitat de les comunitats, es va proposar estimar la singularitat ecològica o evolutiva de cada espècie en una

comunitat. Així, la singularitat d'una espècie es mesura com la seua distància funcional (ecològica) o filogenètica (evolutiva) respecte a la resta d'espècies de la comunitat. La combinació de la singularitat funcional i/o filogenètica de totes de les espècies de la comunitat amb les seues respectives abundàncies ens permet expressar la Diversitat Funcional (FD, riquesa i abundància dels trets funcionals en una comunitat) i/o la Diversitat Filogenètica (PD, riquesa i abundància de les entitats genèticament diferents en una comunitat) de forma anàloga i comparable a la TD (Pavoine & Bonsall 2011). FD es basa en els trets funcionals, que són característiques mesurables en cada individu, afecten la seua eficàcia biològica i reflecteixen la seua execució (Violle et al. 2007); mentre que PD mesura el grau de diferència entre marcadors genètics (Pavoine & Bonsall 2011).

A més a més, la diversitat pot mesurar-se a diferents nivells d'organització per tal d'obtindre una visió completa dels processos que afecten la diversitat de les comunitats. Així, la diversitat α és la diversitat en un punt de mostreig de la localitat estudiada, mentre que la diversitat γ representa la diversitat total de comunitat, és a dir, quan tots els punts de mostreig de la localitat es consideren conjuntament. Per últim, la diversitat β explica la quantitat de diversitat que és deguda a diferències en la diversitat entre els punts de mostreig d'una localitat, és a dir, β relaciona els nivells α i γ de diversitat (Whittaker 1960).

Els diferents nivells i les diferents facetes de la diversitat (TD, FD i PD) poden donar-nos resultats oposats. Aquest fet reconcilia, en certa manera, les idees de Clements i Gleason. Ja que esperem que les forces que afecten la diversitat depenguen de l'escala a la qual es mesura la diversitat (Cavender-Bares et al. 2006).

A més dels components i les facetes de la diversitat descrites, els patrons d'interacció entre els organismes d'una comunitat també poden causar variabilitat mesurable (Bascompte 2009). De fet, s'ha proposat que l'anàlisi de la diversitat hauria d'incloure informació referent a la força de les interaccions entre organismes o espècies, o a la connectància de la comunitat (Pimm 1994). Per a poder entendre les regles que determinen la complexitat de les interaccions entre organismes de diferent gremi podem emprar l'anàlisi de xarxes. L'anàlisi de xarxes prové de la teoria de grafs i permet estudiar sistemes complexos en la seua totalitat per a, finalment, extraure propietats matemàtiques d'acord amb la distribució de les interaccions entre els organismes de la comunitat (Poulin 2010).

Els paràsits estan presents en tots els ecosistemes, representen la forma de vida més comuna entre les espècies actuals de la Terra (Poulin & Morand 2000) i en són una part fonamental per al funcionament dels ecosistemes (Gómez & Nichols 2013). El fet que els paràsits hagen sigut normalment considerats com a amenaces per als seus hostes (Wood &

Johnson 2015), junt amb certs aspectes de la seua biologia (Gómez & Nichols 2013), ha causat que l'ecologia de comunitats paràsites haja romàs darrere de l'ecologia de comunitats general (Poulin 2015). Així, els paràsits rarament han sigut considerats en estudis de comunitats o ecosistemes (per exemple, Rossiter & Sukhdeo 2014), la qual cosa ha provocat un biaix sistemàtic en l'estudi de les comunitats. A més, els parasitòlegs normalment han pres aproximacions descriptives per estudiar les comunitats paràsites, en compte d'aprofitar els beneficis de les ciències predictives (Pedersen & Fenton 2007, Poulin 2007). Tot i que s'han fet grans esforços per cobrir aquesta falta de coneixement, encara queda marge per millorar l'enteniment dels múltiples processos mediats per paràsits (Poulin et al. 2016).

De moment, existeixen pocs treballs que hagen considerat totes les facetes de la diversitat dels paràsits alhora. Encara que l'anàlisi de xarxes ha estat molt més utilitzat pels parasitòlegs, en poques ocasions s'ha emprat en un context d'invasions biològiques d'hostes i paràsits. És per això, que aquesta tesi està dedicada a l'estudi de les comunitats ecològiques de paràsits.

Objectius

L'objectiu principal d'aquesta tesi és avançar en el coneixement de les comunitats ecològiques d'organismes paràsits. Amb aquesta finalitat, aprofitaré i adaptaré les últimes metodologies i tècniques desenvolupades en ecologia de comunitats per a l'estudi d'organismes paràsits. Em centraré en l'estudi de la diversitat paràsita i en la dinàmica de les interaccions hoste-paràsits.
Els objectius específics d'aquest estudi són:

1. Com a pas previ per assolir l'objectiu 2, desenvolupar i avaluar dos mètodes no destructius per estimar indirectament el tret funcional massa d'individus paràsits. Comparar els dos mètodes no destructius amb la mesura directa (bàscula) i l'enfocament tradicional (aproximacions geomètriques).
2. Establir un marc teòric per a definir els trets funcionals dels paràsits. Definir una llista fonamental de trets funcionals dels paràsits relacionats amb els tres reptes universals als quals s'enfronten els organismes (dispersió, establiment i persistència).
3. Identificar les regles d'agrupació de les comunitats de paràsits a diferents escales espacials i sota la influència de diferents factors. Explicar aquestes normes sobre les tres facetes de la diversitat.

4. Estudiar els les funcions que els hostes desenvolupen per a la seua comunitat de paràsits en funció del seu caràcter natiu o invasor en una comunitat i en relació als atributs de les espècies paràsites. Demostrar que l'anàlisi de xarxes pot ser una ferramenta útil per avaluar l'impacte dels hostes introduïts en la transmissió de paràsits.

Avaluació de tres mètodes per a l'estimació de la biomassa d'invertebrats de mida menuda, utilitzant tres espècies de paràsits diferents de gran mida com a organismes model

Introducció

La biomassa és la massa d'organismes vius d'una determinada zona o ecosistema en un moment. La biomassa es pot trobar en formes líquides, gasoses o sòlides (Yadav et al. 2016). En ecologia, la importància de quantificar la biomassa és fonamental per a comprendre els processos que impulsen els canvis en els ecosistemes (Lohbeck et al. 2015). Els invertebrats solen ser la pedra angular dels ecosistemes (Piroddi et al. 2017, Yebra et al. 2017) i estudis recents han demostrat que la seua biomassa és superior al que es pensava (Ellwood & Foster 2004, Wardhaugh et al. 2012). No obstant això, la seua mida menuda i la seua abundància elevada, sovint dificulten la quantificació directa de la biomassa en molts organismes (Wardhaugh 2013). Encara que s'han proposat diferents estimadors per a la seua massa, molts d'aquests són específics de taxó o moment del cicle vital, destructius, laboriosos o passen per alt la contribució dels apèndixs a la massa individual total.

En aquest treball avaluarem tres mètodes per a estimar de manera indirecta la massa d'invertebrats menuts (μm–mm). Utilitzarem tres espècies de paràsits de morfologies molt diferents (un acantocèfal, un crustaci i un platihelmint) com a organismes model, perquè són bons representants de la diversitat morfològica dels invertebrats de mida menuda, pertanyen a tres fílums diferents, inclouen espècies de cos tou com ara dur i tenen diferents seccions transversals i nivells d'ornamentació.

Malgrat l'ampla utilització de mesures lineals per a aproximar formes biològiques a formes geomètriques (per exemple, Kuris et al. 2008), la seua precisió no ha sigut validada. A causa del nombre creixent d'estudis que demostren la importància tant d'invertebrats menuts de vida lliure (per exemple, Ellwood & Foster 2004, Piroddi et al. 2017) com de paràsits (per exemple, Lagrue & Poulin 2016) per als ecosistemes, aquesta avaluació resulta necessària. Així, l'objectiu del nostre treball ha sigut doble: (i) desenvolupar i avaluar dos mètodes no destructius

per estimar indirectament la massa d'invertebrats a nivell individual que siguen aplicables a una gran varietat d'invertebrats menuts; i (ii) provar la precisió d'aquests dos mètodes en comparació amb l'estimació directa (bàscula) i els enfocaments basats en aproximacions geomètriques àmpliament utilitzades a estudis anteriors.

Material i mètodes

La relativament gran mida (mm–cm) de les espècies model (*Bolbosoma capitatum*, *Caligus elongatus*, *Campula oblonga*) emprades en aquest estudi i la seua disponibilitat en gran quantitat, ens permet mesurar la seua massa de forma directa. Els espècimens estaven conservats en alcohol 70% (*B. capitatum*, *Ca. elongatus*, *C. oblonga*) o muntats en preparacions permanents (*C. oblonga*). En aquest treball hem mesurat la massa dels organismes de forma (1) directa; i indirecta mitjançant (2) modelatge d'argila; (3) anàlisi d'imatges (mitjançant dos mètodes diferents); (4) aproximació de formes corporals a formes geomètriques. Per als mètodes indirectes, primer mesuràrem el volum de 20 individus de cada espècie i després multiplicàrem cada volum per la densitat del teixit estimada per a cada espècie.

Els exemplars de les espècies model eren prou grans per a ser pesats. Utilitzàrem aquests resultats com a valors de referència per provar la precisió dels mètodes indirectes. Els acantocèfals i els crustacis els pesàrem de forma individual. En el cas del platihelmint, per assolir el primer objectiu i demostrar l'aplicabilitat del mètode basat en anàlisi d'imatge, vam pesar 41 individus alhora i calculàrem la massa mitjana d'un individu.

Els mètodes indirectes, basats en el modelatge d'argila i en l'anàlisi d'imatges, requereixen estimacions de la grossor dels animals. Així, amb un microscopi òptic calculàrem les micres desplaçades verticalment en enfocar des de la part dorsal fins a la part ventral d'un individu en una preparació i comptàrem el nombre de divisions avançades en el micromètric.

El mètode basat en el modelatge d'argila (Mètode indirecte 1) va ser adaptat del mètode proposat per Nesterenko & Kovalchuk (1991) per a ciliats. Modelàrem en argila comercial els organismes (×2–39) i els seus apèndixs (×92–217) a gran escala. Després mesuràrem el volum d'aigua desplaçat per cada model (V_m). El volum de l'organisme real (V_s) va ser calculat com: $V_s = V_m * (L_s/L_m)^3$. On L_s i L_m són la longitud de l'organisme real i del model en argila.

El mètode basat en l'anàlisi d'imatges (Mètode indirecte 2) consta de tres submètodes, d'acord amb la morfologia de les seccions transversals dels organismes estudiats. El primer submètode l'aplicàrem a *C. oblonga*, que té una secció transversal plana, i està basat en el mètode de Lambden & Johnson (2013) per a trematodes. Mitjançant un microscopi òptic dibuixàrem el contorn dels organismes, digitalitzàrem els dibuixos i amb Fiji-ImageJ (Schindelin et al. 2012)

mesuràrem l'àrea dels dibuixos. Multiplicàrem l'àrea del cos de cada individu per la seua grossor per a obtindre el seu volum. El segon submètode l'aplicàrem a *B. capitatum*, que té una secció transversal circular. Fotografiàrem els organismes amb una càmera digital. En les fotografies, extraguérem els cossos dels organismes i els col·locàrem davant d'un fons negre. Mitjançant Fiji-ImageJ convertírem les imatges en text binari, és a dir, separàrem els píxels del cos dels píxels del fons de la imatge. Processàrem la imatge binària amb un *script* implementat en R (R Core Team 2017). Aquest *script* tracta la imatge binària com columnes de píxels. Per a cada columna assumeix que la profunditat d'aquesta és idèntica a l'amplària (secció transversal circular). Després, calcula el volum d'una columna de píxels com un cilindre i, per últim, suma els volums de totes les columnes de píxels per a obtindre el volum de l'organisme.

El tercer submètode està dissenyat per a treballar amb morfologies complexes, és a dir, que combinen seccions planes i circulars, com és el cas de *Ca. elongatus*. Per al tractament d'aquestes morfologies complexes, identificàrem les formes de les seccions transversals (plana o circular). Entre les seccions planes, distingírem seccions corporals de diferent grossor i mesuràrem la grossor de cadascuna. Amb un fotomicroscòpi òptic fotografiàrem les superfícies corporals dels individus. Per a cada imatge, separàrem les porcions corporals de diferent secció transversal i grossor. Calculàrem el volum de cada porció segons el tipus de secció transversal amb els dos submètodes descrits en els punts anteriors. Finalment, sumàrem els volums de totes les porcions per a obtindre el volum corporal d'un individu.

Per a l'aproximació geomètrica (Mètode indirecte 3), mesuràrem la longitud i amplària màximes de cada organisme i assimilàrem els cossos dels organismes a formes geomètriques regulars d'acord amb el que hi havien fet treballs anteriors amb espècies dels mateixos fílums als de les espècies model (per exemple, Kuris et al. 2008).

Pel que fa a les anàlisis estadístiques, en el cas de *C. oblonga* utilitzàrem un prova *t* d'una mostra amb correcció de Bonferroni. En el cas de *B. capitatum* i *Ca. elongatus* empràrem models lineals d'efectes mixtos per a comparar els diferents mètodes (factor fixe) en els individus (factor aleatori).

Resultats

En el cas de les tres espècies model, els resultats dels mètodes indirectes 1 i 2 van ser molt pareguts als obtinguts pel mètode directe i no diferiren significativament d'aquest. En canvi, els resultats obtinguts mitjançant l'aproximació geomètrica (Mètode indirecte 3) diferiren significativament de l'estimació directa, ja que van sobreestimar la massa corporal dels organismes.

Discussió

L'estimació de la biomassa d'invertebrats de mida menuda suposa una sèrie de reptes que es poden superar mitjançant mètodes indirectes, encara que rarament s'ha provat la seua precisió. Aquest estudi demostra que els mètodes indirectes proposats ací proporcionen una bona estimació de la massa corporal real dels organismes i són molt més precisos que el mètode tradicional, basat en l'aproximació de les morfologies corporals a figures geomètriques regulars. En particular, el mètode per estimar la biomassa a partir d'imatges sembla més eficaç i requereix menys temps que els mètodes anteriors, per tant atenen la creixent necessitat d'obtindre estimacions fiables de biomassa d'invertebrats (Tackenberg 2007). Hem validat el mètode de modelatge amb argila, descrit originalment per a ciliats unicel·lulars (Nesterenko & Kovalchuk 1991), per a poder aplicar-lo a invertebrats. Aquest mètode pot ser particularment valuós per a organismes amb una morfologia complexa. Els avantatges dels mètodes que ací proposem són tres: permeten recuperar el material biològic després de l'estimació de la seua massa, es poden aplicar tant a exemplars frescos com a exemplars en preparacions permanents i les imatges i els models d'argila generats es poden conservar i utilitzar posteriorment en altres estudis.

Cap a un marc unificat de trets funcionals de paràsits

Una ecologia basada en trets

Els estudis ecològics basats en trets per explicar les propietats dels ecosistemes en diferents entorns o gradients ambientals han augmentat molt durant les tres últimes dècades (Cadotte et al. 2015, Moretti et al. 2017, Weiss & Ray 2019). Entre els múltiples tipus de trets existents (Violle et al. 2007), els trets funcionals han demostrat àmpliament la seua utilitat per explicar o predir una gran quantitat de preguntes ecològiques sobre organismes de vida lliure, en particular, qüestions relacionades amb la faceta funcional de la diversitat (FD) d'una comunitat. Els trets funcionals també han permés desvetllar els mecanismes mitjançant els quals els individus (o la variabilitat intraespecífica en aquests trets, Carmona et al. 2016) causen efectes sobre els ecosistemes (Violle et al. 2007). Tot i això, el nombre d'estudis que considera els trets funcionals dels paràsits encara és baix (Mouillot et al. 2005, Keeney & Poulin 2007, Krasnov et al. 2015, 2016, 2019a, 2019b, Sokolov & Zhukov 2017, Warburton et al. 2017) en comparació amb el nombre d'estudis d'organismes de vida lliure. Considerem que aquest fet es deu a tres motius: (i) una subestimació general de les funcions que exerceixen els paràsits en

els ecosistemes, malgrat una ampla evidència que demostra la seua importància (per a una revisió vegeu Gómez & Nichols 2013), (ii) els escassos coneixements sobre les característiques biològiques dels paràsits en comparació amb altres organismes i (iii) la manca d'un marc unificat per a identificar i mesurar trets funcionals en paràsits.

La selecció de trets funcionals és essencial per tal d'aconseguir conclusions ecològiques sòlides, ja que els trets escollits han de ser informatius d'una funció concreta (Petchey & Gaston 2006) i s'han de mesurar mitjançant protocols normalitzats (per exemple, Weiher et al. 1999, Moretti et al. 2017). Els trets funcionals han d'estar relacionats explícitament amb l'eficàcia biològica dels individus (Violle et al. 2007). Per facilitar les comparacions entre grups de paràsits i promoure la reproductibilitat dels estudis, és necessari un marc unificat amb una terminologia comuna per als trets funcionals de paràsits. Aquest marc milloraria i maximitzaria la utilitat dels estudis funcionals en parasitologia i contribuiria al coneixement de la funció dels paràsits en les comunitats i els ecosistemes de manera més ampla. A més, permetria comparar les diversitats de paràsits i hostes amb els mateixos termes (vegeu Weiss & Ray 2019 per comparar els trets funcionals entre taxons), obrint així el camí perquè els ecòlegs incloguen els paràsits en els estudis d'ecologia de comunitats.

En aquest treball, proposem un marc unificat per als trets funcional de paràsits metazous. El marc està fonamentat en la teoria ecològica actual, i assumeix el repte d'identificar trets funcionals prou generals i aplicables a tàxons paràsits filogenèticament distants, però sense perdre resolució per a respondre a qüestions ecològiques.

Múltiples solucions, un estil de vida únic: trets funcionals de paràsits

Independentment d'aconseguir un consens en la definició de paràsit, els organismes comparteixen els mateixos trets funcionals, encara que aquests organismes diferisquen en la seua forma de vida parasitària.

- Llista fonamental de trets funcionals dels paràsits

Per tal de fer que el marc de trets funcionals siga comparable a escales espacials i temporals, recopile informació funcionalment representativa, compartisca dades i maximitze l'aplicabilitat dels resultats, els trets funcionals dels paràsits s'han d'ajustar a la definició acceptada en l'ecologia de comunitats. Els trets funcionals han de representar l'eficàcia biològica dels organismes, han de mesurar-se a nivell individual i sense fer referència a informació externa a l'individu (Violle et al. 2007). Els trets funcionals proposats ací estan relacionats amb tres reptes universals, als quals s'enfronten els organismes: dispersió, establiment i persistència (Weiher et al. 1999); i la seua influència en l'eficàcia biològica de l'organisme que els presenta ha sigut

demostrada prèviament per altres autors. Igualment important, tots aquests trets funcionals poden mesurar-se a nivell de l'individu, sense fer referència al medi extern o a qualsevol altre nivell d'organització (Violle et al. 2007, Carmona et al. 2016). D'acord amb aquests criteris, proposem una llista de trets funcionals aplicables, virtualment, a qualsevol paràsit metazou i basats en característiques morfològiques, d'estratègia vital i de comportament. El nombre de trets funcionals que considerem és el mínim que pot aplicar-se a qualsevol paràsit metazou i que pot resoldre qualsevol qüestió ecològica. Aquests trets funcionals són: òrgan d'ancoratge, massa corporal, nombre d'ous, mida dels ous, forma dels ous, alimentació i cicle de vida.

- Mesurar els trets funcionals

Reconeguem quatre fonts d'on es pot obtindre informació fiable per a mesurar els trets funcionals. (i) Observació directa; (ii) descripció d'espècies, (iii) protocols estandarditzats i (iv) representants d'un tret funcional.

Consideracions per a un marc de trets funcionals de paràsits

- Seleccioneu un nombre adequat de trets funcionals

El nombre de trets funcionals que poden mesurar-se en un organisme és molt gran. Però la nostra habilitat per a mesurar-los és limitada. La llista de trets funcionals presentada ací no ha de considerar-se tancada. Els trets funcionals inclosos en un estudi, depenen de l'objectiu d'aquest.

- Utilitzeu trets funcionals "suaus"

Si diferents trets funcionals expliquen la mateixa funció, haurem d'elegir el més fàcil i barat de mesurar (Weiher et al. 1999), és a dir, el més "suau". Així podrem obtindre la major quantitat possible d'informació per al nostre estudi.

- Trets d'execució i execucions ecològiques

Quan no disposem de tota la informació per a mesurar un tret funcional a nivell d'individu, però podem extraure aquesta d'un conjunt d'individus per a obtindre un representant del tret funcional en qüestió. A aquest representant l'anomenen tret d'execució. L'execució ecològica és l'habilitat d'un organisme per a respondre a variables ambientals. L'execució ecològica no hauria de ser utilitzada com a tret funcional.

- Absència d'informació

Potser no tinguem al nostre abast tota la informació sobre els trets funcionals de totes les espècies del sistema d'estudi. En eixe cas, recomanen utilitzar la distància de Gower (Gower 1971) per a mesurar la singularitat funcional de cada espècie perquè permet la utilització de valors no disponibles.

- Diferents estats de desenvolupament

Els hostes poden estar parasitats per la mateixa o diferents espècies de paràsits en diferents estats de maduresa sexual. En eixe cas, els investigadors hauran de decidir quines espècies incloure en l'estudi, d'acord amb el seu objectiu.

Conclusions

En aquest estudi establim la base de la selecció de trets funcionals de paràsits d'acord amb la teoria ecològica actual, amb la finalitat d'avançar en els nostres coneixements sobre els mecanismes que dissenyen les comunitats paràsites i les seues dinàmiques. Hem donat les ferramentes necessàries per a definir nous trets funcionals dins d'aquest marc, però som conscients que aquestes definicions poden estar a expenses d'estudis experimentals per a identificar-los. Per últim, proposem la creació d'una base de dades d'accés públic perquè els investigadors tinguen al seu abast tota la informació disponible de la manera més fàcil possible.

Organització de les comunitats dels paràsits helmints en llises: combinació dels components de la diversitat

Introducció

Els organismes s'associen els uns amb els altres i formen comunitats ecològiques. Els ecòlegs s'han preguntat si aquestes associacions són degudes a processos deterministes o, al contrari, si les comunitats no són més que col·leccions aleatòries d'espècies (Clements 1916, Gleason 1917).

La diversitat pot mesurar-se a diferents nivells (α, β i γ) d'escales jeràrquiques d'organització (per exemple, escala geogràfica), i/o en les seues facetes (és a dir, TD, FD i PD) (Pavoine & Bonsall 2011). A més, la diversitat β pot mesurar-se a dos nivells, β1 i β2. La diversitat β1 explica la quantitat de diversitat que és deguda a diferències en la diversitat entre els punts de mostreig d'un mateix nivell d'organització (per exemple, diferències en la diversitat de dos punts de mostreig dins d'una mateixa localitat); mentre que la diversitat β2 explica la quantitat de diversitat que és deguda a diferències en la diversitat entre els punts de mostreig de diferent nivell d'organització (per exemple, diferències en la diversitat de dos punts de mostreig de diferents localitats) (Pavoine et al. 2016).

Com que la diversitat té múltiples components, ambdós processos, deterministes i aleatoris, poden afectar simultàniament a la mateixa comunitat. És a dir, la influència d'un

procés o un altre sobre la diversitat d'una comunitat depén del nivell al qual s'estudie la comunitat.

L'estudi en conjunt de la diversitat a diferents nivells i en les seues facetes, ha resultat útil per conéixer els mecanismes que determinen la diversitat d'organismes de vida lliure (Devictor et al. 2010, González-Maya et al. 2016). No obstant això, el nostre coneixement sobre els processos que determinen les diferents facetes de la diversitat en diferents escales d'organització d'organismes paràsits és limitat (per exemple, Mouillot et al. 2005, Krasnov et al. 2014, 2015, 2016). En aquest estudi examinarem les regles que dirigeixen les diferents facetes de la diversitat en diferents escales d'organització en una comunitat paràsita. Així, mesurarem la diversitat TD, FD i un representant de la PD (PPD) d'una comunitat d'helmints paràsits de llises (Teleostei: Mugilidae) en tres localitats de la mar Mediterrània.

Aquest model és apropiat perquè, en primer lloc, les comunitats de paràsits inclouen espècies de paràsits d'origen filogenètic llunyà i funcionalment diferents. En segon lloc, les comunitats paràsites provenen de tres de les fins a sis espècies de llises simpàtriques que es troben en aquesta zona de la Mediterrània (Blasco-Costa 2009) i de tres localitats que varien en els seus paràmetres mediambientals. Concretament, dues de les espècies d'hostes, *Chelon auratus* i *Chelon ramada*, són filogenèticament més properes entre elles que amb *Mugil cephalus* (Durand et al. 2012), mentre que *M. cephalus* i *C. ramada* presenten majors similituds en les seues estratègies de vida entre elles que amb *C. auratus* (Cardona 2001, Cardona 2006). A més, les comunitats provenen de tres localitats costaneres que es diferencien en les seues condicions d'hàbitat (dues marines: mar del Delta de l'Ebre - EDS i mar de Santa Pola - SPS; i una llacuna salobre: llacuna de Santa Pola - SPL) i en la seua proximitat geogràfica (SPS i SPL estan molt a prop, a 10 km de distància; mentre que l'EDS està més allunyada de les altres dues a uns 290 km). Així, podem avaluar si les diferents facetes de la diversitat d'helmints proporcionen resultats congruents i si els factors de l'hoste (proximitat filogenètica i semblança en les estratègies de vida) i/o els ambientals (ubicació geogràfica i condicions d'hàbitat) seleccionen diferents estratègies vitals entre els paràsits. Finalment, el disseny jeràrquic dels mostreigs ens permet mesurar i comparar la diversitat en la unitat de mostreig (diversitat α o diversitat de paràsits a l'individu hoste) i dins i entre els nivells d'un factor (és a dir, la diversitat de paràsits entre hostes d'una mateixa espècie o localitat ($\beta 1$); o entre hostes de diferent espècie o localitat ($\beta 2$)).

Basant-nos en estudis d'organismes de vida lliure (Cavender-Bares et al. 2006) i estudis previs del nostre model de treball (Blasco-Costa 2009, Blasco-Costa et al. 2012, Míguez-Lozano et al. 2012, Sarabeev et al. 2013), proposem que tant l'origen filogenètic (*Chelon* o *Mugil*)

com l'estratègia vital (marina o costanera) de l'hoste influiran en la comunitat del paràsit, mentre que les condicions de l'hàbitat (marí o llacuna) seran més determinants de les comunitats de paràsits que la distància geogràfica (Delta de l'Ebre o Santa Pola).

Material i mètodes

- Les múltiples facetes de la diversitat

Per a mesurar de la FD, construírem una matriu de distàncies entre les espècies de paràsits d'acord amb els seus atributs (és a dir, valors o categories) per a cinc trets funcionals: massa, grandària de l'ou, nombre d'ous, òrgan d'ancoratge i cicle de vida. Per al càlcul de la PPD, estimàrem les distàncies entre espècies d'acord amb la seua classificació taxonòmica. Per a mesurar la TD, establírem les distàncies entre espècies com a la distància màxima, és a dir, aquesta matriu obtingué el valor 1 (Pavoine et al. 2004).

- L'anàlisi de la diversitat

Organitzàrem les mostres d'acord amb tres factors: espècie d'hoste, localitat i estació. Després dividírem les anàlisis de la diversitat en dos Casos d'Estudi. En el Cas 1, avaluàrem la influència de l'espècie d'hoste en les tres facetes de la diversitat dels paràsits. Així, analitzàrem i comparàrem la TD, FD i PPD de les comunitats d'helmints de les tres espècies d'hostes en SPS. En el Cas 2, avaluàrem la influència de la localitat en les tres facetes de la diversitat dels paràsits. Així, analitzàrem i comparàrem la TD, FD i PPD de les comunitats d'helmints de les tres localitats en hostes de l'espècie *M. cephalus*.

Per a cada cas d'estudi mesuràrem la diversitat mitjançant dos tipus d'anàlisis. Primer mesuràrem la diversitat utilitzant l'Anàlisi Doble de Components Principals (DPCoA –de les seues sigles en anglés). El DPCoA mesura α, β i γ per a TD, FD i PPD per a un sol factor (Pavoine et al. 2004). És a dir, la diversitat influenciada pel factor espècie d'hoste o localitat. Per això, per eliminar la variació de la diversitat deguda al mostreig estacional només consideràrem hostes de la mateixa estació.

Després, mitjançant el DPCoA–creuat (Pavoine et al. 2013), avaluàrem el percentatge de diversitat que explica cadascun dels factors de l'anàlisi: individu hoste, estació i espècie d'hoste (Cas 1) o localitat (Cas 2). És a dir, consideràrem la influència de factors creuats en comunitats paràsites de diferent estació i espècie d'hoste (Cas 1) o localitat (Cas 2).

Resultats

La comunitat de paràsits a l'individu hoste (α) representa un conjunt aleatori de la diversitat total de la comunitat (γ). El factor individu hoste va explicar el major percentatge de diversitat de les comunitats (al voltant del 50% de la diversitat) per a les tres facetes de la diversitat.

La TD a nivell β1 va indicar diferències menors de les esperades entre individus hostes de la mateixa espècie d'hoste (Cas 1) o localitats (Cas 2). Mentre FD i PD a aquest nivell mostraren una agregació aleatòria de la diversitat. A nivell β2, trobàrem diferències majors de les esperades entre les comunitats d'hostes de diferent espècie (Cas 1) o localitat (Cas 2) per a les tres facetes de la diversitat.

El factor espècie d'hoste explicà un 32%, 25% i 18% de la TD, FD, i PPD, respectivament. Mentre que la localitat explicà un 6%, 12% i 12% de la TD, FD, i PPD, respectivament. La interacció entre els factors creuats, espècie d'hoste (Cas 1) o localitat (Cas 2) i estació, i el factor estació contribuïren de forma menys rellevant a la diversitat.

Discussió

Dels nostres resultats extraguérem les següents conclusions. En primer lloc, la diversitat d'aquestes comunitats de paràsits mostra almenys dos patrons oposats. Aquests patrons es troben a diferents nivells (α, β1 i β2) de les dues escales d'organització (espècie d'hoste o localitat). En segon lloc, la diversitat de les dues escales organització està influenciada per diverses variables. En tercer lloc, les tres facetes de la diversitat (és a dir, TD, FD i PPD) no sempre van mostrar resultats congruents entre elles, la qual cosa no és sorprenent, ja que és la tendència general registrada per a diversos grups d'organismes (per exemple, Devictor et al. 2010). Per tant, les conclusions d'un estudi podrien no estar completes si s'omet qualsevol de les facetes de la diversitat (Jarzyna & Jetz 2016).

Als nivells intermedis de l'escala d'organització, la coexistència de paràsits similars està limitada, almenys, per a TD. Mentre que a nivells més elevats, l'entorn filtra la diversitat de paràsits, ja que una influència conjunta d'origen filogenètic d'hoste (*Chelon* vs. *Mugil*) i les preferències mediambientals (marí o costaner) podrien determinar les TD, FD i PPD dels paràsits. Tot i que això és menys clar per a les dues últimes facetes de la diversitat. Finalment, les condicions d'hàbitat de la localitat semblen ser més determinants de la TD, FD i PPD que la distància geogràfica.

Les espècies natives i invasores desenvolupen funcions diferents en les xarxes d'hostes i paràsits

Introducció

Les invasions biològiques són introduccions d'espècies fora de la seua distribució original afavorides per la influència dels éssers humans, que aconsegueixen establir poblacions viables al llarg de l'espai i del temps (Richardson et al. 2000). Les espècies invasores representen una amenaça important per als ecosistemes, ja que no deixen transcórrer el temps necessari perquè les espècies natives desenvolupen adaptacions evolutives a la seua presència (Poulin 2017). Les invasions biològiques tenen el potencial d'alterar les dinàmiques dels paràsits i els hostes (Chalkowski et al. 2018). Així doncs, els hostes natius i invasors i les seues comunitats de paràsits natius i/o adquirits poden interactuar de diferents maneres amb les subseqüents conseqüències per als ecosistemes (Chalkowski et al. 2018).

Les associacions entre paràsits i hostes en una comunitat envaïda han sigut estudiades mitjançant diferents tipus d'anàlisis, encara que pocs treballs han utilitzat l'anàlisi de xarxes biològiques per a explicar aquest tipus d'interaccions ecològiques durant una invasió (Médoc et al. 2017). Aquestes anàlisis permeten explorar la manera en què s'associa una comunitat de paràsits amb el seu hoste (individu) (Poulin 2010). Generalment, aquestes associacions bipartides es caracteritzen per no ser aleatòries (Fortuna et al. 2010), és a dir, estan determinades per processos ecològics i evolutius, i un dels patrons que descriu aquestes organitzacions és la modularitat (Newman & Girvan 2004). En xarxes modulars, s'espera que subconjunts (és a dir, mòduls) d'individus interactuen amb més freqüència entre ells que amb individus d'altres mòduls, i valors més alts de modularitat indiquen una millor segregació dels mòduls (Newman & Girvan 2004). Així, podem classificar la funció de cada individu hoste en una xarxa modular segons el nombre d'interaccions que aquest presenta amb altres dins del mòdul al qual pertany (valor z) i el nombre d'interaccions que presenta amb organismes d'altres mòduls (valor c) (Guimerà & Amaral 2005, Olesen et al. 2007):

1. Organismes centrals en el mòdul (*Modul hub*): són organismes vinculats a molts altres organismes dins del seu propi mòdul (alta z, baixa c).
2. Connectors (*Connectors)*: individus que enllacen diversos mòduls (baixa z, alta c).
3. Organismes centrals en la xarxa (*Network hubs*): organismes que actuen com a connectors de mòduls i com a organismes centrals al seu mòdul (alta z, alta c).
4. Perifèrics (*Peripherals*): organismes que interactuen poc amb altres, tant dins del seu mòdul com amb organismes d'altres mòduls (baixa z, baixa c).

D'aquesta manera podem representar gràficament la posició de cada organisme segons els seus valors de c i z, i esperem que els organismes que tinguen una mateixa posició desenvolupen funcions similars per a determinar l'estructura de la comunitat paràsita.

Per tal de millorar el nostre coneixement de les interaccions hoste-paràsits durant les invasions, estudiarem les funcions (anàlisis de modularitat i cz) desenvolupades per individus de dues espècies d'hostes (una nativa i una invasora) per a les seues comunitats paràsites. Caracteritzarem aquestes xarxes en una localitat nativa per a una de les espècies i en una localitat on una de les espècies és nativa i l'altra invasora. Sovint, la distribució dels paràsits és agregada, aleshores, estudiar les associacions hoste-paràsits a nivell d'individu hoste, ens permet controlar la variació intraespecífica i ens dona una idea de la importància relativa de l'espècie invasora per a mantindre la transmissió de paràsits en l'ecosistema (Godfrey 2013).

Les llises (Teleostei: Mugilidae) són un model excel·lent per a estudiar la variació de les funcions desenvolupades pels individus d'una espècie per a la seua comunitat paràsita segons la seua distribució (nativa o invasora), perquè proporcionen un punt de control per a aquesta variació (Sarabeev et al. 2017). En aquest treball estudiarem les funcions desenvolupades per *Mugil cephalus* i *Planiliza haematocheilus* en una àrea on les dues espècies són natives (mar del Japó) i en una àrea on *M. cephalus* és nativa i *P. haematocheilus* és invasora (mar d'Azov). L'arribada de *P. haematocheilus* a la mar d'Azov va suposar un gran canvi en l'estructura de la seua comunitat paràsita: va perdre les espècies paràsites natives amb cicles de vida complexos, va adquirir les espècies paràsites amb cicles de vida complexos de l'àrea envaïda i va cointroduir alguns dels seus ectoparàsits, amb un cicle de vida senzill.

En aquest estudi utilitzarem les anàlisis de modularitat i de valors cz per a determinar la funció desenvolupada pels individus hostes per a les seues comunitats de paràsits. Primer, esperem que la modularitat siga major en les xarxes de l'àrea nativa que en les de l'àrea envaïda, ja que els paràsits natius poden parasitar a l'espècie invasora en l'àrea envaïda, connectar mòduls existents i provocar una disminució de la modularitat en aquesta àrea. Segon, els hostes de les dues espècies desenvoluparan funcions similars en l'àrea nativa, però en l'àrea envaïda els hostes de l'espècie invasora tendiran a desenvolupar una funció perifèrica per a la comunitat de paràsits, perquè les seues interaccions no han estat modulades per una història evolutiva comuna.

Material i mètodes

Avaluàrem la funció desenvolupada pels individus hostes per a les xarxes que incloïen:

1. Tota la comunitat de paràsits helmints.

2. Els paràsits transmesos activament (Monogenea i metacercàries de Trematoda).
3. Els paràsits transmesos passivament/tròficament (adults i larves de Trematoda i Acanthocephala i adults de Nematoda).
4. Ectoparàsits (Monogenea). Aquest és l'únic grup de paràsits introduïts per *P. haematocheilus* a l'àrea envaïda.

Aquestes quatre xarxes s'analitzaren tant a l'àrea nativa com a l'envaïda, és a dir, en total duguérem a terme huit anàlisis. Després de realitzar les anàlisis de modularitat i de valors cz, avaluàrem si els individus de les dues espècies d'hostes estaven distribuïts d'una manera similar entre les quatre categories (centrals de mòdul, connectors, centrals de xarxa i perifèrics) mitjançant una prova exacta de Fisher. En els casos en què trobàrem diferències significatives, avaluàrem si el nombre d'individus perifèrics de *P. haematocheilus* o *M. cephalus* era major o menor de l'esperat respecte a la proporció observada d'individus perifèrics de *M. cephalus* o *P. haematocheilus*, respectivament.

Resultats

Les xarxes analitzades van ser modulars. Però la modularitat va ser major en les xarxes de l'àrea nativa, excepte en els casos on es considerà la comunitat d'ectoparàsits. En aquests casos la modularitat va ser igual en l'àrea nativa i en l'envaïda.

Discussió

El nostre estudi proporciona un exemple clar de com comparar quantitativament les funcions desenvolupades per individus natius i invasors. Al treballar amb xarxes a nivell d'individu hoste, hem pogut estudiar la repartició de la comunitat de paràsits entre individus de diferents espècies. Així, hem vist com les funcions d'individus de diferents espècies poden solapar-se, mentre que individus de la mateixa espècie poden desenvolupar una funció diferent.

Al fraccionar les comunitats de paràsits en subcomunitats de paràsits que tenen les mateixes característiques, hem pogut observar que les funcions de *P. haematocheilus* són similars en les àrees natives i envaïdes per a les comunitats que inclouen ectoparàsits. De manera que juntament amb la cointroducció d'ectoparàsits (Sarabeev 2015), es va mantindre l'estructura de la comunitat.

A més a més, els individus de *P. haematocheilus* desenvoluparen una funció majoritàriament perifèrica per a la comunitat total i la transmesa passivament/tròficament. Açò podria ser causat per la inexistència d'una història evolutiva i ecològica comuna entre hostes i

paràsits, la qual cosa confirmaria la hipòtesi d'alliberació dels enemics (Torchin & Lafferty 2009). Proposem que amb el pas del temps, els individus de l'espècie invasora adquiriran posicions més rellevants a la xarxa biològica. Per tant, amb el seguiment de les comunitats d'hostes i paràsits al llarg del temps, podem establir la maduresa de l'establiment de l'espècie invasora en una àrea.

Conclusions

Aquesta tesi està dedicada a l'estudi de les comunitats de paràsits des d'una perspectiva ecològica, amb especial èmfasi en les comunitats de paràsits helmints de les llises (Teleostei: Mugilidae). S'han aplicat i adaptat metodologies d'avantguarda de l'ecologia de comunitats per a l'estudi de les comunitats de paràsits. Aquestes metodologies inclouen un enfocament basat en l'índex Rao per a mesurar les diferents facetes de la diversitat i l'anàlisi de xarxes biològiques. Les contribucions d'aquesta investigació són oportunes, ja que s'inscriuen en l'objectiu actual de l'ecologia de comunitats de revelar els processos que determinen la composició de la diversitat. Aquesta tesi aporta diverses consideracions teòriques i troballes noves per a l'estudi i la comprensió de les comunitats de paràsits, que ja he comentat als capítols anteriors. Així que, només en destacaré les principals conclusions en aquest apartat.

Al capítol 3, els meus coautors i jo vam desenvolupar i validar la precisió dels mètodes tradicionals i dels mètodes basats en modelatge d'argila i anàlisi d'imatges per estimar la massa d'individus paràsits de grandària menuda. Els mètodes basats en el modelatge d'argila i l'anàlisi d'imatges van proporcionar la millor aproximació a la mesura directa de la massa dels individus. Mentre que, l'aproximació geomètrica, tradicionalment utilitzada, va mostrar la menor precisió i els resultats diferien significativament de la mesura directa. Per tant, recomanem fermament abandonar el seu ús. La varietat morfològica i l'origen filogenètic divers de les espècies model van demostrar que aquests mètodes poden ser útils per quantificar la massa d'una gran varietat d'invertebrats. Particularment, per a l'objectiu d'aquesta tesi doctoral, l'aproximació de l'anàlisi d'imatges va ser útil per estimar la massa de trets funcionals (capítol 4) de les mostres processades al capítol 5.

Al capítol 4, vam construir un marc teòric per definir els trets funcionals de paràsits basant-nos en les consideracions ecològiques acceptades actualment. A més, vam identificar set trets funcionals pràcticament mesurables a qualsevol individu paràsit metazou i capaç de tractar qualsevol qüestió ecològica. Esperem que aquest marc ajude a desvetllar qüestions

ecològiques i evolutives en parasitologia. A més, millorarà les comparacions entre estudis i, fins i tot, pot inspirar una extensió més a paràsits no metazous. D'altra banda, permetrà comparar la diversitat de paràsits i hostes en els mateixos termes, i així obrirà el camí perquè els ecòlegs incloguen als paràsits en l'ecologia de comunitats general.

Al capítol 5, identificàrem les regles que determinen l'estructura de la diversitat de comunitats de paràsits de llises de la Mediterrània occidental. Hem trobat que aquestes regles depenen del nivell de l'anàlisi i de la faceta de la diversitat considerada. En general, l'origen filogenètic de l'hoste (*Chelon* vs. *Mugil*) i les preferències ambientals dels hostes (costaneres o marines) determinen la comunitat paràsita d'un individu hoste. Mentre que les condicions d'hàbitat i la ubicació geogràfica de les localitats no són tan determinants de les comunitats de paràsits. D'aquest estudi concloem que les comunitats de paràsits no es poden entendre plenament si es deixen de banda algunes de les facetes de la diversitat a l'estudiar les comunitats.

Al capítol 6, aprofitant l'anàlisi de xarxes bipartides i les característiques exclusives del sistema hoste-paràsits de les llises, vam avaluar la funció que juguen els individus hostes de llises natives i invasores per les seues comunitats de paràsits. Els meus coautors i jo trobàrem que els individus d'ambdues espècies d'hostes van desenvolupar una funció similar en l'àrea on les dues espècies d'hostes són natives. Tanmateix, en l'àrea on una espècie d'hoste és nativa i l'altra és invasora, els individus hostes invasors desenvoluparen una funció perifèrica per a les comunitats de paràsits, excepte quan es van considerar els seus paràsits cointroduïts. Este fet suggereix que, juntament amb la cointroducció, es va mantindre l'estructura de les interaccions hoste-paràsits. Proposem que el seguiment a llarg termini de les funcions dels hostes invasors per a les comunitats de paràsits pot ser una ferramenta útil per estimar la maduresa de l'establiment dels hostes invasors en un ecosistema.

Finalment, aquesta tesi mostra com es poden estudiar les comunitats de paràsits a la llum de la teoria de l'ecologia de comunitats actual. Dona ferramentes noves als parasitòlegs per millorar la comprensió de la funció dels paràsits en els ecosistemes i esperem que anime als ecòlegs a considerar els paràsits als seus estudis per a obtindre una visió completa dels processos dels ecosistemes.

Resumen

Resumen

Introducción

Los organismos que forman parte de las poblaciones de una misma área dependen los unos de los otros y su conjunto forma una comunidad ecológica (Krebs 2001). Conocer los procesos que rigen la diversidad de una comunidad ha sido una inquietud recurrente entre los ecólogos. La dicotomía Clements-Gleason ha estado en el centro de la discusión alrededor de la composición biológica de las comunidades durante un siglo (Ricklefs 2008, Liautaud et al. 2019). La teoría determinista de Clements considera las comunidades como superorganismos, donde las especies reaccionan conjuntamente a los cambios, como lo hacen las partes de un organismo (Clements 1916). Según estas ideas, las comunidades están claramente definidas con límites nítidos (concepto unitario de la comunidad). Así, por ejemplo, cambios ambientales suaves pueden producir grandes diferencias entre una comunidad y otra (Clements 1936). En cambio, la visión estocástica de Gleason asume una organización individualista de las comunidades. Las especies (concebidas como las unidades fundamentales a la naturaleza) van sustituyéndose progresivamente a medida que el medio cambia y existen zonas amplias de transición de especies. En consecuencia, las especies simplemente conviven porque toleran el mismo hábitat y si hay límites bruscos entre comunidades, estos se mantienen, casi exclusivamente, por perturbaciones físicas, como por ejemplo una fuerte variación en la composición del suelo (Gleason 1917). A pesar de que las ideas de Gleason prevalecieron durante el siglo XX, actualmente se considera que los paradigmas de Clements y Gleason representan los extremos de un gradiente determinista-estocástico de organización de las comunidades ecológicas (Götzenberg et al. 2012, Liautaud et al. 2019).

Para determinar las reglas que rigen la composición de la comunidad, es decir, para establecer en qué punto del gradiente Clements-Gleason se encuentra una comunidad determinada, tenemos que medir características descriptivas a nivel de comunidad, como es la diversidad de la comunidad (Ives 2007).

La diversidad biológica es la variabilidad de vida en una localidad (Hamilton 2005). Tradicionalmente, la diversidad ha sido medida como Diversidad Taxonómica (TD –de sus siglas en inglés–, número y la abundancia de especies). Este enfoque ha sido criticado puesto que la TD considera que todas las especies son igualmente diferentes unas respecto a otras (Chao et al. 2014). En consecuencia, si una especie de la comunidad fuera reemplazada por otra con la misma abundancia, obtendríamos el mismo valor de diversidad para esta comunidad, independientemente de la singularidad ecológica o evolutiva de la nueva especie (Pavoine et al. 2005). Para estudiar de la forma más realista posible la diversidad de las

comunidades, se propuso estimar la singularidad ecológica o evolutiva de cada especie en una comunidad. Así, la singularidad de una especie se mide como su distancia funcional (ecológica) o filogenética (evolutiva) respecto al resto de especies de la comunidad. La combinación de la singularidad funcional y/o filogenética de todas de las especies de la comunidad con sus respectivas abundancias nos permite expresar la Diversidad Funcional (FD, riqueza y abundancia de los rasgos funcionales en una comunidad) y/o la Diversidad Filogenética (PD, riqueza y abundancia de las entidades genéticamente diferentes en una comunidad) de forma análoga y comparable a la TD (Pavoine & Bonsall 2011). FD se basa en los rasgos funcionales, que son características medibles en cada individuo, afectan su eficacia biológica y reflejan su ejecución (Violle et al. 2007); mientras que PD mide el grado de diferencia entre marcadores genéticos (Pavoine & Bonsall 2011).

Además, la diversidad puede medirse a diferentes niveles de organización para obtener una visión completa de los procesos que afectan la diversidad de las comunidades. Así, la diversidad α es la diversidad en un punto de muestreo de la localidad estudiada, mientras que la diversidad γ representa la diversidad total de comunidad, es decir, cuando todos los puntos de muestreo de la localidad se consideran conjuntamente. Por último, la diversidad β explica la cantidad de diversidad que es debida a diferencias en la diversidad entre los puntos de muestreo de una localidad, es decir, β relaciona los niveles α y γ de diversidad (Whittaker 1960).

Los diferentes niveles y las diferentes facetas de la diversidad (TD, FD y PD) pueden darnos resultados opuestos. Este hecho reconcilia, en cierto modo, las ideas de Clements y Gleason. Puesto que esperamos que las fuerzas que afecten la diversidad dependan de la escala a la cual se mide la diversidad (Cavender-Bares et al. 2006).

Además de los componentes y las facetas de la diversidad descritas, los patrones de interacción entre los organismos de una comunidad también pueden causar variabilidad medible (Bascompte 2009). De hecho, se ha propuesto que el análisis de la diversidad tendría que incluir información referente a la fuerza de las interacciones entre organismos o especies, o a la conectancia de la comunidad (Pimm 1994). Para poder entender las reglas que determinan la complejidad de las interacciones entre organismos de diferente gremio podemos emplear el análisis de redes. El análisis de redes proviene de la teoría de grafos y permite estudiar sistemas complejos en su totalidad para, finalmente, extraer propiedades matemáticas de acuerdo con la distribución de las interacciones entre los organismos de la comunidad (Poulin 2010).

Los parásitos están presentes en todos los ecosistemas, representan la forma de vida más común entre las especies actuales de la Tierra (Poulin & Morand 2000) y son una parte

fundamental para el funcionamiento de los ecosistemas (Gómez & Nichols 2013). El hecho que los parásitos hayan sido normalmente considerados como amenazas para sus hospedadores (Wood & Johnson 2015), junto con ciertos aspectos de su biología (Gómez & Nichols 2013), ha causado que la ecología de comunidades parásitas haya permanecido detrás de la ecología de comunidades general (Poulin 2015). Así, los parásitos raramente han sido considerados en estudios de comunidades o ecosistemas (por ejemplo, Rossiter & Sukhdeo 2014), lo cual ha provocado un sesgo sistemático en el estudio de las comunidades. Además, los parasitólogos normalmente han tomado aproximaciones descriptivas para estudiar las comunidades parásitas, en vez de aprovechar los beneficios de las ciencias predictivas (Pedersen & Fenton 2007, Poulin 2007). A pesar de que se han hecho grandes esfuerzos para cubrir esta falta de conocimiento, todavía queda margen para mejorar el entendimiento de los múltiples procesos mediados por parásitos (Poulin et al. 2016).

De momento, existen pocos trabajos que hayan considerado todas las facetas de la diversidad de los parásitos a la vez. Aunque el análisis de redes ha sido mucho más utilizado por los parasitólogos, en pocas ocasiones se ha empleado en un contexto de invasiones biológicas de hospedadores y parásitos. Es por eso, que esta tesis está dedicada al estudio de las comunidades ecológicas de parásitos.

Objetivos

El objetivo principal de esta tesis es avanzar en el conocimiento de las comunidades ecológicas de organismos parásitos. Con este objetivo, aprovecharé y adaptaré las últimas metodologías y técnicas desarrolladas en ecología de comunidades para el estudio de organismos parásitos. Me centraré en el estudio de la diversidad parásita y en la dinámica de las interacciones hospedador-parásitos.

Los objetivos específicos de este estudio son:

1. Como paso previo para lograr el objetivo 2, desarrollar y evaluar dos métodos no destructivos para estimar indirectamente el rasgo funcional masa de individuos parásitos. Comparar los dos métodos no destructivos con la medida directa (báscula) y el enfoque tradicional (aproximaciones geométricas).
2. Establecer un marco teórico para definir los rasgos funcionales de los parásitos. Definir una lista fundamental de rasgos funcionales de los parásitos relacionados con los tres

retos universales a los cuales se enfrentan los organismos (dispersión, establecimiento y persistencia).

3. Identificar las reglas de agrupación de las comunidades de parásitos a diferentes escalas espaciales y bajo la influencia de diferentes factores. Explicar estas normas sobre las tres facetas de la diversidad.
4. Estudiar los roles que los hospedadores juegan para su comunidad de parásitos en función de su carácter nativo o invasor en una comunidad y en relación con los atributos de las especies parásitas. Demostrar que el análisis de redes puede ser una herramienta útil para evaluar el impacto de los hospedadores introducidos en la transmisión de parásitos.

Evaluación de tres métodos para la estimación de la biomasa de invertebrados de tamaño pequeño, utilizando tres especies de parásitos diferentes de gran medida como organismos modelo

Introducción

La biomasa es la masa de organismos vivos de una determinada zona o ecosistema en un momento. La biomasa se puede encontrar en formas líquidas, gaseosas o sólidas (Yadav et al. 2016). En ecología, la importancia de cuantificar la biomasa es fundamental para comprender los procesos que impulsan los cambios en los ecosistemas (Lohbeck et al. 2015). Los invertebrados suelen ser la piedra angular de los ecosistemas (Piroddi et al. 2017, Yebra et al. 2017) y estudios recientes han demostrado que su biomasa es superior a lo que se pensaba (Ellwood & Foster 2004, Wardhaugh et al. 2012). Sin embargo, su tamaño pequeño y su abundancia elevada a menudo dificultan la cuantificación directa de la biomasa en muchos organismos (Wardhaugh 2013). Aunque se han propuesto diferentes estimadores para su masa, muchos de estos son específicos de taxón o momento del ciclo vital, destructivos, laboriosos o pasan por alto la contribución de los apéndices a la masa individual total.

En este trabajo evaluaremos tres métodos para estimar de manera indirecta la masa de invertebrados pequeños (µm–mm). Utilizaremos tres especies de parásitos de morfologías muy diferentes (un acantocéfalo, un crustáceo y un platelminto) como organismos modelo, porque son buenos representantes de la diversidad morfológica de los invertebrados de tamaño pequeño, pertenecen a tres filos diferentes, incluyen especies de cuerpo blando y esclerotizado y tienen diferentes secciones transversales y niveles de ornamentación.

A pesar del uso extendido de medidas lineales para aproximar formas biológicas a formas geométricas (por ejemplo, Kuris et al. 2008), su precisión no ha sido validada. A causa del número creciente de estudios que demuestran la importancia tanto de invertebrados pequeños de vida libre (por ejemplo, Ellwood & Foster 2004, Piroddi et al. 2017) como de parásitos (por ejemplo, Lagrue & Poulin 2016) para los ecosistemas, esta evaluación resulta necesaria. Así, el objetivo de nuestro trabajo ha sido doble: (i) desarrollar y evaluar dos métodos no destructivos para estimar indirectamente la masa de invertebrados a nivel individual que sean aplicables a una gran variedad de invertebrados pequeños; y (ii) probar la precisión de estos dos métodos en comparación con la estimación directa (báscula) y los enfoques basados en aproximaciones geométricas ampliamente utilizadas a estudios anteriores.

Material y métodos

El relativamente gran tamaño (mm–cm) de las especies modelo (*Bolbosoma capitatum*, *Caligus elongatus*, *Campula oblonga*) empleadas en este estudio y su disponibilidad en gran cantidad, nos permite medir su masa de forma directa. Los especímenes estaban conservados en alcohol 70% (*B. capitatum*, *Ca. elongatus*, *C. oblonga*) o montados en preparaciones permanentes (*C. oblonga*). En este trabajo hemos medido la masa de los organismos de forma (1) directa; e indirecta mediante (2) modelado de arcilla; (3) análisis de imágenes (mediante dos métodos diferentes); (4) aproximación de formas corporales a formas geométricas. Para los métodos indirectos, primero medimos el volumen de 20 individuos de cada especie y después multiplicamos cada volumen por la densidad del tejido estimada para cada especie.

Como los ejemplares de las especies modelo eran bastante grandes para ser pesados, utilizamos estos resultados como valores de referencia para probar la precisión de los métodos indirectos. Pesamos a los acantocéfalos y a los crustáceos de forma individual. En el caso del platelminto, para lograr el primer objetivo y demostrar la aplicabilidad del método basado en análisis de imagen, pesamos 41 individuos a la vez y calculamos la masa mediana de un individuo.

Los métodos indirectos, basados en el modelado de arcilla y en el análisis de imágenes, requieren estimaciones del grosor de los animales. Así, con un microscopio óptico calculamos las micras desplazadas verticalmente al enfocar desde la parte dorsal hasta la parte ventral de un individuo en una preparación y contamos el número de divisiones avanzadas en el micrométrico.

El método basado en el modelado de arcilla (Método indirecto 1) fue adaptado del método propuesto por Nesterenko & Kovalchuk (1991) para ciliados. Modelamos en arcilla

comercial los organismos (×2–39) y sus apéndices (×92–217) a gran escala. Después medimos el volumen de agua desplazado por cada modelo (V_m). El volumen del organismo real (V_s) fue calculado como: $V_s = V_m * (L_s/L_m)^3$. Dónde L_s y L_m son la longitud del organismo real y del modelo en arcilla.

El método basado en el análisis de imágenes (Método indirecto 2) consta de tres submétodos, de acuerdo con la morfología de las secciones transversales de los organismos estudiados. El primer submétodo lo aplicamos a C. *oblonga*, que tiene una sección transversal plana, y está basado en el método de Lambden & Johnson (2013) para trematodos. Mediante un microscopio óptico dibujamos el contorno de los organismos, digitalizamos los dibujos y con Fiyi-ImageJ (Schindelin et al. 2012) medimos el área de los dibujos. Multiplicamos el área del cuerpo de cada individuo por su grosor para obtener su volumen. El segundo submétodo lo aplicamos a *B. capitatum*, que tiene una sección transversal circular. Fotografiamos los organismos con una cámara digital. En las fotografías, extrajimos los cuerpos de los organismos y los colocamos ante un fondo negro. Mediante Fiyi-ImageJ convertimos las imágenes en texto binario, es decir, separamos los píxeles del cuerpo de los píxeles del fondo de la imagen. Procesamos la imagen binaria con un *script* implementado en R (R Core Team 2017). Este *script* trata la imagen binaria como columnas de píxeles. Para cada columna asume que la profundidad de esta es idéntica a la anchura (sección transversal circular). Después, calcula el volumen de una columna de píxeles como un cilindro y, por último, suma los volúmenes de todas las columnas de píxeles para obtener el volumen del organismo.

El tercer submétodo está diseñado para trabajar con morfologías complejas, es decir, que combinan secciones planas y circulares, como es el caso de *Ca. elongatus*. Para el tratamiento de estas morfologías complejas, identificamos las formas de las secciones transversales (plana o circular). Entre las secciones planas, distinguimos secciones corporales de diferente grosor y medimos el grosor de cada una. Con un fotomicroscopio óptico fotografiamos las superficies corporales de los individuos. Para cada imagen, separamos las porciones corporales de diferente sección transversal y grosor. Calculamos el volumen de cada porción según el tipo de sección transversal con los dos submétodos descritos en los puntos anteriores. Finalmente, sumamos los volúmenes de todas las porciones para obtener el volumen corporal de un individuo.

Para la aproximación geométrica (Método indirecto 3), medimos la longitud y anchura máximas de cada organismo y asimilamos los cuerpos de los organismos a formas geométricas regulares de acuerdo con lo que se había hecho en trabajos anteriores con especies de los mismos filos a los de las especies modelo (por ejemplo, Kuris et al. 2008).

En cuanto a los análisis estadísticos, en el caso de C. *oblonga* utilizamos una prueba *t* de una muestra con corrección de Bonferroni. En el caso de *B. capitatum* y *Ca. elongatus* empleamos modelos lineales de efectos mixtos para comparar los diferentes métodos (factor fijo) en los individuos (factor aleatorio).

Resultados

En el caso de las tres especies modelo, los resultados de los métodos indirectos 1 y 2 fueron muy parecidos a los obtenidos por el método directo y no difirieron significativamente de este. En cambio, los resultados obtenidos mediante la aproximación geométrica (Método indirecto 3) difirieron significativamente de la estimación directa, puesto que sobreestimaron la masa corporal de los organismos.

Discusión

La estimación de la biomasa de invertebrados de tamaño pequeño supone una serie de retos que se pueden superar mediante métodos indirectos, aunque raramente se ha probado su precisión. Este estudio demuestra que los métodos indirectos propuestos aquí proporcionan una buena estimación de la masa corporal real de los organismos y son mucho más precisos que el método tradicional, basado en la aproximación de las morfologías corporales a figuras geométricas regulares. En particular, el método para estimar la biomasa a partir de imágenes parece más eficaz y requiere menos tiempo que los métodos anteriores, por lo tanto, atienden la creciente necesidad de obtener estimaciones fiables de biomasa de invertebrados (Tackenberg 2007). Hemos validado el método de modelado con arcilla, descrito originalmente para ciliados unicelulares (Nesterenko & Kovalchuk 1991), para poder aplicarlo a invertebrados. Este método puede ser particularmente valioso para organismos con una morfología compleja. Las ventajas de los métodos que aquí proponemos son tres: permiten recuperar el material biológico después de la estimación de su masa, se pueden aplicar tanto a ejemplares frescos como ejemplares en preparaciones permanentes y las imágenes y los modelos de arcilla generados se pueden conservar y utilizar posteriormente en otros estudios.

Hacia un marco unificado de rasgos funcionales de parásitos

Una ecología basada en rasgos

Los estudios ecológicos que se basan en rasgos para explicar las propiedades de los ecosistemas en diferentes entornos o gradientes ambientales han aumentado mucho durante las tres últimas décadas (Cadotte et al. 2015, Moretti et al. 2017, Weiss & Ray 2019). Entre los múltiples tipos de rasgos existentes (Violle et al. 2007), los rasgos funcionales han demostrado ampliamente su utilidad para explicar o predecir una gran cantidad de preguntas ecológicas sobre organismos de vida libre, en particular, cuestiones relacionadas con la faceta funcional de la diversidad, que es, la diversidad funcional (FD) de una comunidad. Los rasgos funcionales también han permitido desvelar los mecanismos mediante los cuales los individuos (o la variabilidad intraespecífica en estos rasgos, Carmona et al. 2016) causan efectos sobre los ecosistemas (Violle et al. 2007). Aun así, el número de estudios que considera los rasgos funcionales de los parásitos todavía es bajo (Mouillot et al. 2005, Keeney & Poulin 2007, Krasnov et al. 2015, 2016, 2019a, 2019b, Sokolov & Zhukov 2017, Warburton et al. 2017) en comparación con el número de estudios de organismos de vida libre. Consideramos que este hecho se debe a tres motivos: (i) una subestimación general de la función que ejercen los parásitos en los ecosistemas, a pesar de que una amplia evidencia que demuestra su importancia (para una revisión véase Gómez & Nichols 2013), (ii) los escasos conocimientos sobre las características biológicas de los parásitos en comparación con otros organismos y (iii) la carencia de un marco unificado para identificar y medir rasgos funcionales en parásitos.

La selección de rasgos funcionales es esencial para conseguir conclusiones ecológicas sólidas, puesto que los rasgos escogidos tienen que ser informativos de una función concreta (Petchey & Gaston 2006) y se tienen que medir mediante protocolos normalizados (por ejemplo, Weiher et al. 1999, Moretti et al. 2017). Los rasgos funcionales tienen que estar relacionados explícitamente con la eficacia biológica de los individuos (Violle et al. 2007). Para facilitar las comparaciones entre grupos de parásitos y promover la reproductibilidad de los estudios, es necesario un marco unificado con una terminología común para los rasgos funcionales de parásitos. Este marco mejoraría y maximizaría la utilidad de los estudios funcionales en parasitología y contribuiría al conocimiento de la función de los parásitos en las comunidades y los ecosistemas de manera más amplia. Además, permitiría comparar las diversidades de parásitos y hospedadores con los mismos términos (véase Weiss & Ray 2019 para comparar los rasgos funcionales entre taxones), abriendo así el camino para que los ecólogos incluyan los parásitos en los estudios de ecología de comunidades.

En este trabajo, proponemos un marco unificado para los rasgos funcionales de parásitos metazoos. El marco está fundamentado en la teoría ecológica actual, y asume el reto de identificar rasgos funcionales suficientemente generales y aplicables a taxones parásitos filogenéticamente distantes, pero sin perder resolución para responder a cuestiones ecológicas.

Múltiples soluciones, un estilo de vida único: rasgos funcionales de parásitos

Independientemente de alcanzar un consenso sobre la definición de parásito, estos organismos comparten los mismos rasgos funcionales, aunque difieran en su forma de vida parasitaria.

- Lista fundamental de rasgos funcionales de los parásitos

Para conseguir que el marco de rasgos funcionales sea comparable a escalas espaciales y temporales, recopilar información funcionalmente representativa, compartir datos y maximizar la aplicabilidad de los resultados, los rasgos funcionales de los parásitos se tienen que ajustar a la definición aceptada en la ecología de comunidades. Los rasgos funcionales tienen que representar la eficacia biológica de los organismos, tienen que medirse a nivel individual y sin hacer referencia a información externa al individuo (Violle et al. 2007). Los rasgos funcionales propuestos aquí están relacionados con tres retos universales, a los cuales se enfrentan los organismos: dispersión, establecimiento y persistencia (Weiher et al. 1999); y su influencia en la eficacia biológica del organismo que los presenta ha sido demostrada previamente por otros autores. Igualmente importante, todos estos rasgos funcionales pueden medirse a nivel del individuo, sin hacer referencia al medio externo o a cualquier otro nivel de organización (Violle et al. 2007, Carmona et al. 2016). De acuerdo con estos criterios, proponemos una lista de rasgos funcionales aplicables, virtualmente, a cualquier parásito metazoo y basados en características morfológicas, de estrategia vital y de comportamiento. El número de rasgos funcionales que consideramos es el mínimo que puede aplicarse a cualquier parásito metazoo y que puede resolver cualquier cuestión ecológica. Estos rasgos funcionales son: órgano de anclaje, masa corporal, número de huevos, medida de los huevos, forma de los huevos, alimentación y ciclo de vida.

- Medir los rasgos funcionales

Reconocemos cuatro fuentes de donde se puede obtener información fiable para medir los rasgos funcionales. (i) Observación directa; (ii) descripción de especies, (iii) protocolos estandarizados y (iv) representantes de un rasgo funcional.

Consideraciones para un marco de rasgos funcionales de parásitos.

- Seleccionar un número adecuado de rasgos funcionales

El número de rasgos funcionales que pueden medirse en un organismo es muy grande. Pero nuestra habilidad para medirlos es limitada. La lista de rasgos funcionales presentada aquí no tiene que considerarse cerrada. Los rasgos funcionales incluidos en un estudio dependen del objetivo de este.

- Utilizar rasgos funcionales "suaves"

Si diferentes rasgos funcionales explican la misma función, tendremos que elegir el más fácil y barato de medir (Weiher et al. 1999), es decir, el más "suave". Así podremos obtener la mayor cantidad posible de información para nuestro estudio.

- Rasgos de ejecución y ejecuciones ecológicas

Cuando no disponemos de toda la información para medir un rasgo funcional a nivel de individuo, pero podemos extraer esta de un conjunto de individuos para obtener un representante del rasgo funcional en cuestión. A este representante se le denomina rasgo de ejecución. La ejecución ecológica es la habilidad de un organismo para responder a variables ambientales. La ejecución ecológica no tendría que ser utilizada como rasgo funcional.

- Ausencia de información

Quizás no tengamos a nuestro alcance toda la información sobre los rasgos funcionales de todas las especies del sistema de estudio. En ese caso, recomendamos utilizar la distancia de Gower (Gower 1971) para medir la singularidad funcional de cada especie porque permite la utilización de valores no disponibles.

- Diferentes estados de desarrollo

Los hospedadores pueden estar parasitados por la misma o diferentes especies de parásitos en diferentes estados de madurez sexual. En ese caso, los investigadores tendrán que decidir qué especies incluir en el estudio, de acuerdo con su objetivo.

Conclusiones

En este estudio establecemos la base de la selección de rasgos funcionales de parásitos de acuerdo con la teoría ecológica actual, con el fin de avanzar en nuestros conocimientos sobre los mecanismos que diseñan las comunidades parásitas y sus dinámicas. Hemos dado las herramientas necesarias para definir nuevos rasgos funcionales dentro de este marco, pero somos conscientes de que estas definiciones pueden estar a expensas de estudios experimentales para identificarlos. Por último, proponemos la creación de una base de datos

de acceso público para que los investigadores tengan a su alcance toda la información disponible de la manera más fácil posible.

Organización de las comunidades de los parásitos helmintos en lisas: combinación de los componentes de la diversidad

Introducción

Los organismos se asocian los unos con los otros y forman comunidades ecológicas. Los ecólogos se han preguntado si estas asociaciones son debidas a procesos deterministas o, al contrario, si las comunidades no son más que colecciones aleatorias de especies (Clements 1916, Gleason 1917).

La diversidad puede medirse a diferentes niveles (α, β y γ) de escalas jerárquicas de organización (por ejemplo, escala geográfica), y/o en sus facetas (es decir, TD, FD y PD) (Pavoine & Bonsall 2011). Además, la diversidad β puede medirse a dos niveles, β1 y β2. La diversidad β1 explica la cantidad de diversidad que es debida a diferencias en la diversidad entre los puntos de muestreo de un mismo nivel de organización (por ejemplo, diferencias en la diversidad de dos puntos de muestreo dentro de una misma localidad); mientras que la diversidad β2 explica la cantidad de diversidad que es debida a diferencias en la diversidad entre los puntos de muestreo de diferente nivel de organización (por ejemplo, diferencias en la diversidad de dos puntos de muestreo de diferentes localidades) (Pavoine et al. 2016).

Como la diversidad tiene múltiples componentes, ambos procesos, deterministas y aleatorios, pueden afectar simultáneamente en la misma comunidad. Es decir, la influencia de un proceso u otro sobre la diversidad de una comunidad depende del nivel al cual se estudie la comunidad.

El estudio en conjunto de la diversidad a diferentes niveles y en sus facetas, ha resultado útil para conocer los mecanismos que determinan la diversidad de organismos de vida libre (Devictor et al. 2010, González-Maya et al. 2016). Sin embargo, nuestro conocimiento sobre los procesos que determinan las diferentes facetas de la diversidad en diferentes escalas de organización de organismos parásitos es limitado (por ejemplo, Mouillot et al. 2005, Krasnov et al. 2014, 2015, 2016). En este estudio examinaremos las reglas que dirigen las diferentes facetas de la diversidad en diferentes escalas de organización en una comunidad parásita. Así, mediremos la diversidad TD, FD y un representante de la PD (PPD) de una comunidad de helmintos parásitos de lisas (Teleostei: Mugilidae) en tres localidades del mar Mediterráneo.

Este modelo es apropiado porque, en primer lugar, las comunidades de parásitos incluyen especies de parásitos de origen filogenético lejano y funcionalmente diferentes. En segundo lugar, las comunidades parásitas provienen de tres de las hasta seis especies de lisas simpátricas que se encuentran en esta zona del Mediterráneo (Blasco-Costa 2009) y de tres localidades que varían en sus parámetros medioambientales. Concretamente, dos de las especies de hospedadores, *Chelon auratus* y *Chelon ramada*, son filogenéticamente más próximas entre ellas que con *Mugil cephalus* (Durand et al. 2012), mientras que *M. cephalus* y *C. ramada* presentan mayores similitudes en sus estrategias de vida entre ellas que con *C. auratus* (Cardona 2001, Cardona 2006). Además, las comunidades provienen de tres localidades costeras que se diferencian en sus condiciones de hábitat (dos marinas: mar del Delta del Ebro - EDS y mar de Santa Pola - SPS; y una laguna salobre: laguna de Santa Pola - SPL) y en su proximidad geográfica (SPS y SPL están muy cerca, a 10 km de distancia; mientras que EDS está más alejada de las otras dos a unos 290 km). Así, podemos evaluar si las diferentes facetas de la diversidad de helmintos proporcionan resultados congruentes y si los factores del hospedador (proximidad filogenética y parecido en las estrategias de vida) y/o los ambientales (ubicación geográfica y condiciones de hábitat) seleccionan diferentes estrategias vitales entre los parásitos. Finalmente, el diseño jerárquico de los muestreos nos permite medir y comparar la diversidad en la unidad de muestreo (diversidad α o diversidad de parásitos al individuo hospedador) y dentro y entre los niveles de un factor (es decir, la diversidad de parásitos entre hospedadores de una misma especie o localidad (β1); o entre hospedadores de diferente especie o localidad (β2)).

Basándonos en estudios de organismos de vida libre (Cavender-Bares et al. 2006) y estudios previos de nuestro modelo de trabajo (Blasco-Costa 2009, Blasco-Costa et al. 2012, Míguez-Lozano et al. 2012, Sarabeev et al. 2013), proponemos que tanto el origen filogenético (*Chelon* o *Mugil*) como la estrategia vital (marina o costera) del hospedador influirán en la comunidad del parásito, mientras que las condiciones del hábitat (marino o laguna) serán más determinantes de las comunidades de parásitos que la distancia geográfica (Delta del Ebro o Santa Pola).

Material y métodos

- Las múltiples facetas de la diversidad

Para medir la FD, construimos una matriz de distancias entre las especies de parásitos de acuerdo con sus atributos (es decir, valores o categorías) para cinco rasgos funcionales: masa, tamaño del huevo, número de huevos, órgano de anclaje y ciclo de vida. Para el cálculo de la

PPD, estimamos las distancias entre especies de acuerdo con su clasificación taxonómica. Para medir la TD, establecimos las distancias entre especies como la distancia máxima, es decir, esta matriz obtuvo el valor 1 (Pavoine et al. 2004).

- El análisis de la diversidad

Organizamos las muestras de acuerdo con tres factores: especie de hospedador, localidad y estación. Después dividimos los análisis de la diversidad en dos Casos de estudio. En el Caso 1, evaluamos la influencia de la especie de hospedador en las tres facetas de la diversidad de los parásitos. Así, analizamos y comparamos la TD, FD y PPD de las comunidades de helmintos de las tres especies de hospedadores en SPS. En el Caso 2, evaluamos la influencia de la localidad en las tres facetas de la diversidad de los parásitos. Así, analizamos y comparamos la TD, FD y PPD de las comunidades de helmintos de las tres localidades en hospedadores de la especie *M. cephalus*.

Para cada caso de estudio medimos la diversidad mediante dos tipos de análisis. Primero medimos la diversidad utilizando el Análisis Doble de Componentes Principales (DPCoA –de sus siglas en inglés). El DPCoA calcula α, β y γ para TD, FD y PPD para un solo factor (Pavoine et al. 2004). Es decir, la diversidad influenciada por el factor especie de hospedador o localidad. Por eso, para eliminar la variación de la diversidad debida al muestreo estacional solo consideramos hospedadores de la misma estación.

Después, mediante el DPCoA–cruzado (Pavoine et al. 2013), evaluamos el porcentaje de diversidad que explica cada uno de los factores del análisis: individuo hospedador, estación y especie de hospedador (Caso 1) o localidad (Caso 2). Es decir, consideramos la influencia de factores cruzados en comunidades parásitas de diferente estación y especie de hospedador (Caso 1) o localidad (Caso 2).

Resultados

La comunidad de parásitos en el individuo hospedador (α) representa un conjunto aleatorio de la diversidad total de la comunidad (γ). El factor individuo hospedador explicó el mayor porcentaje de diversidad de las comunidades (alrededor del 50% de la diversidad) para las tres facetas de la diversidad.

La TD a nivel β1 indicó diferencias menores de las esperadas entre individuos hospedadores de la misma especie de hospedador (Caso 1) o localidades (Caso 2). Mientras FD y PD a este nivel mostraron una agregación aleatoria de la diversidad. A nivel β2, encontramos diferencias mayores de las esperadas entre las comunidades de hospedadores de diferente especie (Caso 1) o localidad (Caso 2) para las tres facetas de la diversidad.

El factor especie de hospedador explicó un 32%, 25% y 18% de la TD, FD, y PPD, respectivamente. Mientras que la localidad explicó un 6%, 12% y 12% de la TD, FD, y PPD, respectivamente. La interacción entre los factores cruzados, especie de hospedador (Caso 1) o localidad (Caso 2) y estación, y el factor estación contribuyeron de forma menos relevante a la diversidad.

Discusión

De nuestros resultados extrajimos las siguientes conclusiones. En primer lugar, la diversidad de estas comunidades de parásitos muestra al menos dos patrones opuestos. Estos patrones se dan a diferentes niveles (α, β1 y β2) de las dos escalas de organización (especie de hospedador o localidad). En segundo lugar, la diversidad de las dos escalas de organización está influenciada por varias variables. En tercer lugar, las tres facetas de la diversidad (es decir, TD, FD y PPD) no siempre mostraron resultados congruentes entre ellas, lo cual no es sorprendente, puesto que es la tendencia general registrada para varios grupos de organismos (por ejemplo, Devictor et al. 2010). Por lo tanto, las conclusiones de un estudio podrían no estar completas si se omite cualquiera de las facetas de la diversidad (Jarzyna & Jetz 2016).

A los niveles intermedios de la escala de organización, la coexistencia de parásitos similares está limitada, al menos, para TD. Mientras que, a niveles más elevados, el entorno filtra la diversidad de parásitos, puesto que una influencia conjunta de origen filogenético de hospedador (*Chelon* vs. *Mugil*) y las preferencias medioambientales (marino o costero) podrían determinar las TD, FD y PPD de los parásitos. A pesar de que esto está menos claro para las dos últimas facetas de la diversidad. Finalmente, las condiciones de hábitat de la localidad parecen ser más determinantes de la TD, FD y PPD que la distancia geográfica.

Las especies nativas e invasoras desempeñan funciones diferentes en las redes de hospedadores y parásitos

Introducción

Las invasiones biológicas son introducciones de especies fuera de su distribución original favorecidas por la influencia de los seres humanos, que consiguen establecer poblaciones viables a lo largo del espacio y del tiempo (Richardson et al. 2000). Las especies invasoras representan una amenaza importante para los ecosistemas, puesto que no dejan transcurrir el tiempo necesario para que las especies nativas puedan desarrollar adaptaciones evolutivas a su

presencia (Poulin 2017). Las invasiones biológicas tienen el potencial de alterar las dinámicas de los parásitos y los hospedadores (Chalkowski et al. 2018). Así pues, los hospedadores nativos e invasores y sus comunidades de parásitos nativos y/o adquiridos pueden interactuar de diferentes maneras con las subsecuentes consecuencias para los ecosistemas (Chalkowski et al. 2018).

Las asociaciones entre parásitos y hospedadores en una comunidad invadida han sido estudiadas mediante diferentes tipos de análisis, aunque pocos trabajos han utilizado el análisis de redes biológicas para explicar este tipo de interacciones ecológicas durante una invasión (Médoc et al. 2017). Estos análisis permiten explorar la manera en que se asocia una comunidad de parásitos con su hospedador (individuo) (Poulin 2010). Generalmente, estas asociaciones bipartitas se caracterizan por no ser aleatorias (Fortuna et al. 2010), es decir, están determinadas por procesos ecológicos y evolutivos, y uno de los patrones que describe estas organizaciones es la modularidad (Newman & Girvan 2004). En redes modulares, se espera que subconjuntos (es decir, módulos) de individuos interactúen con más frecuencia entre ellos que con individuos de otros módulos, y valores más altos de modularidad indican una mejor segregación de los módulos (Newman & Girvan 2004). Así, podemos clasificar la función de cada individuo hospedador en una red modular según el número de interacciones que este presente con otros dentro del módulo al cual pertenece (valor z) y el número de interacciones que presenta con organismos de otros módulos (valor c) (Guimerà & Amaral 2005, Olesen et al. 2007):

1. Organismos centrales en el módulo (*Modul hubs*): son organismos vinculados a otros muchos organismos dentro de su propio módulo (alta z, baja c).
2. Conectores (*Connectors)*: individuos que enlazan varios módulos (baja z, alta c).
3. Organismos centrales en la red (*Network hubs*): organismos que actúan como conectores de módulos y como organismos centrales a su módulo (alta z, alta c).
4. Periféricos (*Peripherals*): organismos que interactúan poco con otros, tanto dentro de su módulo como con organismos de otros módulos (baja z, baja c).

De este modo podemos representar gráficamente la posición de cada organismo según sus valores de c y z, y esperamos que los organismos que tengan una misma posición realicen funciones similares para determinar la estructura de la comunidad parásita.

Para mejorar nuestro conocimiento de las interacciones hospedador-parásitos durante las invasiones, estudiaremos las funciones (análisis de modularidad y cz) desarrolladas por individuos de dos especies de hospedadores (una nativa y una invasora) para sus comunidades parásitas. Caracterizaremos estas redes en una localidad nativa para una de las especies y en una localidad donde una de las especies es nativa y la otra invasora. A menudo, la distribución

de los parásitos es agregada, entonces, estudiar las asociaciones hospedador-parásitos a nivel de individuo hospedador, nos permite controlar la variación intraespecífica y nos da una idea de la importancia relativa de la especie invasora para mantener la transmisión de parásitos en el ecosistema (Godfrey 2013).

Las lisas (Teleostei: Mugilidae) son un modelo excelente para estudiar la variación de las funciones desarrollados por los individuos de una especie para su comunidad parásita según su distribución (nativa o invasora), porque proporcionan un punto de control para esta variación (Sarabeev et al. 2017). En este trabajo estudiaremos las funciones desempeñadas por *Mugil cephalus* y *Planiliza haematocheilus* en un área donde las dos especies son nativas (mar del Japón) y en un área donde *M. cephalus* es nativa y *P. haematocheilus* es invasora (mar de Azov). La llegada *de P. haematocheilus* al mar de Azov supuso un gran cambio en la estructura de su comunidad parásita: perdió las especies parásitas nativas con ciclos de vida complejos, adquirió las especies parásitas con ciclos de vida complejos del área invadida y cointrodujo algunos de sus ectoparásitos, con un ciclo de vida sencillo.

En este estudio utilizaremos los análisis de modularidad y de valores cz para determinar la función desempeñada por los individuos hospedadores para sus comunidades de parásitos. Primero, esperamos que la modularidad sea mayor en las redes del área nativa que en las del área invadida, puesto que los parásitos nativos pueden parasitar a la especie invasora en el área invadida, conectar módulos existentes y provocar una disminución de la modularidad en este área. Segundo, los hospedadores de las dos especies desempeñarán funciones similares en el área nativa, pero en el área invadida los hospedadores de la especie invasora tenderán a desempeñar una función periférica para la comunidad de parásitos, porque sus interacciones no han sido moduladas por una historia evolutiva común.

Material y métodos

Evaluamos la función desempeñada por los individuos hospedadores para las redes que incluían:

1. Toda la comunidad de parásitos helmintos.
2. Los parásitos transmitidos activamente (Monogenea y metacercarias de Trematoda).
3. Los parásitos transmitidos pasivamente/tróficamente (adultos y larvas de Trematoda y Acanthocephala y adultos de Nematoda).
4. Ectoparásitos (Monogenea). Este es el único grupo de parásitos introducidos por *P. haematocheilus* en el área invadida.

Estas cuatro redes se analizaron tanto en el área nativa como la invadida, es decir, en total llevamos a cabo ocho análisis. Después de realizar los análisis de modularidad y de valores cz, evaluamos si los individuos de las dos especies de hospedadores estaban distribuidos de una manera similar entre las cuatro categorías (centrales de módulo, conectores, centrales de red y periféricos) mediante una prueba exacta de Fisher. En los casos en que encontramos diferencias significativas, evaluamos si el número de individuos periféricos de *P. haematocheilus* o *M. cephalus* era mayor o menor del esperado respecto a la proporción observada de individuos periféricos de *M. cephalus* o *P. haematocheilus*, respectivamente.

Resultados

Las redes analizadas fueron modulares. Pero la modularidad fue mayor en las redes del área nativa, excepto en los casos donde se consideró la comunidad de ectoparásitos. En estos casos la modularidad fue igual en el área nativa y en la invadida.

Discusión

Nuestro estudio proporciona un ejemplo claro de como comparar cuantitativamente las funciones desempeñadas por individuos nativos e invasores. Al trabajar en redes a nivel de individuo hospedador, hemos podido estudiar la repartición de la comunidad de parásitos entre individuos de diferentes especies. Así, hemos visto como las funciones de individuos de diferentes especies pueden solaparse, mientras que individuos de la misma especie pueden desempeñar una función diferente.

Al fraccionar las comunidades de parásitos en subcomunitades de parásitos que tienen las mismas características, hemos podido observar que las funciones de *P. haematocheilus* son similares en las áreas nativas e invadidas para las comunidades que incluyen ectoparásitos. De forma que junto con la cointroducción de ectoparásitos (Sarabeev 2015), se mantuvo la estructura de la comunidad.

Además, los individuos de *P. haematocheilus* realizaron una función mayoritariamente periférica para la comunidad total y la transmitida pasivamente/tróficamente. Esto podría ser causado por la inexistencia de una historia evolutiva y ecológica común entre hospedadores y parásitos, lo cual confirmaría la hipótesis de liberación de los enemigos (Torchin & Lafferty 2009). Proponemos que, con el paso del tiempo, los individuos de la especie invasora adquirirán posiciones más relevantes en la red biológica. Por lo tanto, con el seguimiento de las comunidades de hospedadores y parásitos a lo largo del tiempo, podemos establecer la madurez del establecimiento de la especie invasora en un área.

Conclusiones

Esta tesis está dedicada al estudio de las comunidades de parásitos desde una perspectiva ecológica, con especial énfasis en las comunidades de parásitos helmintos de las lisas (Teleostei: Mugilidae). Se han aplicado y adaptado metodologías de vanguardia de la ecología de comunidades para el estudio de las comunidades de parásitos. Estas metodologías incluyen un enfoque basado en el índice Rao para medir las diferentes facetas de la diversidad y el análisis de redes biológicas. Las contribuciones de esta investigación son oportunas, puesto que se inscriben dentro del objetivo actual de la ecología de comunidades de revelar los procesos que determinan la composición de la diversidad. Esta tesis aporta varias consideraciones teóricas y hallazgos nuevos para el estudio y la comprensión de las comunidades de parásitos, que ya he comentado a los capítulos anteriores. Así que, solo destacaré las principales conclusiones en este apartado.

En el capítulo 3, mis coautores y yo desarrollamos y validamos la precisión de los métodos tradicionales y de los métodos basados en modelado de arcilla y análisis de imágenes para estimar la masa de individuos parásitos de tamaño pequeño. Los métodos basados en el modelado de arcilla y el análisis de imágenes proporcionaron la mejor aproximación a la medida directa de la masa de los individuos. Mientras que, la aproximación geométrica, tradicionalmente utilizada, mostró la menor precisión y los resultados diferían significativamente de la medida directa. Por lo tanto, recomendamos firmemente abandonar su uso. La variedad morfológica y el origen filogenético diverso de las especies modelo demostraron que estos métodos pueden ser útiles para cuantificar la masa de una gran variedad de invertebrados. Particularmente, para el objetivo de esta tesis doctoral, la aproximación del análisis de imágenes fue útil para estimar la masa de rasgos funcionales (capítulo 4) de las muestras procesadas en el capítulo 5.

En el capítulo 4, construimos un marco teórico para definir los rasgos funcionales de parásitos basándonos en las consideraciones ecológicas aceptadas actualmente. Además, identificamos siete rasgos funcionales prácticamente medibles en cualquier individuo parásito metazoo y capaz de tratar cualquier cuestión ecológica. Esperamos que este marco ayude a desvelar cuestiones ecológicas y evolutivas en parasitología. Además, mejorará las comparaciones entre estudios e incluso puede inspirar una extensión a parásitos no metazoos. Por otro lado, permitirá comparar la diversidad de parásitos y hospedadores en los mismos términos, y así abrirá el camino para que los ecólogos incluyan a los parásitos en la ecología de comunidades general.

En el capítulo 5, identificamos las reglas que determinan la estructura de la diversidad de comunidades de parásitos de lisas del Mediterráneo occidental. Hemos encontrado que estas reglas dependen del nivel del análisis y de la faceta de la diversidad considerada. En general, el origen filogenético del hospedador (*Chelon* vs. *Mugil*) y las preferencias ambientales de los hospedadores (costeras o marinas) determinan la comunidad parásita de un individuo hospedador. Mientras que las condiciones de hábitat y la ubicación geográfica de las localidades no son tan determinantes de las comunidades de parásitos. De este estudio concluimos que las comunidades de parásitos no se pueden entender plenamente si se deja de lado alguna de las facetas de la diversidad al estudiar las comunidades.

En el capítulo 6, aprovechando el análisis de redes bipartitas y las características exclusivas del sistema hospedador-parásitos de las lisas, evaluamos la función que desempeñan los individuos hospedadores de lisas nativas e invasoras para sus comunidades de parásitos. Mis coautores y yo encontramos que los individuos de ambas especies de hospedadores desempeñaron una función similar en el área donde las dos especies de hospedadores son nativas. Aun así, en el área donde una especie de hospedador es nativa y la otra es invasora, los individuos hospedadores invasores realizaron una función periférica para las comunidades de parásitos, excepto cuando se consideraron sus parásitos cointroducidos. Este hecho sugiere que, junto con la cointroducción, se mantuvo la estructura de las interacciones hospedador-parásitos. Proponemos que el seguimiento a largo plazo de las funciones de los hospedadores invasores para las comunidades de parásitos puede ser una herramienta útil para estimar la madurez del establecimiento de los hospedadores invasores en un ecosistema.

Finalmente, esta tesis muestra cómo se pueden estudiar las comunidades de parásitos a la luz de la teoría de la ecología de comunidades actual. Da herramientas nuevas a los parasitólogos para mejorar la comprensión de la función de los parásitos en los ecosistemas y esperamos que anime a los ecólogos a considerar los parásitos en sus estudios para obtener una visión completa de los procesos de los ecosistemas.

Summary

Summary

Organisms and populations at a place compose a biological community. The assemblage of populations in a community can be influenced by stochastic or deterministic processes. It generally depends on the level at which the community is studied. To ascertain the rules that manage the community composition, one should focus on measuring features meaningful at the community level, such as the diversity of the community. Diversity is the variability of life at a place. Diversity is multifaceted because it includes taxonomic, phylogenetic or functional information about the evolutionary and ecological histories of the organisms and populations. Furthermore, diversity has multiple components because it can be partitioned across hierarchical scales (e.g. spatial scales) composed by levels (i.e. α, β and γ). The simultaneous measurement of the three facets of diversity in combination with its measurement at different levels of an organisational scale is relevant to understand the composition of the diversity in a community. However, other definitions propose to include the strength of interactions among organisms or populations in the measurement of diversity, since the patterns of interactions among organisms can also produce measurable variability between communities.

The study of parasite communities has always lagged behind general community ecology, even though parasites are ubiquitous in all ecosystems, parasitism is the most extended life strategy among extant species and parasites play key roles in ecological processes. I attribute this fact to two main causes. First, the parasitic lifestyle complicates the quantification of the effects of these organisms on the community. Second, parasitologists have commonly adopted a descriptive approach, despite the unrivaled benefits of moving forward a predictive science.

This doctoral thesis aims to increase our knowledge of the community ecology of parasites, with special attention to the helminth parasite communities of grey mullets (Teleostei: Mugilidae). I will not only take advantage of the last analytical methodologies and techniques of general community ecology, but I will also adapt and optimise their use in communities of free-living organisms to organisms with parasitic life strategies. I will focus on the study of parasite diversity and host–parasite dynamics by means of Rao's index of diversity and the bipartite network analysis. With these objectives in mind, I have reached the following conclusions.

First, the two methods based on Clay Modelling and Image Analysis developed in Chapter 3 to estimate mass of small organisms were accurate and did not significantly differ from the direct methods, whereas the traditional Geometric Approximation approach overestimated mass. Consequently, I strongly recommend abandoning its use.

Second, I expect that the theoretical framework and the core list of seven functional traits presented in Chapter 4 will help future researchers to unveil ecological and evolutionary questions in parasitology. Moreover, I hope it will facilitate ecologists to include parasites in their studies.

Third, taking advantage of the methods (Chapter 3) and theoretical considerations (Chapter 4) I found in Chapter 5 that the diversity of the parasite communities from grey mullets from the Western Mediterranean is dependent on the level of the analysis and the facet of diversity considered. Thus, parasite communities cannot be fully understood if any of the facets of diversity is neglected in a study.

Fourth, I conclude in Chapter 6 that grey mullet individuals of two species play different roles for their parasite communities regarding the native or invasive status of the host individuals and the characteristics of the parasite community considered. I propose that long-term monitoring of the roles of invasive hosts in parasite communities can be a useful proxy for estimating the maturity of the establishment of the invasive hosts in an ecosystem.

Overall, this thesis shows how parasite communities can be studied under the light of the current community ecology theory. It gives novel tools to parasitologists to improve our understanding of the role of parasites in ecosystems and we expect it will encourage ecologists to consider parasites in their studies to get a broader comprehension of ecosystem processes.

1.General Introduction

1. General introduction

1.1 Background in community ecology

Neither organisms nor populations live in isolation. They are part of an assemblage of populations in the same area, and all together form an ecological community (Krebs 2001). With the aim of understanding the processes governing diversity, ecologists have recurrently questioned whether communities should be considered as tightly integrated webs composed of species or, on the contrary, as haphazard collections of individual species that merely interact as a consequence of coinciding at the same habitat (Ricklefs 2008, Liautaud et al. 2019). This dichotomy is the basis of the Clements-Gleason controversy, that has pervaded ecological thought for nearly a century. The Clements' deterministic theory views communities as superorganisms, where species jointly react to changes similarly as parts of an organism do (Clements 1916). Accordingly, communities would be clearly defined with sharp boundaries (community-unit concept). Thus, for example, small environmental changes would produce huge shifts from one community to another (Clements 1936). In contrast, Gleason's stochastic view rests on an individualistic community organisation. Species (conceived as real fundamental units in nature) are progressively replaced as the environment changes and there exist broad transition zones of species. Consequently, species simply coexist because they tolerate the same habitat and sharp boundaries between communities can almost exclusively be maintained by physical disturbances, such as sharp variation in soil composition (Gleason 1917). Although Gleason's ideas prevailed over the 20th century, in part thanks to the great number of studies about gradual variation of vegetation in relation to environmental factors (e.g. Whittaker 1967), it is currently accepted that Clements and Gleason paradigms represent the opposite ends of a single deterministic-stochastic continuum of community organisation (Götzenberg et al. 2012, Liautaud et al. 2019).

Steaming from this constant need for understanding community composition, new perspectives have appeared (see Pavoine & Bonsall 2011 for a review) to finally predict the effect of changes in communities produced by, for example, the arrival of new species, the consequences of exploitation of resources or the current climatic change. To ascertain the rules that manage community composition, we have to measure features meaningful at the community level, such as the diversity of the community (Ives 2007). For example, if diversity is reduced and this fact causes several cascade effects, it would suggest that the target community is closest to a Clements community model. In contrast, if the decline does not cause such effects and the interactions of the disappeared species are assumed by others in the

community (i.e. these are redundant species), the community would be close to the Gleason's view (Krebs 2001).

1.1.1 The facets of diversity

Biological diversity or biodiversity (hereafter diversity for simplicity) is the variability of life at a place (Hamilton 2005). Traditionally, ecologists have considered diversity as species diversity, this is Taxonomic Diversity (TD), which combines the number of species (richness) and number of individuals of each species (abundance) in a community (Hamilton 2005, Jarzyna & Jetz 2016). A myriad of indices and mathematical approaches have been developed to quantify TD, mainly looking for the relationship between species richness and the distribution of abundance between species (evenness) (Hamilton 2005). A common objection to this approach is that TD treats all species equally different from (or similar to) each other (Chao et al. 2014) and, therefore, if a species of the community was replaced with another equally abundant, the same diversity values would arise. In other words, TD does not evaluate the ecological or evolutionary redundancy of each species in the community (Pavoine et al. 2005). The incomplete ability of researchers to characterise the ways in which communities were assembled led to develop novel approaches that consider the functional (ecological) and phylogenetic (evolutionary) originality or uniqueness of the individuals or species, to realistically study communities (see references in Pavoine et al. 2005, Pavoine & Bonsall 2011) (Figure 1. 1)

The relative uniqueness of each species in a community can be widely estimated in terms of phylogenetic or functional distance of a species to the others in the community. The combination of the phylogenetic or functional uniqueness of all species in the community with their abundances allows to express Phylogenetic Diversity (PD, richness and abundance of genetically different entities in a community) and/or Functional Diversity (FD, richness and abundance of functional traits in a community) in an analogous way to TD and make them comparable. PD emerged with the idea that the degree of difference in genetic markers among individuals can be included in diversity estimators (Pavoine & Bonsall 2011). Whereas FD rests on the assessment of functional traits that are features measurable at the individual level which impact the fitness of the individual and reflect the individual performance at the ecosystem (Violle et al. 2007). Thus, the main advantages of PD and FD indices are that they provide diversity not only considering the abundance of the taxonomic entities (usually species) in the community, but also according to the relative functional or evolutionary uniqueness of each of them (Pavoine & Bonsall 2011) (Figure 1. 1). The usefulness of combining of TD with FD

and PD to understand the assembly of communities has been demonstrated over the last years, mainly in communities of free-living organisms. For example, studies on birds across France (Devictor et al. 2010) and mammals across Costa Rica (González-Maya et al. 2016) revealed discrepancies between TD, FD and PD and highlighted the complementarity of taking the three facets of diversity into account to take management decisions that cover a larger spectrum of diversity (Devictor et al. 2010, González-Maya et al. 2016).

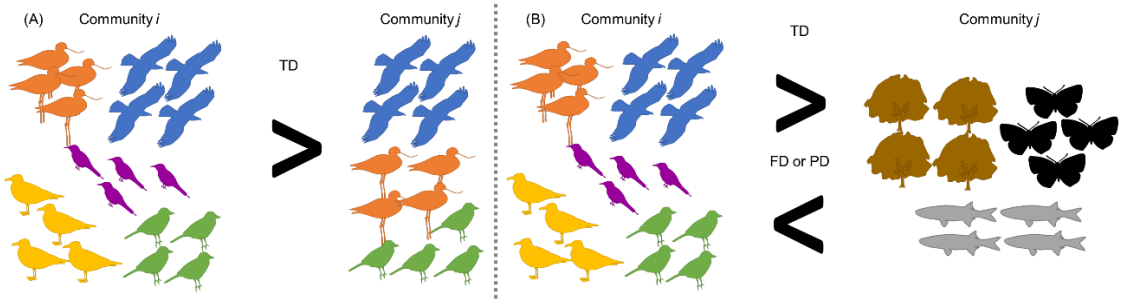

Figure 1. 1. A and B show two communities each with the same abundance for all the species present, but with higher Taxonomic Diversity (TD, species diversity) in community *i* than in community *j* in both cases. Species in community *j* (B) have been replaced with species phylogenetically (evolutionary) and functionally (ecologically) distant from those of community *i*. According to TD measurements, community *i* is more diverse than community *j* both in (A) and (B). However, according to Phylogenetic Diversity (PD) and Functional Diversity (FD) measurements, community *j* is more diverse than community *i* in (B). (Partially modified from Llopis-Belenguer et al. 2019).

Functional traits should be measured at the individual level, but in practice species (or population) mean trait values have been usually employed as surrogates of the original trait. Thus, ongoing research is focused on the development of individual functional variability measures of diversity (e.g. Carmona et al. 2016). Furthermore, some researchers in the field have questioned whether abundance is always the best measure of the relative contribution of different species to the diversity of a community. For instance, two species with equal abundance can differ by one or more orders of magnitude in terms of their biomasses (i.e. mass of living organisms from a species) (Poulin 2015). Hence, incorporating species biomass in diversity indices might be more relevant than using the species abundances (Pavoine et al. 2013).

The three facets of diversity defined above are often partitioned across hierarchical scales and the level at which they are measured is also relevant to understand the composition of the diversity in a community (Figure 1. 2). So α diversity refers to the diversity found at the sampling unit; γ diversity describes the total diversity in a community (sampling units are pooled together); and β diversity indicates the amount of diversity due to differences between sampling units from the same community (Whittaker 1960). Thus, β diversity relates the other

two levels of diversity in a multiplicative ($\beta = \gamma/\alpha$) or additive ($\beta = \gamma-\alpha$) decomposition (Whittaker 1960, Lande 1996). Among the multiple approaches to measure diversity, Rao's index (Rao 1982) provides a framework for partitioning diversity into α, β and γ components. It combines a matrix of species abundances with a distance matrix of pairwise species dissimilarity (functional or phylogenetic), which is set as 1 (maximum dissimilarity) when aiming at studying TD. Hence, the Rao index of Diversity provides a standardised methodology applicable to study diversity at its different levels and facets (de Bello et al. 2010). However, researchers realised that, although this mathematical reasoning is correct, β diversity leads to biological incongruences (Jost 2006, 2007). This is because β is dependent on α (Jost 2007), namely β decreases (like in an identical composition) as α becomes larger, even getting negative values that are biologically inacceptable (Villéger & Mouillot 2008, de Bello et al. 2010). In consequence, the β partitioning is only correct when the different sampling units hold exactly the same total abundance, which is rarely the case in ecology (Villéger & Mouillot 2008). To resolve this issue the "equivalent numbers" correction of diversity was proposed (Jost 2007). The equivalent number of a diversity measure is the theoretical value of diversity that we would obtain if all the species were equally likely and maximally dissimilar (Ricotta & Szeidl 2009, Pavoine et al. 2016). Hence, the equivalent numbers correction is the diversity found in a null community that is neutral with respect to the effect of an uneven abundance distribution on the calculation of diversity (Ricotta & Szeidl 2009). Consequently, Jost (2007) proposed to transform α and γ results into their equivalent numbers: $\alpha_{eqv} = 1/(1-\alpha)$ and $\gamma_{eqv} = 1/(1-\gamma)$; prior to computing β: $\beta_{eqv} = \gamma_{eqv}/\alpha_{eqv}$ or $\beta_{eqvAdd} = \gamma_{eqv}-\alpha_{eqv}$. Note that in α_{eqv} and γ_{eqv} abundances are expressed as relative abundances (de Bello et al. 2010). Finally, de Bello et al. 2010 proposed to normalise β_{eqv} to the interval [0, 1] to make it comparable across studies.

Currently, it is well appreciated that diversity need to be integrated across spatial scales (Pavoine & Bonsall 2011). The contrasting results obtained at different organisational levels can reconcile the ideas of Clements and Gleason up to a point, since we would expect that forces affecting diversity are scale-dependent (Cavender-Bares et al. 2006). However, the mathematical framework on which diversity is partitioned allows other types of decompositions that are equally revealing but not so commonly used. For example, Pavoine et al. (2009) partitioned diversity across temporal scales to study fish PD across time.

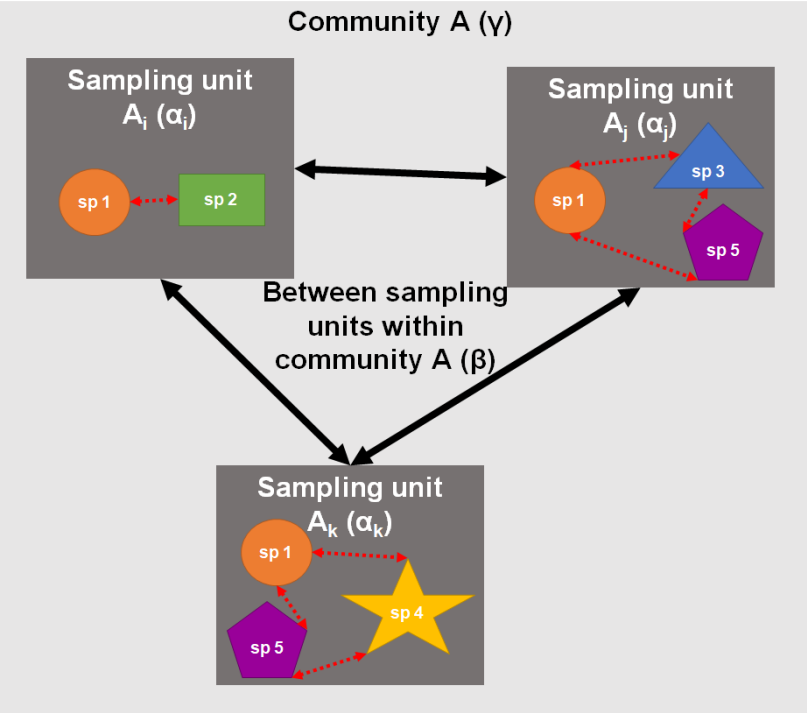

Figure 1. 2. Biodiversity can be measured at different levels of a hierarchical scale, such as spatial scales. In community A, the species (sp) occur in three sampling units (i, j, and k). Gamma diversity (γ) is the diversity of species in the community. Alpha diversity (α) represents the diversity at the sampling unit. Beta diversity (β) is the dissimilarity in species composition between sampling units within the community. Black arrows refer to the differences measured between the sampling units required to calculate the β component. Red arrows indicate the possibility of calculating the distance between species (in terms of genetic or functional distances) to compute the phylogenetic and functional facets of biodiversity. (Partially modified from Llopis-Belenguer et al. 2019).

1.1.2 Ecological networks

In addition to the multiple facets of diversity described, the patterns of interactions among organisms in a community can also produce measurable variability between communities (Bascompte 2009). In fact, it has been proposed that the analysis of diversity should also include information about the strength of interactions among organisms or species, or the connectance of the community (i.e. proportion of realised ecological interactions among the potential ones) (Pimm 1984). Extending things further, a recent definition, considers ecological communities as networks of biological interactions that vary in strength and integration (Poisot et al. 2015).

Indeed, biological interactions are among the main determinants of diversity composition and dynamics in a community, since organisms rely on others from the same or different guild to make the best of their performance (Fontaine et al. 2011). The fact that interactions between pairs of organisms can be, for instance, (i) abundant because of the vast numbers of interactors (Poulin 2010), (ii) asymmetric due to the relative unequal dependence

between the two mutual interactors (Bascompte et al. 2006), (iii) modular because of the existence of subsets of organisms that interact more frequently among them than with others at the community (Newman & Girvan 2004) and/or (iv) variable throughout time (Poisot et al. 2015) reflects the complexity of biological communities. In order to understand and describe the rules that determine the complexity of interactions between organisms of different guilds (e.g. hosts–parasites) in a community, we can use the bipartite network analysis (Poulin 2010).

Traditional approaches to studying interactions in communities have relied either on experiments or on mathematical models. Both approaches suffer from serious shortcomings (Poulin 2010). The former can be logistically challenging, the latter depends on simplifying assumptions, and both can only address interactions among a few species at a time. Derived from graph theory and first used in other disciplines, the bipartite network analysis allows to entirely study complex systems, such as ecological communities, to finally extract their mathematical properties according to the distribution of links between interactors (Poulin 2010). Hence, it represents interactors (i.e. nodes, e.g. hosts or parasites) and actual interactions (i.e. links) between sets of organisms from different guilds (Dormann et al. 2009). In a biological context, it has been applied to disentangle ecological associations in communities. For example, Tylianakis et al. (2007) found that, despite little variation in species richness in bee, wasp and their parasitoids, habitat modification affected interactions among these species, with one or few interactions dominating the community in intensively managed agricultural habitats. These results highlighted that conventional species-composition descriptors failed to discriminate adequately among habitats, and that, when the structure of communities is overlooked, an important effect of habitat modification by humans remains hidden (Tylianakis et al. 2007).

1.2 Parasite communities: current status

Due to the extraordinary complexity of most communities, usually, ecologists make their studies feasible by focusing on, for instance, species from the same functional group (those engaged in similar processes, such as parasitism), on species that directly interact (e.g. hosts and their parasites) and/or limit their scopes to specific spatial and temporal scales (Morin 2011).

Parasites are ubiquitous in all ecosystems and they represent the most common lifestyle among extant species on Earth (Poulin & Morand 2000). However, parasites are most often viewed as threats to their hosts and the loss of parasites might be understood as an unrivalled

benefit for free-living biodiversity (Wood & Johnson 2015). Contrary to this view, it has been shown that parasites constitute a vital part of ecosystem functioning (Gómez & Nichols 2013). For instance, many studies have reported parasite-mediated ecological processes, such as host-parasite interactions as drivers of diversification in a Red-Queen-race manner (Hudson et al. 2006). Parasites also affect the abundance, richness and evenness components of host diversity (Frainer et al. 2018). Moreover, they are enhancers of the connectivity and nestedness parameters of food webs (Lafferty et al. 2006), transformers of the nutrient cycling (Vannatta & Minchella 2018) and predictors of the success of invasive host species (Sarabeev et al. 2017). From a technical point of view, the study of parasite communities has at least two unrivalled advantages over communities of free-living organisms. First, ecological studies of free-living organisms repeatedly ask to precisely define the sampling units according to the aim of the study, because the community found will broadly depend on the spatial extent and ecological variability of the sampling units (Götzenberg et al. 2012). In contrast, in parasitology, the sampling unit is clearly defined, since it usually consists of an individual host. So, the parasite species found in/on a host represent a community by themselves (infracommunity *sensu* Bush et al. 1997). This fact leads us to the second advantage. Since the sampling unit can be precisely defined, it also makes relatively easy to obtain replicates of the sampling unit (i.e. host) to get a complete representation of the whole parasite community of one or several host species in a locality (component community *sensu* Bush et al. 1997).

However, despite their key role in ecological processes and the benefits of their study design, parasite community ecology has always lagged behind general community ecology (Poulin 2015). I principally attribute this fact to two causes. First, parasites have been traditionally ignored in mainstream community ecology, partly because different factors (e.g. small size, complex life cycles, generalised taxonomic impediments) complicate quantifying parasite effects on the community (discussed in Gómez & Nichols 2013), and all these complexities become even more difficult in the marine realm because of, for example, the uncertainty of the community bounds (Poulin 2010). Hence, ecologists have seldom considered parasites in studies concerning all of the species in communities or ecosystems (e.g. Rossiter & Sukhdeo 2014). This lack of knowledge might lead to systematic biases, which pose a problem in the current situation where global climate change puts ecosystems under pressure (Vitousek et al. 1997). In fact, it has already been shown that climate change is driving a loss of parasite diversity that can alter ecosystems at different scales (Cizauskas et al. 2017). Second, parasitologists studying communities have commonly adopted a descriptive approach, despite the benefits of moving towards a predictive science (Pedersen & Fenton 2007, Poulin 2007).

Although great effort has been made recently to solve this gap, there is still much to be done to improve our understanding on the huge amount of parasite-mediated ecological processes, especially in marine communities (Poulin et al. 2016). To this end, one can take advantage of the approaches and techniques developed from community ecology of free-living organisms (Pedersen & Fenton 2007).

The earliest ecological studies of parasitic organisms were principally concerned with human disease agents. The field of epidemiology considerably progressed, although it had little impact on ecological studies of animal and plant parasites (Anderson 1981). Late in the 80s, a couple of studies by Bush & Holmes (1986a, 1986b) triggered off the discipline. These authors were the first to rigorously quantify the presence and intensity of parasite species within individual hosts, and to place parasite communities (also called parasite assemblages, arguing that the parasites in and/or on the same host do not always interact, Poulin & Morand 2004) into the conceptual framework of mainstream community ecology (reviewed by Goater et al. 2014). These early studies were followed by a prominent increase in the number of publications since the 90s (Poulin & Morand 2004).

The study of parasite diversity has provided insight into parasite species history and biogeography, structure of ecosystems and processes behind the diversification of life (Poulin & Morand 2004). However, the most obvious manifestation of diversity, that is the number of species, made parasitologists to traditionally consider TD as the almost exclusive component of diversity, and thus to ignore its functional and evolutionary facets (Poulin & Morand 2004, Poulin 2015). To date, few studies have investigated patterns of TD, PD and FD independently or in combination, in comparison to those of plants, mammals, birds or insects. Studies about the different facets of diversity of metazoan parasites come exclusively from parasite communities of fish and mammals to best of our knowledge. The first attempt was conducted by Poulin & Mouillot (2004). These authors studied how PD of parasite communities was driven by mammal host traits. This was followed by a study aimed at looking for the rules that explained the assemblage of nine congeneric species of monogeneans of fish (Mouillot et al. 2005). These authors based their analysis on a null model which tested the probability of coexistence and considered the phylogenetic and functional singularity of each species. In this line, Luque et al. (2004) and Luque & Poulin (2008) observed that PD of fish parasite communities was more sensitive to host features than TD. Keeney and Poulin (2007) illustrated how to quantify FD in terms of functional richness and functional evenness considering a single functional trait (the position of the parasite along the intestine of elasmobranchs) and four databases of cestodes as representatives of communities. After that,

a fruitful collection of papers were published aiming at understanding the rules that manage ectoparasite communities of small mammals at different spatial scales and using different facets of diversity (Krasnov et al. 2005, 2012, 2014, 2015, 2016, 2019a, 2019b, Warburton et al. 2017, Vinarski et al. 2019, Maestri et al. 2020). From them, we can conclude that TD, PD and FD differently vary with host traits, host phylogenetic background, environmental variables and geographic distance, and that these factors can simultaneously affect the same community but at different organisational levels (i.e. α, β and γ). Lately, Sokolov & Zhukov (2017) studied FD of the parasite assemblages of a fish species considering a single functional trait, the path of infestation, in a native and an invaded area. These authors found that FD was lower in the invaded area than in the native one. Despite all these advances in the field, there is still room to improve our knowledge of parasite diversity, get stronger conclusions and make results comparable only if future studies are grounded on a common framework.

The study host-parasite interactions as bipartite networks has received much more attention than the study of the facets of diversity, especially after Poulin (2010). In this article, the author introduced the bipartite network approach to parasitologists and encouraged them to use this tool to shed light on parasite ecology and diversity. In this regard, network analysis was employed to understand the roles played (i.e. position occupied) by endoparasites at different developmental stages and ectoparasites in fish parasite communities (Bellay et al. 2011, 2013, 2015). It was also used to study latitudinal (Guilhaumon et al. 2012, Morris et al. 2014), seasonal (Samsing et al. 2017) and temporal (Pilosof et al. 2013) dynamics in parasitic arthropods associated with mammal or fish hosts. Network analysis has also enabled to draw the evolutionary forces behind different host-parasite systems (Mouillot et al. 2008, Brito et al. 2014) and disentangle the dynamics of hosts in social networks by tracking parasitic loads (MacIntosh et al. 2012, Fenner et al. 2011). In this sense, it was proposed that behaviour-altering parasites can modify the role of individual hosts within their social network (Poulin 2018), and network analysis is considered as an effective tool to model epidemic spread (Pilosof et al. 2017). Furthermore, this approach can be useful in applied conservation, since it has been shown that host fish-parasite coextinction can cause faster loss of diversity and structure of communities than expected under random extinction scenarios (Dallas & Cornelius 2015). However, despite the usefulness of the network analysis to predict changes in communities, it has been scarcely applied to the study of host-parasite associations in an invasion context. One of the few examples is the study of Amundsen et al. (2013) in which the authors evaluated how the introduction of two fish species, followed by the co-introduction

of five parasite species and four predatory bird species, altered the topology of a native food web.

1.3 This study

This study has been made possible through a predoctoral contract (ACIF/2016/374), three visiting studentships to the Muséum National d'Histoire Naturelle (France) (BEFPI/2017/062), to the University of Canterbury (New Zealand) (BEFPI/2018/012) from Conselleria d'Educació, Investigació, Cultura i Esport (Generalitat Valenciana, Spain) and the European Social Fund, and to the Muséum d'Histoire Naturelle-Ville de Genève (Switzerland) (UV-MOACDOC14_15-285269) from the University of Valencia (Spain); and a research grant funded by MINECO-FEDER, EU (Spain) (CGL201571146-P).

This doctoral thesis is devoted to the study of parasite community ecology. In an early stage of the thesis my co-authors and I realised that the trait mass is a functional trait for parasites (e.g. Koehler et al. 2012, Cramer & Cameron 2006, Poulin & Latham 2003), since it can be measured at individual level and it affects individual fitness. However, we lacked satisfactory methods to estimate individual mass of small parasites and other invertebrates (from μm to a few mm). Hence, we developed non-destructive methods to indirectly estimate individual parasite's mass and tested their accuracy (Chapter 3). Then, we aimed at elaborating a core list of functional traits of parasites and a framework for future studies that defines functional traits of parasites under a common terminology and facilitates comparisons between groups of parasites and promotes reproducibility (Chapter 4).

Taking advantage of the methods (Chapter 3) and theoretical considerations proposed (Chapter 4), we studied the helminth parasite communities of the grey mullets (Teleostei: Mugilidae) from the Mediterranean Basin. We studied the rules that manage the facets of diversity at different organisational levels of these communities from the Western Mediterranean Sea (Chapter 5). These communities include parasite species from distant phylogenetic origins and that are functionally disparate. Furthermore, they come from three of the up to six sympatric grey mullet species that coexist in this area of the Mediterranean (Blasco-Costa 2009). Particularly, two of the host species, *Chelon auratus* and *Chelon ramada*, are phylogenetically closer to each other than *Mugil cephalus* (Durand et al. 2012), whereas *M. cephalus* and *C. ramada* show greater similarities in their life strategies than *C. auratus* (Cardona 2001, Cardona 2006).

In Chapter 6, we studied the host-parasite networks in an invasion context. Grey mullets and their helminth parasites represent an excellent system to study and compare the

role of individuals of a host species depending on its distribution (native or invasive) because it provides a benchmark to control for such variation (Sarabeev et al. 2017). Since 1972 a grey mullet species, *Planiliza haematocheilus*, was repeatedly introduced from its native area (Sea of Japan) into the Black Sea and the Sea of Azov (Sabodash & Semenenko 1998, Occhipinti-Ambrogi & Savini 2003), whereas *M. cephalus* s.l. is a species native to both the Sea of Japan and the Black – Azov basin (Whitfield et al. 2012). The arrival of *P. haematocheilus* at its new habitat entailed a deep structural change in its parasite community (Sarabeev et al. 2017). Here (Chapter 6), we used bipartite networks to study the roles played by individuals of *P. haematocheilus* and *M. cephalus* s.l. for their parasite communities in the native area for both hosts (Sea of Japan) and in an area where *P. haematocheilus* is invasive and *M. cephalus* is native (Sea of Azov).

2. Objectives

2. Objectives

Aim

The aim of the present thesis is to advance the current knowledge of parasite community ecology. I will not only take advantage of the last analytical methodologies and techniques of general community ecology, but I will also adapt and optimise them from their use in communities of free-living organisms to organisms with parasitic life strategies. I will focus on the study of parasite diversity and host–parasite dynamics. On the one hand, I wish to provide theoretical and analytical tools to parasitologists to understand the rules that determine parasite diversity in communities. On the other hand, I hope to encourage ecologists to consider parasites in their community ecology studies to obtain fully grounded conclusions in their studies.

Specific objectives

In order to accomplish the main objective, I undertook the following specific objectives:

1. To develop and evaluate two non-destructive approaches to indirectly estimate the functional trait mass of parasite individuals, as well as to test the accuracy of the two non-destructive methods in comparison with the direct measurement (weighted with a scale) and the traditional approach (geometric approximation).
2. To establish a theoretical framework to define functional traits of parasites by setting a core list of functional traits of parasites related to three universal challenges faced by organisms (dispersal, establishment and persistence).
3. To identify the assembly rules that determine parasite communities at different spatial scales and under the influence of different factors. To explain these rules regarding the three facets of diversity.
4. To study the roles that host species play to its parasite community depending on its native or invasive status in a community and relying on the attributes of the parasite species. To show that the network analysis can be a useful tool to evaluate the impact of introduced hosts on parasite transmission.

3. Evaluation of three methods for biomass estimation in small invertebrates, using three large disparate parasite species as model organisms

3. Evaluation of three methods for biomass estimation in small invertebrates, using three large disparate parasite species as model organisms

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3.1 Abstract

Invertebrate biomass is considered one of the main factors driving processes in ecosystems. It can be measured directly, primarily by weighing individuals, but more often indirect estimators are used. We developed two indirect and non-destructive approaches to estimate biomass of small invertebrates in a simple manner. The first one was based on clay modelling and the second one was based on image analysis implemented with open-source software. Furthermore, we tested the accuracy of the widely used geometric approximation method (third method). We applied these three different methods to three morphologically disparate model species, an acanthocephalan worm, a crustacean and a flatworm. To validate our indirect estimations and to test their accuracy, we weighed specimens of the three species and calculated their tissue densities. Additionally, we propose an uncomplicated technique to estimate thickness of individuals under a microscope, a required measurement for two of the three indirect methods tested. The indirect methods proposed in this paper provided the best approximation to direct measurements. Despite its wide use, the geometric approximation method showed the lowest accuracy. The approaches developed herein are timely because the recently increasing number of studies requiring reliable biomass estimates for small invertebrates to explain crucial processes in ecosystems.

3.2 Introduction

Biomass is the mass of living organisms from a given area or ecosystem at a point in time that can be found in liquid, gas and solid forms (Yadav et al. 2016). In ecology, the importance of quantifying biomass stems from understanding the processes that drive changes in ecosystems (Lohbeck et al. 2015). For instance, vegetal biomass has been considered as the principal factor promoting the first phase of ecological succession in forests (Lohbeck et al. 2015), high loads of soil microbial biomass reduces the efficacy of a biological agent on plant pathogens (Bae & Knudsen 2005), species with greater biomass are expected to have lower probabilities to become extinct, which might reduce the impact on ecosystem functioning under extinction scenarios (Schläpfer et al. 2005) and the variation in abundance (as proxy of biomass), not in richness, in few species of bees drive ecosystem services (Winfree et al. 2015). Furthermore, it has been suggested that measuring diversity using biomass in community ecology studies might be more insightful than using species abundances (Pavoine et al. 2013). Although biomass is an extremely important attribute, its estimation represents often a challenge, among other reasons, because of the difficulty in identifying the unit measured (Bao et al. 2000), the need to manipulate or destruct samples (Postel et al. 2000), the lack of resolution in large-scale studies (Broadbent et al. 2008, Réjou-Méchain et al. 2017) or the impossibility to discern dead from alive individuals (Zetsche & Meysman 2012).

Invertebrates are often the cornerstone of ecosystems (Piroddi et al. 2017, Yebra et al. 2017) and recent studies have shown that their biomass is greater than that previously thought (Ellwood & Foster 2004, Wardhaugh et al. 2012). Different methods have been proposed to study the allocation of biomass between various taxonomic groups of invertebrates, mostly arthropods, but also considered groups include sponges, cnidarians, platyhelminths, annelids, acanthocephalans, nematodes, molluscs, nemerteans, echinoderms, bryozoans and urochordates have been considered (Aznar et al. 2001, Ellwood & Foster 2004, Kuris et al. 2008, Novack-Gottshall 2008, Cedergreen et al. 2013, Lambden & Johnson 2013, Wardhaugh 2013, Martins et al. 2014, Reed et al. 2016, Eklöf et al. 2017). As direct measurements of biomass of small invertebrates (i.e. body length from μm to a few mm), common practices include weighing wet (Heine et al. 1991, Postel et al. 2000, Yebra et al. 2017) dry (Richardson et al. 2000) or ash-free dry masses (Oosterhuis et al. 2000); and measuring elements or biomolecules in a sample (Cedergreen et al. 2013, Yebra et al. 2017). However, small body size and high abundance often hampers direct quantification of biomass in many organisms (Wardhaugh 2013). Therefore, indirect estimators have been proposed, such as using body surface areas or volumes as proxies of individual mass based on linear measurements (George-

Nascimento et al. 2002, Alcaraz et al. 2003, George-Nascimento et al. 2004, Hernández-León & Montero 2006, Poulin & George-Nascimento 2007, Kuris et al. 2008, Novack-Gottshall 2008, Hernández-Orts et al. 2012, Koehler et al. 2012, Lagrue & Poulin 2016), linear lengths of different features converted into biomass through generalised regression equations (Sample et al. 1993, Lambden & Johnson 2013, Wardhaugh 2013, Martins et al. 2014, Reed et al. 2016, Eklöf et al. 2017), displacement of water volume in a graduated cylinder (Postel et al. 2000, George-Nascimento et al. 2002, Poulin & George-Nascimento 2007, Santoro et al. 2013, Yebra et al. 2017), or biovolume estimated using confocal microscopy and image analysis (Roselli et al. 2013). Nonetheless, most of these methods are taxon- or age-specific, destructive, laborious and time consuming or overlook the contribution of appendages to the total individual mass.

In the present paper, we evaluate three different approaches to estimate biomass in small invertebrates, using three notably dissimilar in shape parasite species (an acanthocephalan, a crustacean and a flatworm) as model organisms. Although neglected at first (see references in: Lagrue & Poulin 2016), the increasing number of studies pointing at the importance of parasite biomass in ecosystem functioning (George-Nascimento et al. 2004, Poulin & George-Nascimento 2007, Kuris et al. 2008, Lafferty 2008, Preston et al. 2013, Lagrue & Poulin 2016, Soldánová et al. 2016) demand accurate and easy-to-apply procedures to estimate this component of biodiversity. We contend that, although the three model species analysed here, each have a parasitic mode of life, they are good representatives of morphological diversity of small invertebrates in general, because they represent three different phyla, cover both soft and hard-body species, with different transversal sections and levels of ornamentation (Figure 3. 1).

Despite the wide use of linear measurements to implement geometric approximations (see references above), to the best of our knowledge, their accuracy has not been validated with alternative methods for size/biomass estimators before. Due to the growing number of studies testing functions of both free-living small invertebrates (e.g. Ellwood & Foster 2004, Piroddi et al. 2017) and parasites (e.g. Lagrue & Poulin 2016) in ecosystems this real critical appraisal is long overdue. Using three phylogenetic and morphologically disparate invertebrate species as models, the aim of our work was twofold: (i) to develop and evaluate two non-destructive approaches to indirectly estimate individual mass, which can be applied to a wide range of small invertebrate and entails the challenge of being applicable to a huge diversity of forms; and (ii) to test the accuracy of these two methods in comparison with direct estimation and the approaches based on geometric approximations widely used in previous studies.

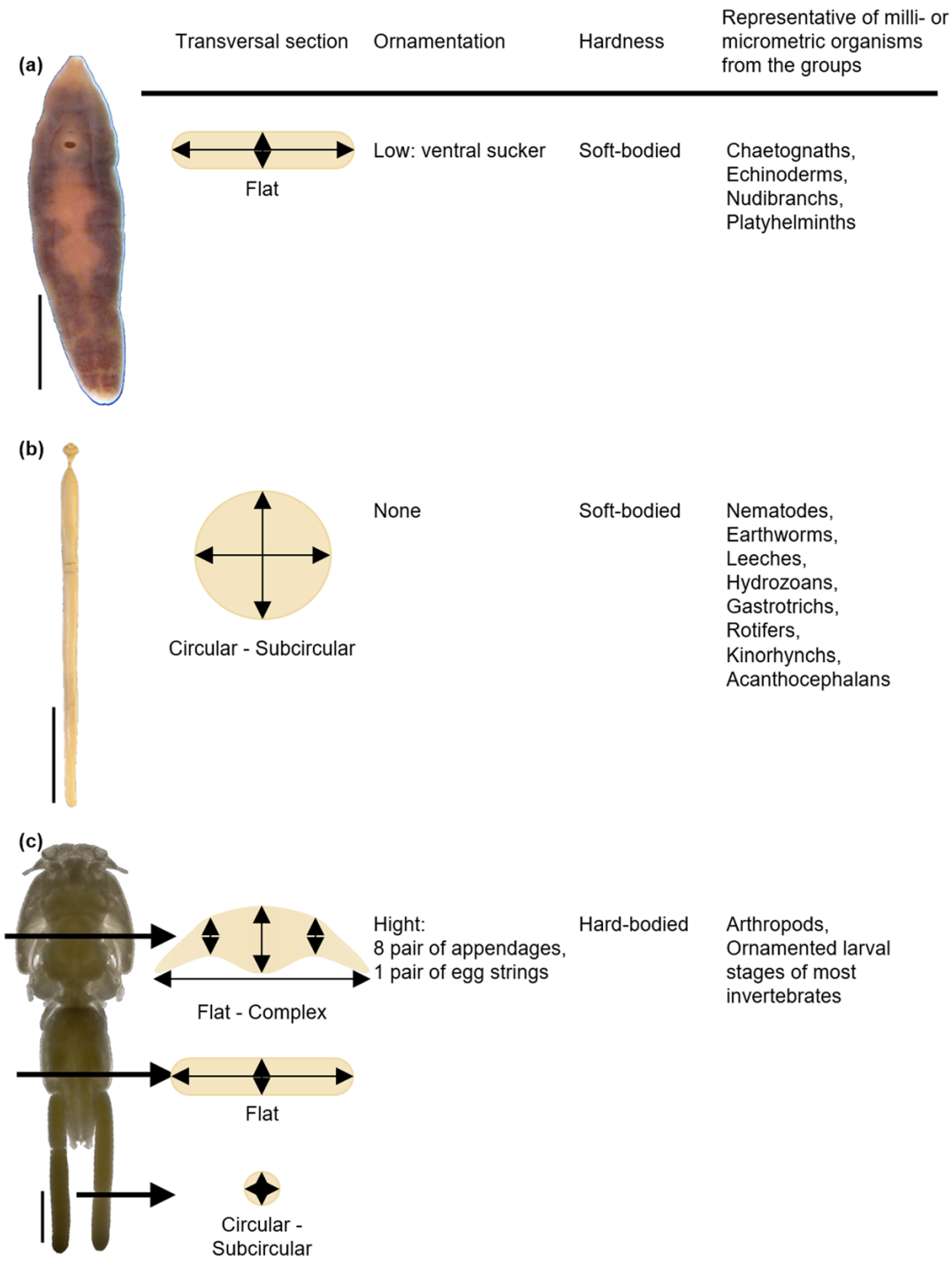

Figure 3. 1. Phenotypic traits that justify the use of the model species. (a) *Campula oblonga*, (b) *Bolbosoma capitatum* and (c) *Caligus elongatus* as model species of the biomass indirect estimation methods. Scale bars 2, 20 and 1 mm, respectively.

3.3 Material and Methods

Model Specimens

We based our analyses on three disparate species (Figure 3. 1): the flatworm *Campula oblonga* Cobblod (Platyhelminthes, Trematoda), the acanthocephalan *Bolbosoma capitatum* Porta and the

crustacean *Caligus elongatus* von Nordmann. These were selected as models because of their relatively large size (mm) and their availability in sufficient numbers for the present study, thereby allowing to estimate their biomass directly. *C. oblonga* is a relatively large trematode (4–8 mm long × 1–2 mm wide), which inhabits the hepatic and bile ducts of small toothed whales (mostly Phocoenidae) in the northern hemisphere (Adams et al. 1998). *B. capitatum* is a large acanthocephalan (34–99 mm × 1.5–3.5 mm) found in the intestine of large, pelagic toothed whales all over the world (Balbuena 1991). *Ca. elongatus* (body length 5–6 mm) is an extremely common parasitic copepod in the North Atlantic, which has been reported on over 80 species of teleosts and elasmobranchs (Piasecki 1996, Jackson et al. 2000).

The specimens used herein are part of our research institute parasite collection's and have been collected over the years in necropsies of cetaceans and fishes. *C. oblonga* individuals were collected from *Phocoena phocoena* (Linnaeus), *B. capitatum* from *Globicephala melas* (Traill) and *Pseudorca crassidens* (Owen) and *Ca. elongatus* from *Gadus morhua* Linnaeus. The parasite specimens were in good condition at the time of collection, i.e. no sign of degradation of lysis was observed, and either preserved in ethanol 70% (*B. capitatum, C. oblonga* and *Ca. elongatus*) or in microscope slides mounted in Canada balsam (*C. oblonga*). Since there is a marked sexual dimorphism in *Ca. elongatus*, the specimens used herein for the sake of demonstration of the methods were all females. The reader is referred to the Discussion for guidelines for dealing with intraspecific morphological differences.

In this paper, we performed (1) direct measurements of mass of parasites; and indirect measurements based on (2) clay modelling, (3) image analysis (two approaches) and (4) approximation of the actual body shapes to regular geometric shapes. For direct measurements, in *C. oblonga* we weighed a group of 41 individuals to calculate the mean individual body mass; whereas in *B. capitatum* and *Ca. elongatus* we weighed 20 specimens of each species individually to measure individual weights. For indirect measurements, we estimated first body volume. In *C. oblonga*, we used 20 individuals mounted on permanent slides; whereas in *B. capitatum* and *Ca. elongatus*, we used the same 20 individuals each mentioned above. Then, we multiplied body volume by tissue density estimated for each species to estimate individual body mass for each indirect approximation. A flowchart of the process is given in Figure 3. 2.

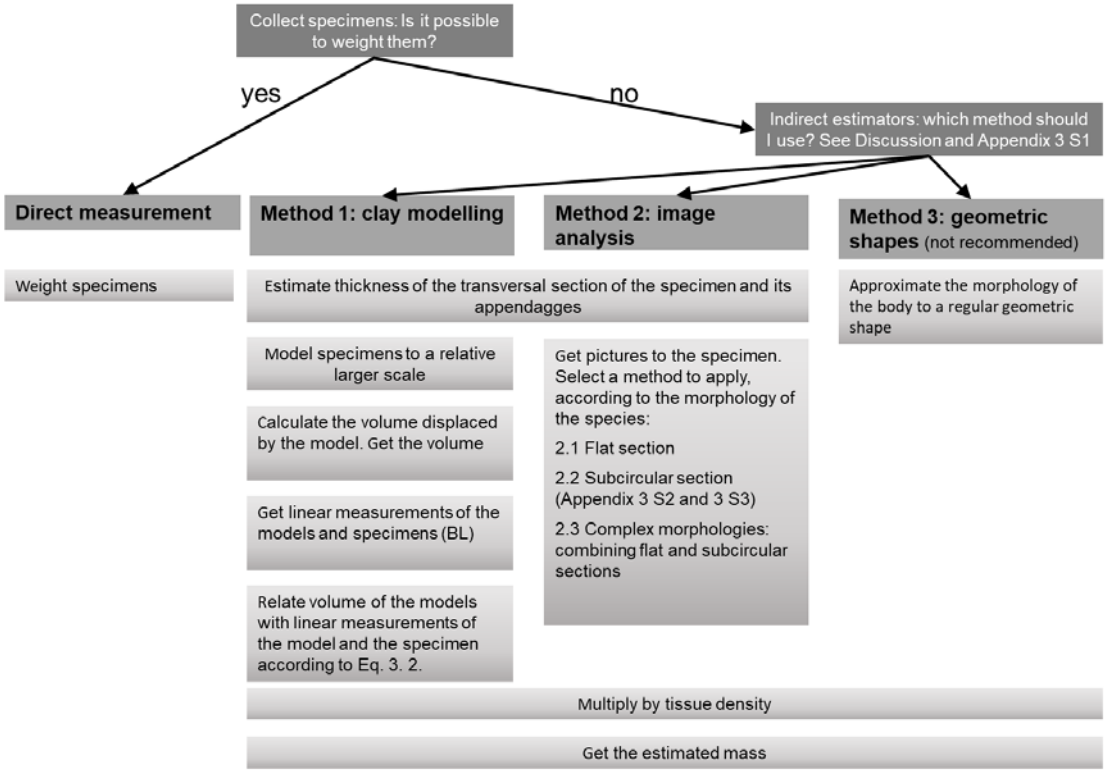

Figure 3. 2. Flowchart summarising the steps of each method.

Direct Estimation

Direct biomass estimates of the specimens were used as a benchmark for the other methods, enabling quantifying the error associated with indirect methods. Mean biomass was estimated using the method described in the literature (Aznar et al. 2001). Since specimens had been stored in 70° ethanol for 10–30 years, they were placed in 0.9% saline solution (9 g NaCl per 1 L distillate water) for 1 to 5 days to allow the tissues to re-hydrate. Then mean individual mass was calculated as follows: the excess of water was removed by leaving the individuals briefly on blotting paper. For *C. oblonga*, we weighed two sets of 20 (w_{set1}) and 21 (w_{set2}) individuals from two different host individuals and calculated the mean individual weight ($w_{individual}$) as follows (Eq. 3. 1):

$$w_{individual} = ((w_{set1}/20) + (w_{set2}/21))/2 \qquad \text{(Eq. 3. 1)}$$

For *B. capitatum* and *Ca. elongatus*, we chose 20 individuals of each species. *B. capitatum* were collected from 12 and *Ca. elongatus* from 6 different host individuals. Individuals of both species were weighed individually. The specimens were weighed to the nearest milligram twice for each species.

Given that the indirect methods described herein are based on estimation of body volume, an estimate of tissue density is required for conversion to biomass. For this purpose, we weighed and measured the volume displaced in a graduated cylinder by a mass of new sets of several hundreds (*C. oblonga*) or tens (*B. capitatum* and *Ca. elongatus*) of re-hydrated specimens. We did these procedures twice and used the averaged quotient of mass to volume as density of each species.

Thickness Estimation

The indirect methods presented here require expert predictions about the transversal section of specimens. In the simplest case, as in *B. capitatum*, it can be assumed to be subcircular (Figure 3. 1b) and, thus, thickness and width are expected to be nearly equivalent along the longitudinal axis.

In other cases, as in our flatworm or crustacean models, the transversal section is far from circular, which requires its modelling based on body thickness estimates (Figure 3. 1a and c). In the published descriptions, measurements of thickness are often not available (Teo et al. 2010) as specimens are viewed and depicted frontally (dorso-ventrally rather than laterally). In the present study, the thickness of specimens in permanent mounts was measured individually under a microscope. First, we marked both sides of a microscope slide 100 µm thick, placed it under the microscope and focused on one of the sides. For a given magnification, we recorded the number of divisions of the micrometre knob taken to focus on the opposite side. This operation was repeated ten times and the mean number of divisions was used to establish the vertical displacement accounted by each knob division. Following the same approach, we measured the thickness of the specimens of *C. oblonga* mounted in Canada balsam on slides and *Ca. elongatus* mounted on non-permanent slides in saline solution at 20× and 10× magnification, respectively. For *C. oblonga*, body thickness was measured at the levels of pharynx, ventral sucker and posterior end of vitellarium. We also measured the thickness of the ventral sucker to improve the accuracy of our proposed method (Table 3 S1). For *Ca. elongatus*, body thickness was measured at the lateral and central areas of the cephalothorax, fourth pedigerous somite, genital segment and abdomen. Additionally, we measured the thickness of one appendage of each of the 8 pairs occurring in adult specimens of *Ca. elongatus*: Antennae 1–2, maxillae, maxilliped and legs 1–4 (Table 3 S 2 and Table 3 S 3).

Indirect Method 1: Clay Modelling

We adapted the method initially proposed by Nesterenko & Kovalchuk (1991) to determine the individual mass of ciliates. We modelled with commercial air-drying clay the body of the selected specimens of *C. oblonga, B. capitatum* and *Ca. elongatus* to approximate scales of 16–19, 2–9 and 26–39, and the appendages of *Ca. elongatus* to 92–217 times larger than the real structures, respectively (Figure 3. 2 and Figure 3. 3). Then, we measured the volume of water displaced by each model in graduated cylinders to the nearest 0.05 ml for *C. oblonga* and 0.5 ml for *B. capitatum* and *Ca. elongatus*, respectively. The volume of the specimen was calculated as (Eq. 3. 2):

$$V_s = V_m * (L_s/L_m)^3 \qquad \text{(Eq. 3. 2)}$$

Where V_m and L_m are the clay model volume and length respectively; and V_s and L_s are the specimen's volume and length respectively.

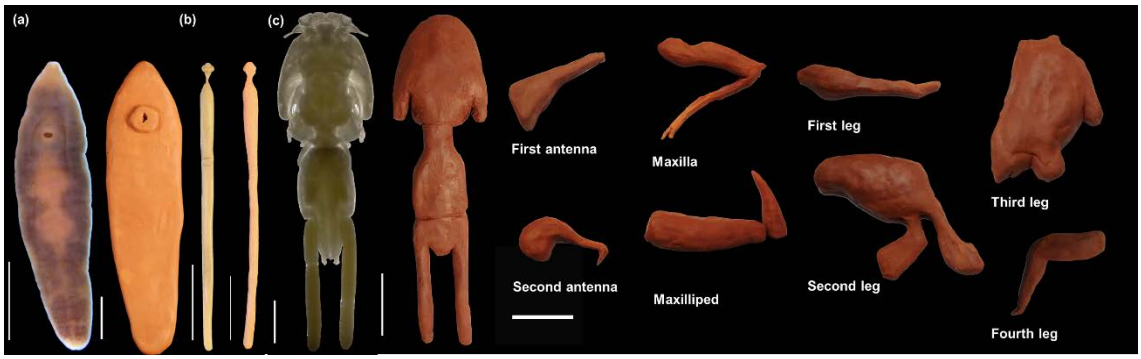

Figure 3. 3. Specimens and clay figurines of the model species. (a) *Campula oblonga*, scale bars 2 and 20 mm, respectively; (b) *Bolbosoma capitatum*, scale bars 20 and 50 mm, respectively; (c) *Caligus elongatus*, scale bars 1, 50 and 50 mm, respectively.

Indirect Method 2: Extracting Mass From Images

Image analysis is a suitable tool to indirectly estimate biomass because it is non-destructive, time- and cost-effective (Appendix 3 S1) and allows continuous observation of individual development (Tackenberg 2007). Estimating mass of single individuals from images is a common concern in distant disciplines of biology and different solutions have arisen (e.g. aquaculture Lines et al. 2009; zooplanktology Alcaraz et al. 2003, Hernández-León & Montero 2006; palaeontology Motani 2001; or botany Tackenberg 2007).

The indirect method 2 was divided into 3 submethods according to the morphology of the transversal sections of the individuals understudy (Figure 3. 1).

Figure 3. 4 Two steps in image analysis process to estimate individual mass from images as volume of revolution. (a) *Bolbosoma capitatum* picture with Feret diameter aligned with image wide margin (i.e. angle minimised); (b) binary conversion of the specimen after thresholding, white pixels represent the background and black pixels the animal surface. Scale bar 5 cm.

Area By Depth By Density (Flat Section) (Indirect Method 2.1)

This method was applied to *C. oblonga* and is based on Lambden & Johnson (2013). These authors squashed specimens in a microwell of known depth to obtain the ventral area of the organism by means of image acquisition and analysis software. Individual volume was then estimated as the product of microwell depth by the ventral area and converted into biomass after multiplying by tissue density. We drew in ventral view the outline of the body, pharynx, and ventral sucker of the 20 selected individuals under a microscope fitted with a drawing tube (Nikon Optiphot-2 at 10× magnification). Drawings were scanned at 600 ppi and were saved in TIFF format. We measured the area (μm^2) of individuals in ventral view with Fiji-ImageJ version 1.51n (Schindelin et al. 2012). As our specimens were mounted on permanent slides, their depth (*sensu* Lambden & Johnson 2013) was the mean thickness of each individual (measured as indicated above). To obtain individual body volume, we multiplied body area by the mean thickness of each individual (Figure 3. 1a). The advantage of this approach compared with that of Lambden & Johnson (2013) is that it can be applied to both fresh and permanent mounted material. Additionally, using the same approach, we added the volume of the ventral sucker to the body mass of each individual.

Volume Of Revolution By Density (Subcircular Section) (Indirect Method 2.2)

In *B. capitatum*, we photographed the 20 selected individuals with a digital camera (Canon EOS 700D EFS 15–85 mm) held by a camera stand (Kaiser RSX). Pictures were taken at 5184 dpi. By means of GIMP version 2.8.18 (The GIMP Team 2016), we extracted the individual from the picture and placed it on a black background (Figure 3. 4a). Using ImageJ, pictures were scaled to convert linear measurements into μm. Then, pictures were thresholded to make them binary (i.e. tell apart object pixels from background pixels) (Figure 3. 4b) and rotated to render

the Feret diameter of the object horizontal. Images were then saved as text image (Appendix 3 S2). After thresholding, ImageJ saves object pixels as 255 and background pixels as 0. Lastly, we processed text images with a R (R Core Team 2017) script (Appendix 3 S3). Parameters included in the script were:

ratio: $\mu m\ px^{-1}$

- As the script initially expressed the body volume in $pixels^3$, we converted body volume into μm^3 using the scale computed above with ImageJ.
- Based on the text image, each column of object pixels was treated as a one-pixel-wide slice (i.e. transversal section) with a regular circular shape. Thus, the volume of each individual was computed as the sum of volumes of each slide.

rho: tissue density as g ml^{-}

- To calculate body mass, volume was multiplied by the estimated tissue density to obtain body mass in mg.

Complex Morphologies (combining flat and subcircular sections) (Indirect Method 2.3)

To deal with more complex morphologies, as in *Ca. elongatus* (Figure 3. 1c), we processed each specimen dividing the body into portions according to (i) their transversal section (flat vs subcircular) and (ii) when flat, according to similar mean thickness. In *Ca. elongatus*, the body can be easily divided as per (i) into main body and appendages (flat sections), and egg strings (subcircular section). Following (ii), three large body areas with similar mean thickness, were recognized: the cephalothorax, the fourth pedigerous somite and the genital-abdominal complex. Additionally, we measured the area of one appendage of each of its 8 pairs (Table 3 S 2 and Table 3 S 3). We photographed the 20 selected individuals with a Nikon Fotomicroscope E800 at 4× magnification to obtain the body surface and at 10× to obtain the surfaces of appendages. Pictures were taken at 5184 dpi.

To estimate volumes of main body areas and appendages (i.e. flat section pieces) we applied the method described in section (Indirect Method 2.1), analogously to *C. oblonga*. For egg strings (i.e. subcircular section), we used the method explained in section (Indirect Method 2.2), analogously to *B. capitatum*. Finally, volumes of pieces were added up.

Indirect Method 3: Approximation To Regular Geometric Shapes

We measured maximum body length (BL) and width (BW) of individuals by approximating body volume to simple geometric shapes (e.g. Kuris et al. 2008, Roselli et al. 2013). In *C.*

oblonga, body volume was approximated to an ellipsoid (e.g. George-Nascimento et al. 2004, Poulin & George-Nascimento 2007) (Method 3a in Figure 3. 5 and Table 3 S 4) and to a cylinder (e.g. George-Nascimento et al. 2002, Roselli et al. 2013) (Method 3b in Figure 3. 5 and Table 3 S 4). For *B. capitatum*, body volume was calculated assuming a cylindrical shape (e.g. George-Nascimento et al. 2002, George-Nascimento et al. 2004, Lagrue & Poulin 2016). In *Ca. elongatus*, body volume was approximated to an ellipsoid (e.g. Alcaraz et al. 2006) and egg strings to a cylinder. In the three model organisms, we based our measurements on total BL, maximum BW and body depth equal to BW (Figure 3. 1).

Statistic Analyses

Due to the nature of our samples, we performed two kinds of statistical analyses to test for differences between the estimates obtained directly and those computed indirectly. In *C. oblonga*, we compared the average individual mass obtained directly for a sample of individuals with the corresponding mean weight obtained for each individual with each indirect method (i.e. individuals mounted on permanent slides) (methods 1, 2, 3a and 3b) using *t*-tests for one sample with Bonferroni correction (i.e. alpha = 0.05/4). In *B. capitatum* and *Ca. elongatus*, we used Linear Mixed Effect Models to compare the different methods (fixed factor) across individual specimens (random factor). All statistical analyses were carried out with R packages lme4 (Bates et al. 2015) and stats (R Core Team 2017).

Data Availability

Collection of the Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia. Accession numbers of samples: *Campula oblonga* mounted specimens: CN491122, CN491158, CN610121, CN610128, CN610140, CN675077, CN677015, CN677090-92, CN677117, CN677120, CN680012, CN681007, CN687088, CN687094, CN696110, CN696123, CN716016-17; weighed specimens: CN707, CN716; *Bolbosoma capitatum*: 04013, 04150, 04192, 04196, 04199, 04202, 04209, 04299, 04305, 08823, 08826, 08830; *Caligus elongatus*: CT1E035, CT2C039, CT2C051, CT3B017, CT3B035, CT3B037. ImageJ and R scripts can be downloaded as online supporting information (Appendix 3 S2 and 3 S3). All data analysed during this study is included in this article (Tables 3 S1–6).

3.4 Results

Tissue Densities

The estimated tissue densities (± absolute errors) were 1.06 ± 0.03 g ml⁻ for *C. oblonga*, 1.05 ± 0.10 g ml⁻ for *B. capitatum* and 1.15 ± 0.01 g ml⁻ for *Ca. elongatus*.

Thickness Estimation

Mean body and mean thickness of appendages (±standard error) of the species are shown in Tables 3 S1–3.

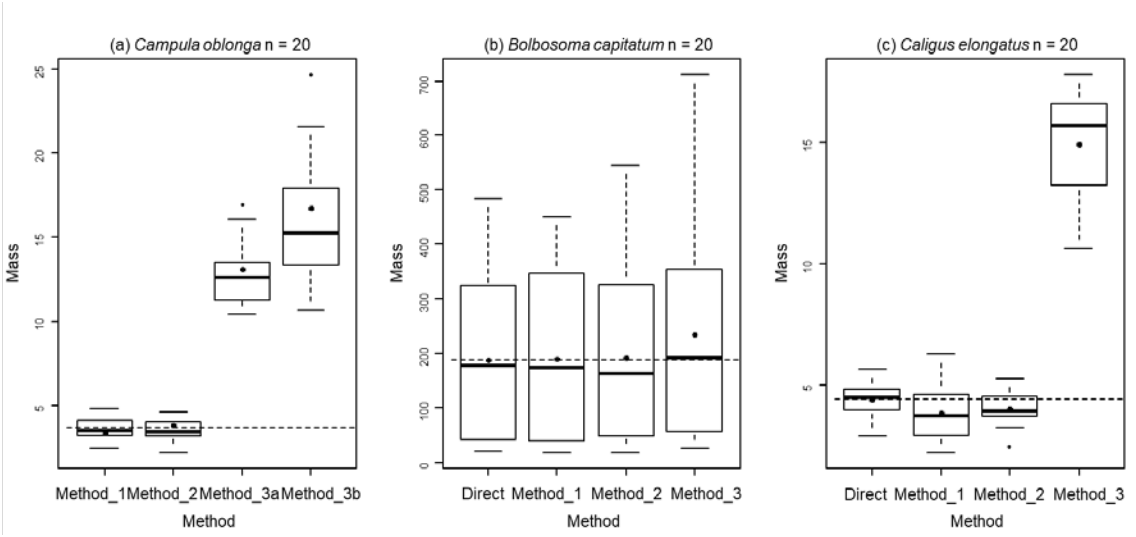

Figure 3. 5. Boxplot of mass (mg) estimated by different methods for (a) *Campula oblonga*, (b) *Bolbosoma capitatum* and (c) *Caligus elongatus*. Continuous line indicates the median value for each method. Fill point represents the mean individual mass for each method. Dashed line represents the mean mass value of a single individual obtained by direct method. Method 1: clay model; Method 2: image analysis; Method 3: geometric approximation, 3a ellipsoid, 3b cylinder.

Estimations of Masses

Individual mass estimated for each specimen is presented in Table 3 S 4, Table 3 S 5 and Table 3 S 6 for *C. oblonga, B. capitatum* and *Ca. elongatus* respectively. A comparison of the accuracy of the methodologies is provided in Figure 3. 5. For *C. oblonga*, the average individual mass estimated by indirect methods 1 and 2 were very similar to and not significantly different from that estimated with the direct method ($t = -1.86$, $P = 0.08$; $t = 0.74$, $P = 0.47$). In contrast, means obtained by methods 3a and 3b differed significantly from that computed directly ($t = 20.02$, $P < 0.001$; $t = 13.73$, $P < 0.001$). These methods overestimated mean individual mass by 3.5 to 4.4 times. For *B. capitatum* and *Ca. elongatus*, the mean values obtained with methods 1 and 2 were very close to and not significantly different from those of the direct method

(Table 3. 1). In contrast, method 3 showed a higher and significantly different value of mean individual mass (Table 3. 1).

Table 3. 1 Linear Mixed Model analyses between different methods for individual mass estimation of *Bolbosoma capitatum* and *Caligus elongatus*.

	Estimate	Std. Error	t value	P value
Bolbosoma capitatum				
Intercept	0.19	0.04	4.74	0.00
Method 1	0.00	0.01	0.29	0.77
Method 2	0.00	0.01	0.03	0.97
Method 3	0.05	0.01	3.51	0.00
Caligus elongatus				
Intercept	4.43	0.30	14.77	0.00
Method 1	-0.53	0.37	-1.44	0.15
Method 2	-0.36	0.37	-0.99	0.32
Method 3	10.50	0.37	28.65	0.00

3.5 Discussion

In this paper, we evaluated the accuracy of indirect methods estimating individual parasite mass. Our results showed that the indirect methods proposed herein provided the closest approximation to the direct estimation of average individual mass. Despite the extensive use of approximation to geometrical shapes (e.g. George-Nascimento et al. 2002, Alcaraz et al. 2003, George-Nascimento et al. 2004, Koehler et al. 2012), method 3 was far from satisfactory in all situations as it grossly overestimates biomass.

Regarding species tissue densities, although they are available in the literature (e.g. Kuris et al. 2008), we decided to measure density independently as additional validation of our biomass measurements. Our density results agree with that published previously for adult flatworms (Kuris et al. 2008) (1.1 g ml^{-}) and crustaceans (Spaargaren 1979) (1.098–1.506 g ml^{-}). According with this, we can assume that our specimens were fully rehydrated. However, it is worth saying that if a researcher wanted to know the tissue density of a species from stored specimens, they would check the completely rehydration of the specimens.

Classical approaches to estimate biomass of small invertebrates have relied on approximations to regular geometric shapes, in most cases cylinders or ellipsoids (George-Nascimento et al. 2002, Alcaraz et al. 2003, George-Nascimento et al. 2004, Pitois & Fox 2006,

Roselli et al. 2013). However, these regular geometric structures might be quite different from the real morphology of organisms (Hernández-León & Montero 2006) and this could often lead to misinterpretations. Particularly, when extrapolating biomass results to community and/or ecosystem studies, the effect of these biases can be additive. As shown in Figure 3. 5, classical approaches (i.e. indirect method 3) provided estimators well over the reference values. Furthermore, when assuming a regular geometric shape, the contribution of salient structures, for example ventral sucker of flatworms and paired appendages of crustaceans in our case, or tail of cercaria (e.g. Lagrue & Poulin 2016), or expansions of the tegument of molluscs (e.g. Novack-Gottshall 2008), among others, is neglected. Furthermore, we would also like to emphasise that if a researcher plans to estimate the biomass of a population using any of the methods proposed in this paper: (1) they should consider the phenotypic variability of their population (identifying if required morphological categories according to life stage, sex etc.), (2) estimate the mean weight of a representative number of organisms of each category and (3) multiply the mean weight of an individual of a category to the observed proportion of the category in the population.

Comparing the indirect method 1 (modified from Nesterenko & Kovalchuk 1991) with 2, both require estimation of body thickness and yielded similar results. Both approaches are time- and cost-effective and easy to apply in most situations. In addition, they are non-destructive and the new estimations of individual mass from images are based on open-source software. Note also that the boxplots shown in Figure 3. 5 convey the variation of the sample for each method, which results from the inherent sample variance ± the measurement error. This facilitates assessment of the measurement error between methods. Overall the error committed in methods 1 and 2 seem fairly similar to each other and to those incurred in the direct estimation of weights. The exception is apparent larger variation associated to method 1 when applied to *Ca. elongatus*. As this species was the most morphologically complex, this observation suggests that measurement error is probably dependent of species shape and skill of the modeler. So, although for more morphologically complex organisms, clay modelling (i.e. method 1) could be the best option, it may require the intervention of a qualified artist to render realistic representations of model organisms, thereby minimizing measurement error. In any case the average value of the biomass estimator of *Ca. elongates* obtained was not significantly different from those obtained directly or applying method 2.

Method 2.2 would work best with straight and symmetrical organisms with convex contours. For asymmetric and/or extremely appendage-ornamented organisms, one-pixel thick slices will not add-up correctly, leading to overestimation of individual mass.

Nonetheless, the inaccuracy for estimating mass of complex morphologies can be solved by dividing the specimens into parts as demonstrated herein with the crustacean model species (method 2.3). We would like to highlight the importance of scanning images at high resolution to minimise the error associated to image acquisition.

To fill the gap of invertebrate descriptions (Teo et al. 2010), we developed an easy technique to measure thickness of mounted individuals using a light microscope, the commonest way to study morphology of small invertebrates. We foresee that our thickness estimator will be very useful to measure thickness of any kind of small invertebrate (e.g. plankton or soil-dwelling species) or structures on a slide. There are three main advantages of our method: (1) It allows estimating thickness of organisms previously stored in collections as it can be applied to specimens on permanent and non-permanent mounts. (2) The specimens can be recovered after measuring and used in further applications. (3) In comparison to Lambden & Johnson (2013) estimation of body thickness, our measurement can be applied to specimens thicker than 0.127 mm, which cannot be squashed into a plate. Additionally, our estimation of mass from images is less expensive as the use of a special plate is not required. Novack-Gottshall (2008) found that the ATD method (i.e. the product of lengths of the three major axes of invertebrate fossil bodies) was the best predictor of body volume as representative of body mass. Thus, Lambden & Johnson (2013) and our 2.1 indirect method proposed represent similar strategies to estimate mass of small invertebrate individuals, but more elaborated than that of Novack-Gottshall (2008). Lagrue & Poulin (2016) measured thickness of specimens placing them in lateral view under a stereomicroscope. Although this approach is straightforward, it might be tedious and inaccurate to apply to very thin and/or small invertebrates. A limitation of method 2.1 lies in the availability of material to measure thickness. However, this could be solved by measuring thickness from morphologically similar species.

Conclusion

Estimating biomass of small invertebrates poses a series of challenges that can be overcome by using indirect methods that have been rarely tested for accuracy. Our study shows that the indirect methods proposed in this paper provide a good approximation to the real body mass and are much more accurate than approximating body morphology to regular geometric figures, as previously applied to small invertebrates in the literature. In particular, our method for estimating biomass from images seems more time- and cost-effective than previous approaches, catering for the growing need of obtaining reliable estimates of invertebrate

biomass (Tackenberg 2007). We validated the shaping methodology originally described for unicellular ciliates (Nesterenko & Kovalchuk 1991) to be generally applied to small invertebrates. This clay shaping-based method may be particularly valuable for organisms with complex morphology, although with the cost of time and skills investment, that may render this approach only useful for model species. The benefit of our proposed methods is threefold. They allow recovering the material after use, can be applied to both fresh and mounted specimens on permanent slides and the images and figurines generated can be permanently archived and used in further studies.

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Author Contributions

Conceived the ideas and designed methodology: C.L.B., I.B.C., J.A.B. Collected the data: C.L.B. Analysed the data: C.L.B., J.A.B. Contributed reagents/materials/analysis tools: C.L.B. Wrote the manuscript: C.L.B., I.B.C., J.A.B.

Additional Information

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3.7 Supplementary Information

Table 3 S1. Thickness (µm) of *Campula oblonga* specimens at pharynx level, ventral sucker level, posterior end of vitellarium and thickness of the ventral sucker.

	Pharynx	VS level	Posterior end	VS
Co_1	484	465	435	254
Co_2	517	342	362	230
Co_3	435	414	383	200
Co_4	460	378	394	236
Co_5	399	403	394	204
Co_6	494	427	471	212
Co_7	412	352	339	297
Co_8	352	424	399	241
Co_9	438	318	342	261
Co_10	452	297	404	266
Co_11	317	207	370	228
Co_12	383	234	388	266
Co_13	457	255	406	241
Co_14	444	354	404	207
Co_15	278	311	411	258
Co_16	437	373	407	219
Co_17	351	287	366	210
Co_18	417	432	434	194
Co_19	414	438	389	166
Mean	419	350	392	231
SD	59	74	33	31

VS: ventral sucker.

Table 3 S 2. Thickness (µm) of *Caligus elongatus* specimens at lateral area of the cephalothorax, cephalothorax, fourth pedigerous somite, genital segment and abdomen

	Lateral (cephalotorax)	Thorax (cephalotorax)	Fourth pedigerous somite	Genital segment	Abdomen
Ce_1	452	729	231	345	266
Ce_2	398	588	297	463	192
Ce_3	327	549	280	336	164
Ce_4	428	592	192	497	246
Ce_5	306	484	296	463	292
Ce_6	283	490	178	521	258
Ce_7	223	357	152	423	213
Ce_8	358	484	280	444	250
Ce_9	445	687	275	466	385
Ce_10	444	662	219	374	212
Ce_11	400	651	232	482	237
Ce_12	234	448	221	437	258
Ce_13	278	562	210	332	308
Ce_14	323	444	209	438	259
Ce_15	337	447	182	306	201
Ce_16	379	543	192	388	118
Ce_17	237	278	157	222	293
Ce_18	287	460	253	305	240
Ce_19	333	518	226	302	181
Ce_20	348	639	281	305	259
Mean	341	531	228	392	242
SD	72	113	45	82	57

Table 3 S 3. Thickness (µm) of appendages of *Caligus elongatus*.

	1st antenna	2nd antenna	Maxilla	Maxilliped	1st leg	2nd leg	3rd leg	4th leg
Ce_1	74	157	160	149	185	115	116	201
Ce_2	83	133	110	157	155	152	129	144
Ce_3	118	168	129	44	165	141	154	132
Ce_4	49	154	104	138	129	115	64	93
Ce_5	113	146	94	181	148	165	74	140
Ce_6	82	110	126	144	143	174	71	119
Ce_7	113	129	96	130	171	107	80	102
Ce_8	126	122	96	149	104	96	63	105
Ce_9	110	82	116	143	140	173	155	118
Ce_10	89	108	108	141	132	110	89	148
Ce_11	94	94	97	146	115	126	86	148
Ce_12	91	126	88	144	113	151	130	141
Ce_13	122	111	89	162	144	137	115	144
Ce_14	110	91	97	135	121	127	119	126
Ce_15	94	124	83	129	121	121	100	133
Ce_16	122	135	82	108	96	111	105	135
Ce_17	88	83	86	132	99	111	102	99
Ce_18	113	116	91	129	99	105	107	93
Ce_19	108	107	104	165	127	129	138	121
Ce_20	110	129	121	108	149	137	143	138
Mean	101	121	104	137	133	130	107	129
SD	19	24	19	28	25	23	29	25

Table 3 S 4. Individual mass (mg) of specimens of *Campula oblonga*. Note that after obtaining body volume for each method, it was multiplied by tissue density.

INDIVIDUAL (BL=6532 μm; BW=1748 μm)	Direct	Method 1	Method 2	Method 3a	Method 3b
n = 20	3.43				
n = 21	3.95				
Co_1		4.50	3.89	12.69	13.98
Co_2		4.32	4.54	15.18	18.87
Co_3		4.04	3.58	12.26	14.52
Co_4		3.43	3.12	10.85	12.20
Co_5		3.17	3.09	10.53	10.63
Co_6		4.61	4.13	11.67	15.34
Co_7		3.74	4.19	12.61	17.63
Co_8		4.49	3.98	14.65	15.87
Co_9		3.48	3.42	12.30	13.37
Co_10		3.28	2.79	10.73	11.78
Co_11		2.44	2.17	10.38	13.52
Co_12		3.15	3.37	13.17	16.75
Co_13		3.45	2.92	12.92	15.18
Co_14		3.80	3.11	12.79	18.09
Co_15		3.25	2.53	13.41	18.80
Co_16		4.63	3.27	16.90	21.59
Co_17		3.34	3.39	13.53	16.81
Co_18		4.83	4.02	16.02	24.65
Co_19		5.16	4.34	17.81	27.89
Co_20		3.08	2.35	11.35	16.29
MEAN	3.69	3.81	3.41	13.09	16.69
SD	0.37	0.72	0.67	2.10	4.25

BL and *BW*: body length and width respectively; *n* number of individuals; Method 1: clay model; Method 2: image analysis; Method 3: geometric assumption: 3a ellipsoid. 3b cylinder.

Table 3 S 5. Individual mass (mg) of specimens of *Bolbosoma capitatum*. Note that after obtaining body volume for each method, it was multiplied by tissue density.

INDIVIDUAL BL = 59268 μm BW = 1904 μm	Host species	Direct	Method 1	Method 2	Method 3
Bc_1	Gm	183	173	196	191
Bc_2	Gm	205	178	186	213
Bc_3	Gm	216	205	184	220
Bc_4	Gm	187	160	165	191
Bc_5	Gm	172	165	251	227
Bc_6	Pc	312	331	343	349
Bc_7	Pc	426	436	445	544
Bc_8	Pc	386	397	450	474
Bc_9	Pc	336	320	349	359
Bc_10	Pc	445	545	415	711
Bc_11	Pc	485	434	438	705
Bc_12	Gm	85	88	84	101
Bc_13	Gm	58	50	65	64
Bc_14	Gm	36	41	39	53
Bc_15	Gm	43	47	38	45
Bc_16	Gm	69	79	77	77
Bc_17	Gm	41	45	37	57
Bc_18	Gm	19	17	18	25
Bc_19	Gm	42	48	42	55
Bc_20	Gm	22	18	22	26
MEAN		188	189	192	234
SD		158	164	160	221

BL and *BW*: body length and width respectively; *n* number of individuals; Method 1: clay model; Method 2: image analysis; Method 3: geometric assumption. Gm *Globicephala melas*; Pp *Pseudorca crassidens*.

Table 3 S 6. Individual mass (mg) of specimens of *Caligus elongatus*. Note that after obtaining body volume for each method, it was multiplied by tissue density.

INDIVIDUAL BL = 5620 μm BW = 2321 μm	Direct	Method 1	Method 2	Method 3
Ce_1	4.60	3.80	4.94	14.50
Ce_2	4.93	3.28	4.67	15.93
Ce_3	4.97	3.58	3.97	15.13
Ce_4	4.53	2.74	4.15	11.67
Ce_5	4.50	5.63	4.00	15.46
Ce_6	3.97	2.85	3.72	10.66
Ce_7	4.50	2.24	3.29	17.50
Ce_8	4.33	2.52	3.76	11.11
Ce_9	3.95	6.31	4.68	12.26
Ce_10	4.65	5.44	4.66	16.69
Ce_11	5.00	4.08	5.31	16.44
Ce_12	5.65	5.61	4.35	17.39
Ce_13	4.10	2.50	3.95	16.28
Ce_14	5.33	4.86	4.29	17.77
Ce_15	4.53	3.83	3.85	16.80
Ce_16	4.77	4.26	4.46	16.52
Ce_17	2.93	3.20	2.47	12.71
Ce_18	3.57	3.09	3.38	13.89
Ce_19	3.67	3.75	3.54	16.14
Ce_20	4.07	4.43	3.84	13.79
MEAN	4.43	3.90	4.06	14.93
SD	0.33	1.17	0.65	2.23

BL and *BW*: body length and width respectively; *n* number of individuals; Method 1: clay model; Method 2: image analysis; Method 3: geometric assumption.

Appendix 3 S1. Relative costs and time required of the indirect methods reviewed.

When working on biomass of invertebrates, the final method applied mainly depends on the aim or the requirements of precision of the study, the technical support of laboratory and the cost of the method (Postel et al. 2000). The common physicochemical indirect methods (e.g. wet, dry, ash-free dry, protein and carbon weights) have high costs (i.e. thousand €) and time (i.e. days) associated (Postel et al. 2000, Sandak et al. 2017). Thus, these methods may be unaffordable for scientists with time or budgetary limitations (Meyer et al. 2011).

In this Appendix, we aim to present the table below (Table Appendix 3 S1) of the relative costs and time required of the indirect methods reviewed: clay modelling, extracting mass from images and geometric approximation. As initial steps of three methods require the same laboratory equipment and consumables we have not considered such costs in the table of comparisons. So, depending on the studied species, laboratory equipment required will be a microscope gearing a camera or a drawing tube or a digital camera held by a camera stand. For the consumables, the three methods need for saline solution, petri dish, slide and cover, tweezers, graduated cylinder and scale.

Table Appendix 3 S1. Relative costs and time required of the indirect methods reviewed.

	Clay modelling	Image analysis	Geometric approximation
Price/ individual assessed	Very low (Clay and a ruler are needed)	Low (A computer and free software are required)	Low (A computer and free software are required)
Time/ individual assessed*	High (1 to 1.5 workdays/ individual assessed)	Medium (5 hours/ individual assessed)	Low (30 minutes to 1 hour/ individual assessed)
Precision (see Results section)	High	High	Very low

* The "time/ individual assessed" factor rely on the complexity of the morphology of the species assessed. We have considered the most complex species in our study (i.e. *Caligus elongatus*).

References in Appendix 3 S1

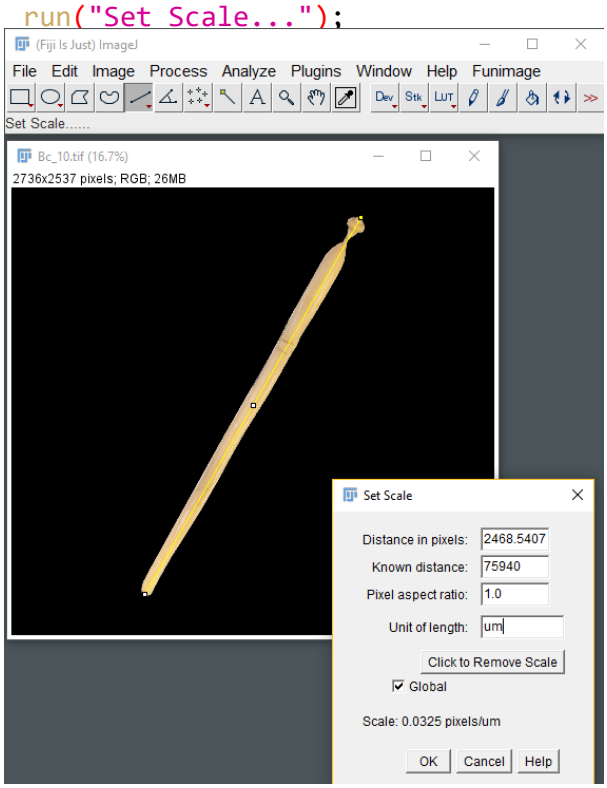
Postel, L., Fock, H. & Hagen, W. (2000) 4 - Biomass and abundance. In R. Harris, P. Wiebe, J. Lenz, H.R. Skjoldal & M. Huntley (Eds.), *ICES Zooplankton Methodology Manual* (1st ed., pp. 83-192). London, UK: Academic Press.

Sandak, A., Sandak, J., Waliszewska, B., Zborowska, M. & Mleczek, M. (2017) Selection of optimal conversion path for willow biomass assisted by near infrared spectroscopy. *iForest - Biogeosciences and Forestry*, 10, 506.

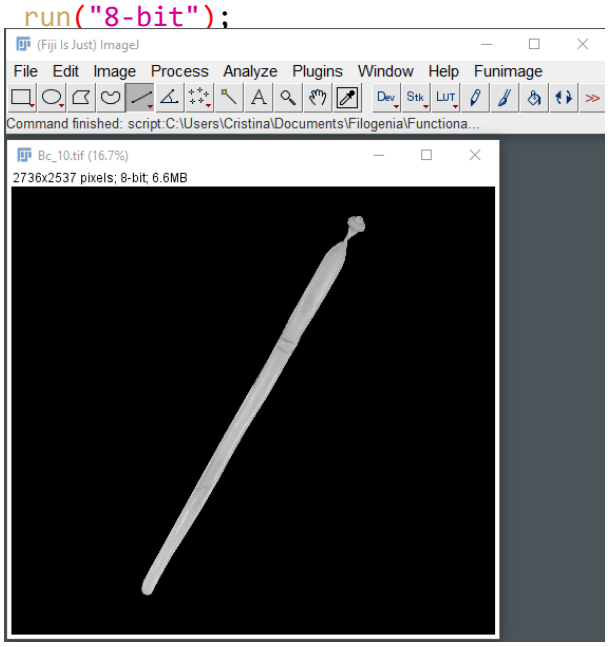
Meyer, C.K., Peterson, S.D. & Whiles, M.R. (2011) Quantitative Assessment of Yield, Precision, and Cost-Effectiveness of Three Wetland Invertebrate Sampling Techniques. *Wetlands (Wilmington)*, 31, 101-112.

Appendix 3 S2. ImageJ script for extracting individuals from images.

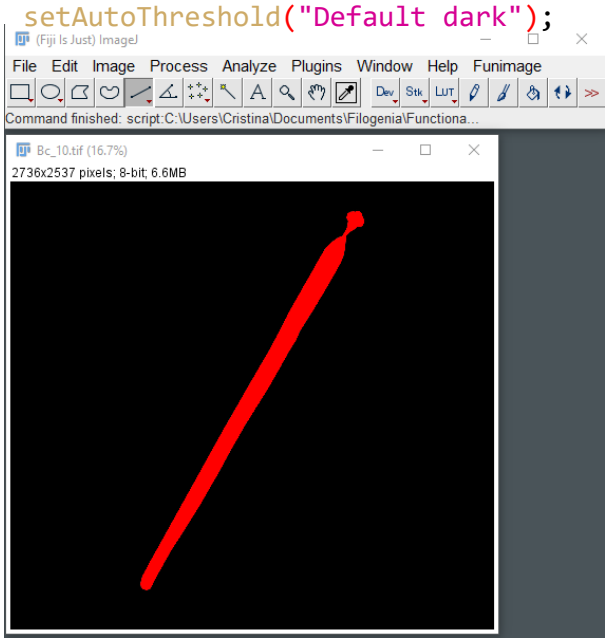
```
open() //To select and open the input image file

//Step 1
      run("Set Scale...");
//Step 2
      run("8-bit");
//Step 3
      setAutoThreshold("Default dark");
      // Alternatively, the user may threshold the image
      // interactively by running the next two lines
      //run("Threshold...");
      //setOption("BlackBackground", true);
//Step 4
      run("Convert to Mask");
//Step 5
      run("Analyze Particles...", "display");
//Step 6
      run("Rotate... ");//Rotate to Feret angle
                        // obtained in Results Step 5
run("Make Binary");
run("Auto Crop (guess background color)");
//Step 7
saveAs("Text Image...");

 // Step 1
 run("Set Scale...");
```



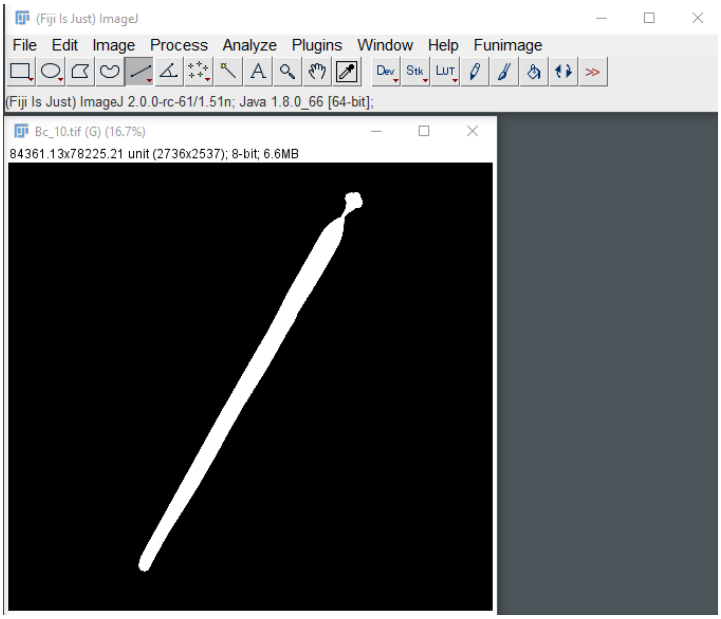
```
// Step 2

run("8-bit");
```



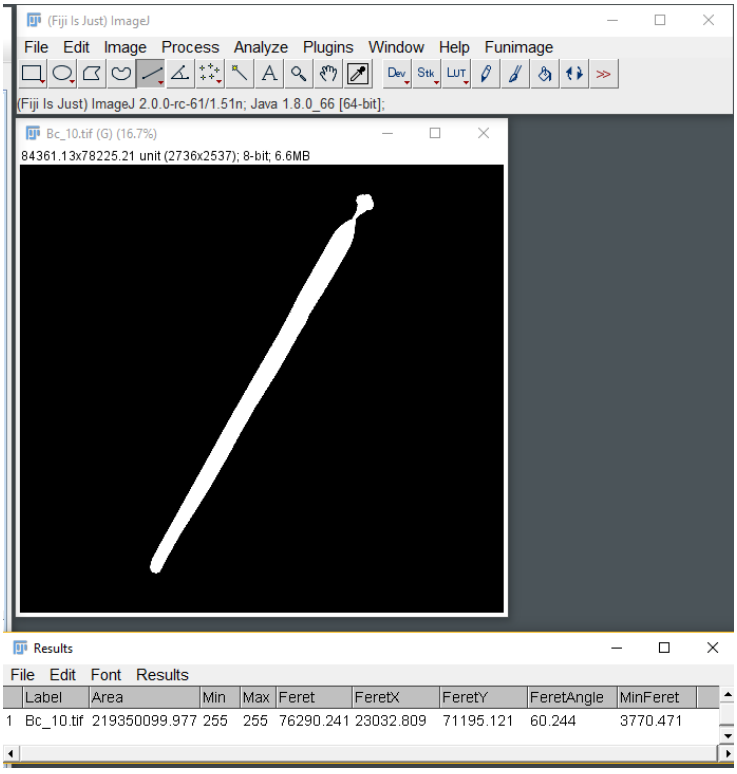

```
// Step 3

setAutoThreshold("Default dark");
```



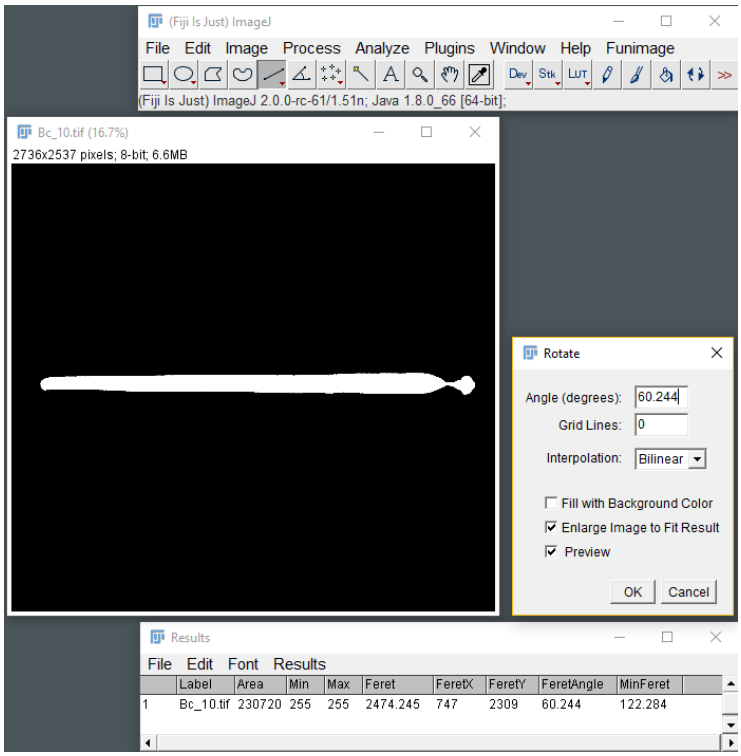
```
// Step 4
run("Convert to Mask");
```



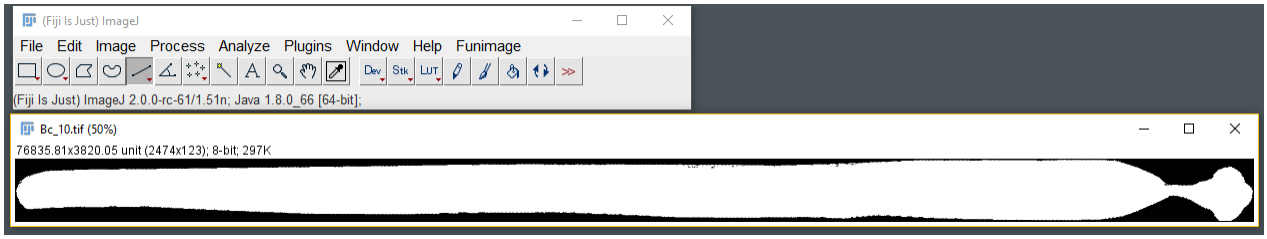

```
// Step 5
run("Analyze Particles...", "display");
```



```
// Step 6
run(""Rotate... ");
run("Make Binary");
```



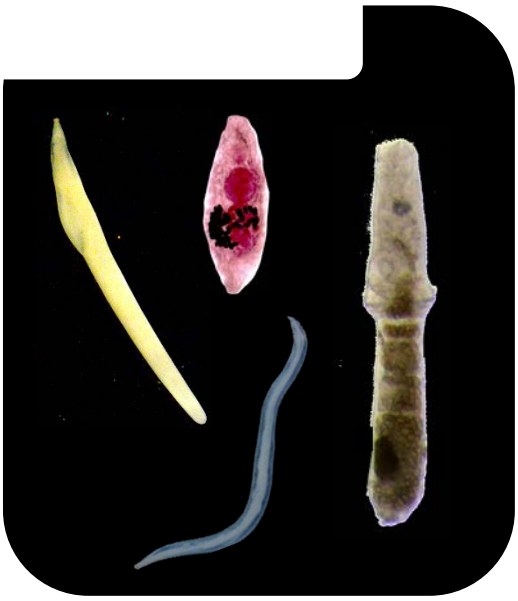

```
// Step 7
run("Auto Crop (guess background color)");
saveAs("Text Image...");
```



Appendix 3 S3. R script for estimating mass of an individual with a circular transversal section as volume of revolution.

```
# It reads text images and computes their revolution volume
# The script is intended for inputs in µm (length) and g/ml (tissue density)
# and computes the specimen's mass in mg
################## SCRIPT STARTS HERE #
#Read images and compute their revolution volume

ratio = 0.0119 # pixels/µm -- scale obtained with ImageJ (Set Scale)
rho = 1.05 # g/ml -- tissue density
bin.image <- as.matrix(read.table(file.choose())) # select text image to
analyze interactively

#read image as matrix and convert to binary matrix
vol.image <- function (x, sc, dens) {
x <- x/255 # sets matrix to binary
if (all(x %in% 0:1)==FALSE) {stop("The image matrix is not binary")} else {
x <- x[which(rowSums(x) != 0), which(colSums(x) != 0)] #crop image
width <- colSums(x)
sc <- 1/sc # invert scale to transform pixels into µm
M <- pi*sum((width/2)**2) * sc**3 * dens
M <- M*1e-9 # returns body mass in mg
}
return (M)
}

body_mass <- vol.image (bin.image, ratio, rho)

print(paste("The specimen's mass is ", round(body_mass,2), " mg"))
```

4. Towards a unified functional trait framework for parasites



4. Towards a unified functional trait framework for parasites

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Highlights

- Functional traits are morphological, physiological, phenological, and behavioural characteristics of organisms which impact their fitness and are measurable at the level of the individual without using information external to it.
- Parasitology is lagging behind in the application of a functional trait approach to the study of parasite diversity and community ecology. To bridge both disciplines, we introduce a core list of functional traits for parasites.
- In order to cover a large variety of ecological questions, we relate functional traits of parasites with the main challenges faced by organisms: dispersal, establishment, and persistence.

Keywords

functional traits, functional diversity, dispersal, establishment, persistence, community ecology

4.1 Abstract

Trait-based research holds high potential to unveil ecological and evolutionary processes. Functional traits are fitness-related characteristics of individuals, which are measured at individual level and defined without using information external to the individual. Despite the usefulness of the functional approach to understand the performance of individuals in ecosystems, and parasitism being the most common life-history strategy on Earth, studies based on functional traits of parasites are still scarce. Since the choice of functional traits is a critical step for any study, we propose a core list of seven functional traits of metazoan parasites, related to three universal challenges faced by organisms (dispersal, establishment, and persistence), and give guidelines to define appropriate functional traits in future parasite community studies.

4.2 A Trait-Based Ecology

Ecological studies based on **traits** (see Glossary) have greatly increased over the last three decades to explain ecosystem **properties** under different environments or environmental gradients (Cadotte et al. 2015, Moretti et al. 2017, Weiss & Ray 2019). Among the multiple types of traits that exist (Violle et al. 2007), **functional traits** have widely demonstrated their usefulness to explain or predict a variety of ecological questions about free-living organisms, in particular questions related to the functional facet of diversity, that is, **functional diversity (FD)** of a community (Box 4. 1). Functional traits have also allowed to unveil the mechanisms by which individuals (or their **intraspecific trait variability**, Carmona et al. 2016) scale up to effects on ecosystems and ecosystem processes (Violle et al. 2007). However, the number of studies using functional traits of parasites is still low (Mouillot et al. 2005, Keeney & Poulin 2007, Krasnov et al. 2015, 2016, 2019a, 2019b, Sokolov & Zhukov 2017, Warburton et al. 2017) in comparison with those of free-living organisms. This is perhaps due to three reasons: (i) a general underestimation of the roles played by parasites in ecosystems, despite ample evidence showing their importance (for a review see Gómez & Nichols 2013), (ii) the scarce knowledge on fitness-related traits of parasites in comparison with other organisms, and (iii) the lack of a unified framework of functional traits in parasites.

The selection of functional traits is essential to draw sound ecological conclusions, as the traits chosen must be informative of the target function (Petchey & Gaston 2006) and should be measured using standardised protocols (e.g. Weiher et al. 1999, Moretti et al. 2017). To unveil or predict ecosystem properties and interactions between organisms (even from different trophic levels), functional traits should be explicitly related to individual **performance** (Violle et al. 2007). Our review of the published studies on functional traits of parasites, mainly FD-related studies, suggests that the choice has been often dictated by their

relationship with the research questions being asked and/or by their availability, without explicit consideration of their functional value and repeatability in future studies (Table 4. 1). To facilitate comparisons between groups of parasites and promote reproducibility, a unified framework with a common terminology for parasite functional traits is absolutely needed and would parallel frameworks developed for other groups of organisms (e.g. plants Weiher et al. 1999; terrestrial invertebrates Moretti et al. 2017; crustacean zooplankton Barnett et al. 2007; algae Lange et al. 2016). Such a framework would improve and maximise the utility of functional trait approaches in parasitology and contribute to the delineation of the roles of parasites in communities and ecosystems more broadly. Furthermore, it will allow for comparing parasite and host diversities on common terms (see Weiss & Ray 2019 for how to compare functional traits across taxa), thereby paving the way for general ecologists to widely include parasites in community ecology.

Box 4. 1. Taxonomic and Functional Diversity

The way in which diversity is measured is key to understanding species assemblages. Community ecology has relied on species-based measurements (taxonomic diversity, TD), which leads to a loss of ecological (functional) and evolutionary (phylogenetic) information. To solve this limitation, two alternative frameworks to study biodiversity were developed: **phylogenetic diversity** (**PD**) measures the diversity of evolutionary histories of organisms in communities; and functional diversity (FD) quantifies the relative originality of functions provided by each organism in a community (Pavoine & Bonsall 2011). The combined study of these three facets of diversity provides a more complete picture of ecosystem properties. Among the many approaches developed to quantify TD and FD (and PD), we focus on Rao's diversity index (Rao 1982) and Jost's correction of diversity (Jost 2007) given their widespread use in community ecology.

Rao's index is derived from Simpson's index and allows comparisons between TD and FD within the same mathematical framework (Rao 1982). This distance-based diversity measure relies on a matrix of pairwise dissimilarities (d) between species. Species are plotted in an n-dimensional functional trait space (n: number of functional traits) and the pairwise distances between them are calculated (Petchey & Gaston 2006) (Figure 4. IA).

Diversity can be partitioned into spatial components (i.e. α, β, γ), which is key to describe species composition and ecosystem functioning. The α level represents diversity at the sampling unit (usually the host individual), and the Rao's index incorporates the distance between species, i and j (d_{ij}), and the relative abundance (p) of each one with respect to the total number of species in the sampling unit (s) (Figure 4. IB). d_{ij} depends on the kind of data considered, such as functional traits (FD). For TD, $d_{ij} = 1$ for all $i \neq j$ and $d_{ii} = 0$ for all i, so the Rao's index is equal to the Simpson's index of diversity and reaches its potential maximum value (Botta-Dukát 2005).

At γ level, the locality is studied as a single unit by pooling units (hosts) together. P_i and P_j are the local relative abundances for species *i* and *j*, and *S* the total number of species in the locality (de Bello et al. 2010) (Figure 4. IB). β diversity measures the amount of diversity due to differences between units from the same locality (Figure 4. IB). To make β diversity comparable across localities, α and γ diversity values are usually transformed into their **equivalent numbers** (α_{eqv} and γ_{eqv}) (Jost 2007). β Diversity is expressed as a proportion of the local (γ) diversity across all units (α) within a locality. These proportions can be normalised between (0,1) (β_{norm}) to account for a different number of units (*x*) from each locality (de Bello et al. 2010).

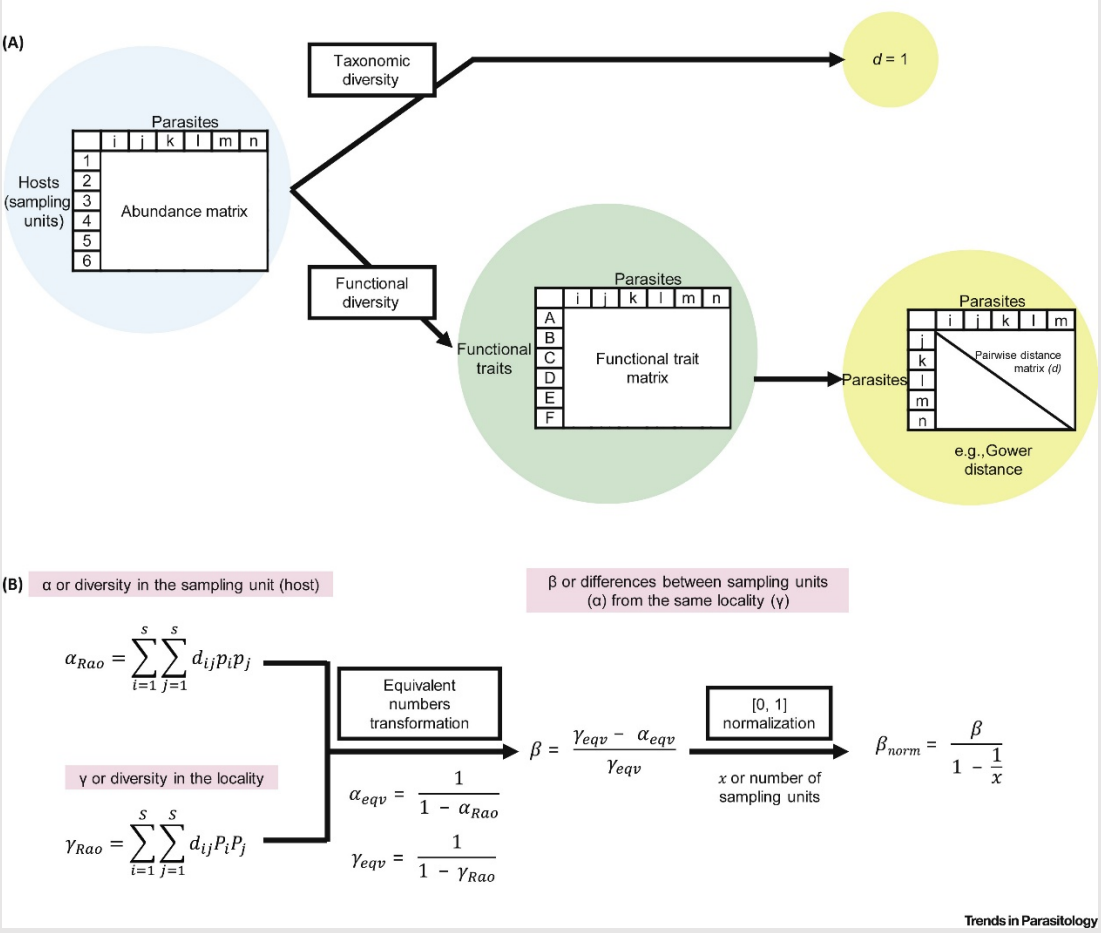

Figure 4. I. Taxonomic and Functional Diversity According to Distance-Based Indices (A) and Rao's Equations for α, β and γ Diversity (B).

Here, we propose a unified functional trait framework for metazoan parasites grounded in the current ecological theory, with the challenge of identifying traits sufficiently general and applicable across phylogenetically distant parasite taxa without losing resolution when characterising the functional groups of parasites.

Table 4. 1. Traits used as functional traits in previous parasitological studies.

Trait	Type of trait[1]	Type of measurement	Level of measurement	External information	References
Body length or size	Functional trait	Quantitative (continuous linear measurements: maximal body length; midline length of the dorsal shield)	Individual	No	Mouillot et al. 2005, Krasnov et al. 2016, 2019a, 2019b
Attachment	Functional trait	Quantitative (continuous standardised linear measurements: haptor's sclerotised parts; ordinal: number of combs)	Individual	No	Mouillot et al. 2005, Krasnov et al. 2016, 2019b
Reproductive organs	Functional trait	Qualitative (categorical: relative length; shape of the vagina armament; shape of the copulatory tube; shape of the accessory piece of the copulatory organ)	Individual (identical for all individuals from the same species)	No	Mouillot et al. 2005
Niche space	Ecological performance	Quantitative (distribution of individuals or biomass of a species in a niche space)	Species	Yes	Keeney & Poulin 2007
Mean characteristic abundance	Demographic parameter	Quantitative (mean number of parasites per individual host; mean abundance on the principal host)	Species	Yes	Krasnov et al. 2015, 2016, 2019a, 2019b
Path of infestation	Ecological performance	Qualitative (cutaneous, percutaneous or alimentary)	Individual (identical for all individuals from the same species)	Yes	Sokolov & Zhukov 2017
Niche preference	Ecological performance	Qualitative (body, burrow/ nest or both)	Individual (identical for all individuals from the same species)	Yes	Krasnov et al. 2015, 2016, 2019b, Warburton et al. 2017
Feeding mode	Functional trait	Qualitative (binary: facultative or obligatory haematophagy; categorical: facultative, non-exclusive obligatory, obligatory haematophagy)	Individual (identical for all individuals from the same species)	No	Warburton et al. 2017, Krasnov et al. 2019a

Seasonality in reproduction	Ecological performance	Qualitative (main reproduction period: warm, cold season or year-round)	Individual (identical for all individuals from the same species)	Yes	Krasnov et al. 2015, 2016, 2019b, Warburton et al. 2017
Host specificity	Ecological performance	Quantitative (mean number of host species on which a given flea species was recorded; mean phylogenetic distinctness of the regional and continental host spectrum; number of hosts on which an ectoparasite species was recorded significantly correlated with the number of individual parasites collected)	Species	Yes	Krasnov et al. 2015, 2016, 2019a, 2019b, Warburton et al. 2017
Degree of sexual dimorphism	Demographic parameter	Quantitative (logarithmic female-to-male body size ratio)	Species	No	Krasnov et al. 2019a, 2019b
Geographic range size	Ecological performance	Quantitative	Species	Yes	Krasnov et al. 2019b
Geographic range latitude	Ecological performance	Quantitative (latitude of the centre of the geographic range)	Species	Yes	Krasnov et al. 2019b

[1]Traits classified following definitions in Violle et al. 2007.

4.3 Multiple Solutions, One Lifestyle: Parasite Functional Traits

Since parasitism has arisen several times independently throughout the tree of life, it is difficult to find a common definition, and this leads researchers to disagree on considering some particular groups of organisms as parasites. Regardless of achieving a consensus, the fact is that the same functional traits can be shared by organisms with different life strategies. Hence, our unified framework can be applied to a wide range of metazoans differing in their parasitic way of life and can inspire further extension to non-metazoan parasites.

Core List of Parasite Functional Traits

In order to make the functional trait framework comparable across spatial and temporal scales, collect functionally representative information, share data, and maximise the applicability of results, functional traits in parasitology should conform to the accepted definition in community ecology. They should be fitness-related, measured at the individual level, and without referring to information external to the individual (Violle et al. 2007). The functional traits proposed herein are related to three universal challenges faced by organisms: dispersal, establishment, and persistence (Weiher et al. 1999) (Table 4. 2, Figure 4. 1) and influence fitness through its effects on performance. Most importantly, a requirement for any functional trait is that it can be measured at the individual level, without reference to the environment or any other level of organisation (Violle et al. 2007, Carmona et al. 2016), although in practice **species (or population) mean trait values** are usually employed as surrogates of the original trait (Moretti et al. 2017) (Box 4. 1). In agreement with these criteria, we propose a framework applicable to metazoan parasites based on morphological, life-history, and behavioural characteristics (Table 4. 2, Figure 4. 1).

The following core list includes seven functional traits, which we consider the minimum that can be applied to any metazoan parasite and to address any ecological question.

Attachment

Related to persistence (Table 4. 2, Figure 4. 1). Categorical, continuous, or discrete. As categorical, it is coded as the type of organ used to hold on to their host, whereas as continuous or discrete, metric measurements, (e.g. sucker diameter), or number of attaching structures (e.g. clamps), can be used respectively. Each type of measurement could be combined in a **nested functional trait**.

Egg shape

Related to dispersal and establishment (Table 4. 2, Figure 4. 1). Categorical or continuous. As categorical, it can be approximated to geometrical bodies. As continuous, different shape factors (i.e. dimensionless metrics that depend on the relationship between geometric elements) can be used.

Feeding

Related to persistence (Table 4. 2, Figure 4. 1). Categorical or continuous. As categorical, type of food ingested (e.g. blood). As continuous, examples include amount of food eaten, values of $\delta^{13}C$ and $\delta^{15}N$ isotopes, and time spent feeding.

Life Cycle

Related to dispersal, establishment, and persistence (Table 4. 2, Figure 4. 1). Binary, discrete, or continuous. As binary, it can be coded as organisms with a "one-host" (i.e. monoxenous) or "several-host" (i.e. heteroxenous) life cycle. As discrete, it can be assessed as the number of intermediate, paratenic, or dormant stages, episodes of reproduction or times actively transmitted, among others. Examples of continuous traits include estimates of the longevity of developmental stages. See discussion below.

Egg Size

Related to dispersal and persistence (Table 4. 2, Figure 4. 1). Continuous (Table 4. 3).

Number of Eggs

Related to dispersal, establishment, and persistence (Table 4. 2, Figure 4. 1). Discrete (Table 4. 3).

Body Mass

Related to establishment and persistence (Table 4. 2, Figure 4. 1). Continuous (Table 4. 3).

Table 4. 2. Relationship between functional traits of metazoan parasites and three primary challenges faced by organisms (dispersal, establishment and persistence) found in the literature.

Traits	Dispersal	Establishment	Persistence
Morphological			
Attachment			Different strategies depending on the likelihood of being dislodged. More elaborated organs in individuals with higher risk of being detached (Poulin 2007).
Egg size	Positive relationship. Larger eggs (or transmission stages) enhance the probability of transmission, therefore dispersal (Koehler et al. 2012).		Positive relationship. Larger eggs (or transmission stages) have greater food reserves and thus they can spend longer searching for a suitable host (Costello 2006).
Egg shape	Negative relationship with higher density or presence of appendages in eggs or transmission stages. Individuals with complex egg morphologies are less dispersed (Chambers & Ernst 2005). Positive relationship with complex morphologies when appendix-like structures enhance the transmission to an intermediate host (e.g. Pfenning & Sparkes 2019)	Positive relationship with complex morphologies (Yoshida 1920).	
Mass		Negative relationship with available space. More difficult for larger species (Cramer & Cameron 2006, Koehler et al. 2012).	Positive relationship with fecundity in adult individuals (Poulin & Latham 2003).

Behavioural			
Feeding			Negative relationship. More aggressive habits could damage the host and cause the die-off the parasite. Different degrees of persistence depending on pathogenicity (Poulin & Morand 2004). However, it may be advantageous in terms of competition with other parasites.
Life-history			
Life-cycle	Positive relationship with number of intermediate stages through host migration (e.g. Koehler et al. 2012).	Positive relationship with individuals without intermediate stages when there is a high probability of reaching the definitive host (Parker et al. 2003).	Positive relationship with individuals with intermediate stages counteracting environmental stress (Poulin 1992).
Number of eggs	Positive relationship. Individuals that produce more eggs will be more widespread (Costello 2006).	Positive relationship. More likely to succeed in forming a new generation (Lagrue et al. 2011).	Positive relationship. High number of eggs increases the possibilities of persistence over time (Croll et al. 1982, Roughgarden & Iwasa 1986).

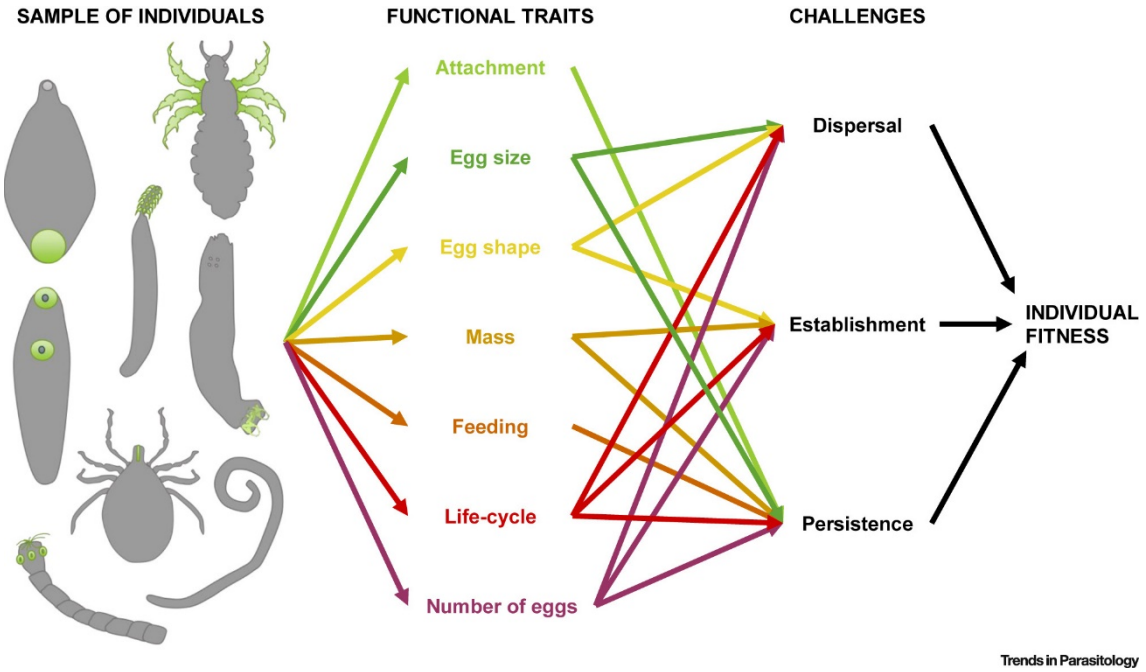

Figure 4. 1. Key Figure. Core list of metazoan parasite functional traits. The term "parasite" can be used to define organisms of distant phylogenetic origins. However, the same functional traits can be shared by organisms with different parasitic strategies. Functional traits are morphological, physiological, phenological, and behavioural characteristics of individuals, measurable at the individual level and that reflect individual performance in ecosystems and its fitness (adapted from Violle et al. 2007).

Measuring Functional Traits

Availability and quality of functional trait data are always an issue, especially for parasites for which information about traits has always been scarce (e.g. Morand 1996). Reliable trait information can be obtained from at least four sources: (i) direct observation, for example, researchers can notice the digestive content of the parasites to establish feeding categories (Moravec 1980); (ii) species descriptions, for example, number of eggs reported (Presswell & Blasco-Costa 2019); (iii) standardised protocols to measure continuous or categorical functional traits, for example, estimation of body mass (Llopis-Belenguer et al. 2018); (iv) proxies, for example, the product of egg length and width as egg size (Poulin 1997). In Table 4. 3, we summarise different approaches previously used in the literature to easily measure some of the proposed traits above.

Analyses based on mean trait values per population or species are acceptable solutions to measure FD in a community (Box 4. 1), albeit they neglect intraspecific trait variability, which is most appropriate to address questions related to responses to environmental gradients, such as climate change (Cadotte et al. 2015, Carmona et al. 2016). In any case, we

recommend getting functional trait information from a reasonable number of individuals for reliable estimates of the species (or population) mean trait value (Moretti et al. 2017) and intraspecific trait variability. This is particularly relevant for traits that vary widely among conspecifics, as, for example, the morphology of attachment organs (Rodríguez-González et al. 2015).

Parasites versus Free-Living Organisms

The study of parasite communities entails an obvious difference with respect to free-living organisms: hosts are discrete and natural sampling units. This advantage can lead to the establishment of comparisons and studies being more easily reproducible in parasitology than in other disciplines. Furthermore, we can get a reliable representation of the community by sampling an adequate number of hosts (Walther et al. 1995). However, as any biological organism, hosts differ in their genetic and physiological condition to prevent parasitic infections (Krist 2004) and these are additional effects to control for in the study of natural assemblages of parasites. Although the characteristics of the environment should not be used as traits (see below), providing detailed information about the host where the **attribute** (i.e. value or category) for a functional trait has been measured is essential to interpret its ecological and evolutionary meaning (Violle et al. 2007). Thus, such information should be considered in the analyses and in the discussion of the results.

4.4 Caveats for a Functional Trait Framework for Parasites

Select Appropriate Number of Functional Traits

An important challenge is to identify an adequate number of key functional traits (Petchey & Gaston 2002) that reflect the performance of organisms in their ecosystems or in their interaction with other organisms in the community (e.g. Weiher et al. 1999, Lavorel et al. 2013). The number of functional traits that can potentially be assessed in any organism is large (Carmona et al. 2016). But our ability to measure functional traits of parasites is often limited due to sampling biases, lack of information about their life history (Poulin 2010), and unclear evidence of a link between the trait and its impact on individual fitness, among other reasons. The core list of functional traits proposed herein should not be considered complete or closed. In studies of phylogenetically closely related species, for example, traits related to reproductive organs can be used as they tend to diverge to avoid cross-fertilisation among closely related

species and are likely to have direct impacts on fitness (e.g. Mouillot et al. 2005). Conversely, when distant phylogenetic groups are studied, traits that tend to converge into few categories should be preferred (e.g. those related to transmission behavior, Thomas et al. 2005).

Furthermore, in some situations, functional traits can be correlated to each other. For instance, a trade-off between egg size and egg numbers has been reported for some parasites (Herreras et al. 2007a, Cavaleiro & Santos 2014). So, when a trade-off is suspected, the opposed traits should be included in the analysis to avoid biased predictions of fitness.

Use Soft Traits

Functional traits should be easy and cheap to measure (soft functional traits *sensu* Weiher et al. 1999). So, when several functional traits are related to the same processes, we should select the softest (Weiher et al. 1999, Violle et al. 2007). For example, the longevity of egg or larval stages relates to persistence (Costello 2006), but longevity is a difficult-to-measure (hard) trait, especially in the wild. In contrast, egg and larval size often reflect longevity and thus represent easy-to-measure (soft) traits. Otherwise, we may not be able to get all the functional information for each species in our sample or we could preclude replication in future studies (Table 4. 3; see matrix of pairwise distances, d_{ij}, in Box 4. 1).

Examples of hard functional traits of parasites include those that involve the life cycle as longevity, since it would require tracking the lifespan of the parasite from hatching to death (e.g. Morand 1996). Likewise, functional traits such as voltinism (number of generations an individual completes in a single year), metabolic rate, or parity (number of times a female lays eggs or gives birth) proposed recently for terrestrial free-living invertebrates (Moretti et al. 2017), are often unknown and very difficult to measure in most parasitic organisms. Researchers should, therefore, focus on soft traits that are correlated with the function to be assessed (e.g. persistence) but easier to measure than the function itself (e.g. egg size). These often have been referred to as **functional markers** (Garnier et al. 2004).

Performance Traits and Ecological Performances

One should abstain from considering as functional traits, features based on information external to the individual or measured at a higher level than the individual (Violle et al. 2007). Particularly, specificity (Table 4. 1), widely used in parasitology, could be considered as a proxy of parasite survival ability (a **performance trait**). As classically defined, specificity cannot be measured at the individual level because it represents the number of different hosts (environmental habitats) in which a parasite species can survive, instead of habitats which every

single individual in a sample could inhabit. In addition, it relies on information external to the individual (range of hosts) to be defined. Thus, specificity does not conform to the concept of functional trait currently accepted in ecology (Violle et al. 2007). The range of hosts a parasite can infect is just an environmental variable. Likewise, habitat/niche (e.g. host tissue or location on or in the host, the ecto- and endoparasite dichotomy), the taxonomic identity or features of hosts, and macrohabitat (e.g. freshwater, marine, terrestrial, mixed) are environmental variables (i.e. external to the individual) commonly employed in our field. The response of an organism to these environmental variables can be measured as an **ecological performance**. For instance, given an array of potential hosts in a locality (environmental variable), the survival ability (performance trait) of a parasite can be assessed as classical host specificity, that is, the host range (ecological performance), for the parasite species at that locality.

Table 4. 3. Standardised methods to measure continuous functional traits.

Functional trait	Methods	
Egg size	Volume of geometric morphologies: sphere for copepods; ellipsoid or prolate spheroid for nematodes, trematodes, acanthocephalans or fleas. Proxy of egg size: product of egg length and width for trematodes; maximum length of eggs for monogeneans.	Kearn 1985, Poulin 1997, Fredensborg & Poulin 2005, Herreras et al. 2007a, Cavaleiro & Santos 2014, Khokhlova et al. 2015
Number of eggs	Counts from individuals mounted on permanent slides for trematodes. Counts from aliquots of dissected individuals for nematodes. Number of eggs laid by an individual for a period for monogeneans, trematodes and fleas. Automated counting methods for nematodes.	Kearn 1985, Fredensborg et al. 2004, Herreras et al. 2007b, Khokhlova et al. 2015, Preswell & Blasco-Costa 2019
Mass	Area by Depth by Density for flatworms. Volume of Revolution by Density for nematodes or acanthocephalans. Direct measurements (weighting large parasites on a scale). Approximating body forms to regular geometric morphologies. Generalised regression equations between body length and mass.	Llopis-Belenguer et al. 2018

Although ecological performances can provide valuable insight into community ecology (e.g. Cizauskas et al. 2017), they depend on the coordinated response of multiple traits to environmental factors (Violle et al. 2007) and thus do not represent functional traits. We are not in favour of using ecological performances under a functional-trait perspective because it hinders our understanding of the actual mechanisms driving the fitness responses of the organisms. However, in the absence of knowledge on the traits influencing individual fitness, an ecological performance can be tentatively used as a surrogate of a complex of functional traits.

The use of other features that refer or apply to different levels of organisation (i.e. **demographic parameters**, such as population size, birth, death, immigration, or emigration rates) is discouraged because they do not affect fitness (Violle et al. 2007).

Handling Missing Information

It is difficult to gather a complete or highly resolved dataset of each trait for each taxon (e.g. Barnett et al. 2007). Often, information for a functional trait is either not available or structurally absent (e.g. number of eggs in larval stages). Nonetheless, as many community ecology analyses (such as FD studies) rely on computing a matrix of pairwise distances between parasite species (Box 4. 1), one very common solution is to use the **Gower distance**: pairwise (dis)similarities among taxa based on traits (Gower 1971). Its application is gaining currency as a measure of the pairwise distances among taxa based on traits because of its ability to combine several types of traits (continuous, categorical, binary, etc.) and to allow for missing data when calculating the pairwise dissimilarity matrix of functional traits between species (Botta-Dukát 2005) (Box 4. 1).

Dealing with Different Developmental Stages

Commonly, hosts (i.e. sampling unit, Box 4. 1) harbour parasite species at different developmental stages and, even a parasite species can be represented by adults and larvae in or on the same host. Depending on the aims of the study, it is acceptable to focus on adults, larvae, or both. For instance, one might be interested in unveiling the forces that select certain functional traits of parasites at their definitive hosts exclusively. So, researchers would exclusively focus on adults. If the study considers incorporating larvae and adults jointly, the first question is whether they represent the same or different functional entities. The answer depends on the previous knowledge on each parasite species involved and the scope of the study. Below, we contemplate three potential scenarios:

(i) Adults and larvae of the same species in/on the same organ. Host role: definitive. Larvae that arrived recently and still have to develop to the adult stage share host with adult stages (e.g. ticks (Parasitiformes)). Adults and larvae can be considered as the same functional entity when computing pairwise dissimilarity matrix. For continuous functional traits, we propose to average individual values (regardless of the adult or larval condition) by the total number of individuals.

(ii) Adults and larvae of the same species in/on the different organs. Host role: definitive and intermediate, respectively.

This can occur in species with multiple developmental stages and complex life cycles (e.g. *Trichinella* spp.). Adults and larvae can be considered as different functional entities holding different functional traits. For instance, feeding can be set as "tissue" or "latent" depending on its developmental stage. Furthermore, functional traits related to adults, such as size and number of eggs, will be set to zero for the larval stages.

(iii) Only larvae of a given species occur. Host role: intermediate.

A species only uses the target host during its larval stage. As in (ii), the larvae would represent an independent entity in terms of functional trait characterization.

Finally, as a note of caution, decisions on what to include must be consistent across both the functional and taxonomic facets of diversity (Box 4. 1). Thus, if, for instance, adults and larvae of species A are considered as separate functional entities, the same criterion should apply in the analysis of **taxonomic diversity**.

4.5 Concluding Remarks

This framework sets the basis for the selection of adequate functional traits (fitness-related, measurable at the individual level, and without information external to the individual, Violle et al. 2007) and promotes novel insights into the mechanisms of parasite community assembly and dynamics. Studying parasite ecology from a functional perspective is lagging behind other disciplines and this is hindering our fine understanding of the multitude of roles that parasites play in ecosystem properties. As in other disciplines, we hope that this ready-to-use core list of functional traits of metazoan parasites inspires further efforts in defining and understanding functional traits of parasitic organisms.

To date, most functional trait studies have been based on species (or population) mean trait values. However, focussing on intraspecific trait variability could be especially insightful (Carmona et al. 2016) because conspecifics can occupy different positions in the **functional trait space** or individuals of different species can overlap in it (see Outstanding Questions).

In consequence, it can prove extremely rewarding for revealing cryptic (i.e. unrelated or overlapped positions) functional roles and mechanisms, which are masked in the mean value approach. As it was previously demonstrated (Carmona et al. 2016), this approach entails computing functional trait information of a statistically meaningful number of individuals in the sample at the same time. This seems especially challenging in parasitology because of the patchy distribution of parasites on or in their hosts. The issue of intraspecific trait variability also brings to light the need to perform more experimental studies to unveil the relationship between potential functional and performance traits and the components of individual fitness. Furthermore, the development of new functional markers can prove fruitful to understand complex ecosystem properties. For example, the proportion of the chemical elements C:N:P in parasite individuals can be a proxy of the performance of the individual nutrient consumption, and, finally, an indicator of the nutrient flux at a locality (Bernott & Poulin 2018). Ultimately, the expansion of this core list of functional traits relies on the availability of species information. Thus, the creation of a public-access database of functional traits of parasites compiled by international and multidisciplinary parasitologists, from taxonomists to ecologists, is highly encouraged, as it exists for other groups of organisms.

Outstanding questions

- How do different environmental filters select for parasite functional traits in communities?
- How do species interactions select for parasite functional traits?
- Can we predict species abundances within communities based on trait variation, environmental conditions, and competitive interactions?
- What are the mechanisms selecting for particular traits at different stages of parasite community assemblies?
- Under a functional trait perspective, what can we learn from parasite biological invasions? Which functional-trait attributes of introduced parasites can foster the success of an invasion? Which attributes of native parasites can hamper host invasions?
- How do functional-trait attributes determine the position and role of parasites in the interaction network of the community?
- How can intraspecific functional trait variability inform of ecoevolutionary responses in parasite communities?

- What hidden patterns in parasite community ecology could be revealed by the combined analysis of the facets of diversity (taxonomic, functional, and phylogenetic diversity)?

4.6 References

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4.7 Glossary

Attribute: value or category taken by a trait at any place and time.

Demographic parameter: population-level feature based on aggregation of features of individuals.

Ecological performance: optimum and/or the breadth of distribution of performance traits along an environmental gradient.

Equivalent numbers (or effective number of species): value of a diversity index that would result if all the species of the assemblage were equally likely (equally abundant and evenly distributed) and maximally dissimilar.

Functional diversity (FD): a measure of richness and abundance of functional traits in a community or ecosystem.

Functional marker: trait correlated with a function that is easier to measure than the function itself. For example, in Acanthocephala the complex-to-measure function dispersal ability of an individual has been assessed by egg morphology (Pfenning & Sparkes 2019).

Functional trait: trait (see below) which impacts the fitness of individuals and reflects their performance in ecosystems.

Functional trait space: *n*-dimensional space where *n* is the number of functional traits considered. Typically, species are plotted in such space according to their species mean functional trait value. Under the recent "intraspecific trait variability" approach (Carmona et al. 2016), individuals are plotted according to their own values, without averaging by species.

Gower distance (d_{ij}): a measure of the pairwise distances among taxa based on traits. It ranges from zero (identical taxa) to one (maximum dissimilarity between taxa). It allows different types of variables and missing data. It is associated with some properties of the Euclidean distance.

Intraspecific trait variability: range of variation in the same trait among conspecifics within a sample.

Nested functional trait: functional trait that can be measured at different self-contained levels. Levels can combine different types of attributes. For example, "attachment", a categorical functional trait can be combined with morphometric (continuous) measures of the organ involved.

Performance: the ability of individuals to grow, reproduce, or survive in a particular ecological habitat.

Performance traits: traits that measure one of three components of the fitness (survival, growth, or reproduction). They can be measured in a cohort that reflect average fitness of individuals.

Phylogenetic diversity (PD): measure of the richness and abundance of genetically different entities in a community or ecosystem.

Property: feature or process at community or ecosystem level.

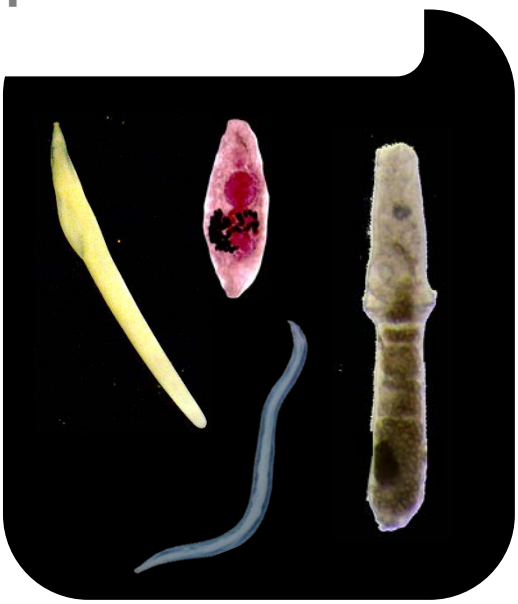
Species (or population) mean trait value: mean value of a trait for a species or population of the species. These values are used in the functional trait matrix (Box 4. 1) to calculate the distances between species/populations.

Taxonomic diversity: a measure of the richness and abundance of taxonomic entities in a community or ecosystem.

Trait: morphological, physiological, phenological, or behavioural feature measurable at the individual level, from the cell to the whole organism, without reference to the environment or any other level of organisation. Traits can be of various types: continuous, discrete, ordinal, categorical, binary, fuzzy, multiple choice, or circular.

$$\alpha_{Rao} = \sum_{i=1}^{s} \sum_{j=1}^{s} d_{ij} p_i p_j$$

5. Assembly rules of helminth parasite communities from grey mullets: combining taxonomic, functional and phylogenetic approaches



5. Assembly rules of helminth parasite communities from grey mullets: combining taxonomic, functional and phylogenetic approaches

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Highlights

- Diversity can be studied at multiple facets and levels.
- We examined the mechanisms driving helminth parasite diversity in a community.
- Random and deterministic processes simultaneously explain diversity in our community.
- Parasite communities cannot be fully understood if a diversity component is ignored.

Keywords

diversity, ecological communities, environmental filtering, helminths, limiting similarity, random processes

5.1 Abstract

Organisms associate themselves in ecological communities. It has been widely debated whether these associations are explained by deterministic or, contrary, haphazard processes. The answer may vary depending on the level of an organisational scale (α, β and γ) and the facet of diversity considered: taxonomic (TD), functional (FD) and phylogenetic (PD). Diversity at the sampling unit (i.e. host individual) is the α diversity; β diversity represents extent of dissimilarity in diversity among sampling units (within a level of an organisational scale, β1; between levels of an organisational scale, β2); and the total diversity of a system is γ diversity. Thus, the combination of facets and levels of scale may be useful to disentangle the mechanisms driving the composition of a parasite community. Using helminth parasite TD,

FD and a Proxy of PD (PPD) of grey mullets (Teleostei: Mugilidae) from the Mediterranean Sea, we show that random and deterministic processes of different nature explain the assemblage of parasite communities. The parasite community at a host individual (α) was invariably a random subset of the total diversity in the community. At β1 TD was lower than expected by chance, whereas FD and PPD were random. This may suggest that, at a given place and in a given host species, some parasite species are more abundant than others, leading to many species shared and a few species unshared with other host individuals. At β2 level, diversity patterns suggested environmental filtering of the parasite assemblage: species, trait, and phylogenetic compositions of parasite communities seem to depend primarily on the species of host, but also on the locality and season. Our study demonstrates that parasite communities are not totally understood if any of the components of diversity is neglected.

5.2 Introduction

Understanding the processes governing the distribution and assembly rules of biological diversity is one of the major challenges in biogeography and macroecology, and a prerequisite for successfully predicting global change impacts on biodiversity (Guisan & Rahbek 2011). However, due to the extreme complexity of communities and ecosystems, we are still far from achieving this goal. The current state of affairs can be described as a schism between ecological and historical biogeography, which stems from the unresolved debate about the nature of the mechanisms governing community assembly. This divide, known as the Clements-Gleason controversy, has pervaded ecological thought for nearly a century. Whereas the Clementsian ecologists view ecological communities as tightly integrated entities consisting of interdependent species (the community-unit concept), the Gleasonians posit that species co-occur largely according to the individualistic response of each species to variable environmental conditions (the individualistic concept) (Liautaud et al. 2019). Accordingly, species coexistence would be accounted for by the coincidence of species that tolerate the same habitat (Götzenberger et al. 2012, Liautaud et al. 2019). To a large degree, the latter view has prevailed in the last decades, based largely on empirical evidence of community composition along environmental gradients, and has eventually led to postulate the "disintegration of ecological community" (Ricklefs 2008). Under this paradigm, species distributions would be mostly, if not exclusively, constrained by local environmental conditions, historical large-scale events and dispersal capacity of the species. However, recent evidence suggests that community structure cannot be fully understood without taking into consideration deterministic processes, such as environmental filtering or limiting similarity (Pavoine & Bonsall 2011).

Stochastic processes can affect diversity, influencing speciation, extinction, colonisation or dispersal to a new community, and these can finally cause a random assemblage of diversity. On the other hand, deterministic processes can lead to either clustering (i.e. convergence) or overdispersion (i.e. divergence) of diversity (not to be confused with spatial clustering and overdispersion, Götzenberg et al. 2012). A clustering pattern of diversity assembly is interpreted as evidence of environmental filtering structuring the diversity of the community, because abiotic factors select individuals with particular environmental tolerances, and it results in similar life strategies converging in a community (Pavoine & Bonsall 2011). In contrast, the opposite pattern, i.e. overdispersion, would reflect a limiting similarity process, in which biotic forces tend to limit the coexistence of very similar life strategies (Pavoine & Bonsall 2011, Götzenberger et al. 2012). Nonetheless, environmental filtering and limiting similarity are not the only potential deterministic processes acting on communities and alternative processes can lead to similar patterns of diversity dispersal. For instance, competition due to biotic interactions excluding the less competitive strategies can also result in diversity overdispersion (Mayfield & Levine 2010).

The understanding of the processes that drive biological communities can be examined for the multiple components of diversity to get a complete resolution of communities. Diversity includes multiple levels (e.g. α, β, γ) nested in organisational scales (e.g. spatial scales) and it has been argued that the importance of ecological processes is probably scale dependent. From the lowest to the highest of level of an organisational scale, three diversity levels are classically defined: α diversity or diversity at the sampling unit; β diversity or extent of dissimilarity in diversity among sampling units (within a level of an organisational scale, β1; between levels of an organisational scale, β2) and γ diversity or total diversity (Pavoine et al. 2016). Moreover, it includes multiple facets, such as the variety in species, traits or evolutionary units (Pavoine & Bonsall 2011). Regardless of the level considered, diversity has been studied under three facets: Taxonomic Diversity (TD, richness and abundance of taxonomic entities in a community), Functional Diversity (FD, richness and abundance of functional traits in a community) and Phylogenetic Diversity (PD, richness and abundance of genetically different entities in a community) (Pavoine & Bonsall 2011). Traditionally, ecologists considered TD as the single measure of diversity. This fact caused a continuous loss of ecological (FD) and evolutionary (PD) information, since TD considers that all the species in a community are equally similar and does not take into account the uniqueness of the functions or the phylogenetic distinctness of each species (Pavoine & Bonsall 2011).

Parasite communities offer excellent models to study the assembly rules of the facets of diversity at different levels of organisation. The sampling unit (i.e. host individual) can be precisely defined (as required by Götzenberger et al. 2012) and it is often relatively easy to sample a large number of communities to eventually get strong statistical conclusions (Poulin & Valtonen 2001). Although pioneering studies exist (e.g. Mouillot et al. 2005, Krasnov et al. 2014, 2015, 2016), up to now little is known about the mechanisms driving the different facets of diversity at different organisational scales of an entire parasite community. In studies of free-living organisms, diversity dispersal patterns can hierarchically be assessed at different spatial levels (e.g. local communities, regions, continents) or even temporal levels (e.g. years, decades, centuries) (Pavoine et al. 2009a). The study of parasite communities provides an additional organisational scale, the host, which also shapes parasite diversity. For example, parasite diversity can be examined at host taxonomic levels (i.e. species, genus, family, and so on).

Here, we will investigate the assembly mechanisms (deterministic vs stochastic processes) of the helminth parasite communities of grey mullets (Teleostei: Mugilidae) from the Western Mediterranean Sea under the influence of host and environmental factors. This model is appropriate because, first, these parasite communities include parasite species from distant phylogenetic origins and are functionally disparate. Second, it comes from three of the up to six sympatric grey mullet species that coexists in this area of the Mediterranean (Blasco-Costa 2009) and from localities that vary in their environmental parameters. This allows us to test whether the different facets of helminth diversity provide congruent results and whether host factors (phylogenetic proximity and similarity in life strategies) and/or environmental factors (geographical location and habitat conditions) select for different parasitic life strategies. Particularly, two of the host species, *Chelon auratus* and *Chelon ramada*, are phylogenetically closer to each other than to *Mugil cephalus* (Durand et al. 2012), whereas *M. cephalus* and *C. ramada* show greater similarities in their life strategies between them than *C. auratus* (Cardona 2001, 2006). Additionally, our samples are from three coastal localities that differ in their habitat conditions (two marine: Ebro Delta, Sea - EDS and Santa Pola, Sea - SPS; and one brackish lagoon: Santa Pola, Lagoon - SPL) and in their geographical proximity (SPS and SPL are very close, ~10 km apart; whereas EDS is more distant from the other two, ~290 km). Finally, our nested sampling design allows us to measure and compare diversity at the sampling unit (α diversity or parasite diversity at the host individual) and within and between levels of a factor (i.e. parasite diversity within (β1) or between (β2) host individuals of a host species or locality).

We asked whether diversity patterns differ between the facets of parasite diversity and across two hierarchical scales (i.e. locality and host), and which are the factors (i.e. host phylogeny vs life strategies; habitat conditions vs geographic proximity) related to such variation. Based on evidence from free-living organisms (Cavender-Bares et al. 2006, Kraft & Ackerly 2010), and previous studies of our host-parasite system (Blasco-Costa 2009, Blasco-Costa et al. 2012, Míguez-Lozano et al. 2012, Sarabeev et al. 2013), we hypothesise that both host phylogeny and host life strategy will influence the parasite community, whereas habitat conditions will be a stronger determinant of the parasite communities than the geographic distance. Parasite communities will be overdispersed at low organisational levels (within a locality or a host species), since similar life strategies will be limited (i.e. not able) to coexist. Conversely, at higher levels, we expect that clustering will be the driver of the parasite community, because the environment filters (i.e. selects for) certain life strategies.

5.3 Material and Methods

5.3.1 Data

Fish were obtained from local harbour markets and surveyed for parasites as described in Blasco-Costa (2009). Adults of helminth parasites were identified following Yamaguti (1958), Gaevskaya & Dmitrieva (1992) and Blasco-Costa (2009) for trematodes; Paperna (1964), Euzet & Combes (1969) and Sarabeev et al. (2013) for monogeneans; Orecchia & Paggi (1987) for nematodes; and Orecchia et al. (1988) and Tkach et al. (2014) for acanthocephalans. The dataset includes 272 host individuals and 30 parasite species from three seasons (two autumns and one spring) of two years (2004 and 2005) (Table 5. 1). Data analysis was entirely performed in R (R Core Team 2019).

Table 5. 1. Sample summary. Fish (host individuals) sample sizes by host species, seasons and localities.

	Santa Pola Sea			Santa Pola Lagoon			Ebro Delta Sea		
	Autumn 2004	Spring 2005	Autumn 2005	Autumn 2004	Spring 2005	Autumn 2005	Autumn 2004	Spring 2005	Autumn 2005
Chelon auratus	12		30						
Mugil cephalus	20	22	30		25	31		28	29
Chelon ramada	30		15						

5.3.2 The multiple facets of diversity

Functional trait information

We used five functional traits based on the framework and core list developed by Llopis-Belenguer et al. (2019): attachment organ, type of life cycle, body mass, egg size and number of eggs. We extracted information of categorial functional traits (i.e. attachment organ and type of life cycle) from direct observations, whereas, we obtained information of continuous functional traits (i.e. body mass, egg size and number of eggs) from species descriptions or as the mean value measured from a varying number of individuals of each species. To estimate individual body mass, we resorted to indirect methods "Area by Depth by Density" (for flatworms: trematodes and monogeneans) and "Volume of Revolution by Density" (for organisms with circular transversal section along their bodies: acanthocephalans and nematodes) (Llopis-Belenguer et al. 2018). Mass of a parasite species was computed as the mean mass of a range of individuals (mean number of individuals per species 9; range 2-12). Egg size was estimated as the mean maximum egg length and mean width from species descriptions, when these measures were available. Otherwise, we measured these features from 3 to 20 eggs (mean 10) from a varying number of individuals from each species. Then egg volume (μm^3) was estimated assuming an ellipsoid shape (i.e. depth equal to width). For the number of eggs of monogeneans and trematodes, we counted eggs from 1 to 20 (mean 9) individuals per species mounted on slides. Since acanthocephalans and nematodes possessed too many eggs to be counted directly, the number of eggs was estimated as follows: 10 females of each species were dissected individually, and their eggs diluted in 1 ml of saline solution each. Then the total number of eggs was estimated from aliquots of 0.1 ml from each specimen. Regardless of the method used, we performed the procedure twice to obtain the mean number of eggs for each individual. Finally, we log-transformed data of continuous traits.

We built a functional trait (columns) by parasite species (rows) matrix with the dist.ktab function from package ade4 (Dray & Dufour 2007) and calculated the Gower's distance (Gower 1971) between species. This distance allows combining several types of traits (continuous and categorical as described above) and incorporating observations with missing data (4% in our dataset). Then, we transformed the Gower matrix of pairwise distances into Euclidean pairwise distances (function lingoes in ade4) and divided the resulting matrix by its maximum to bound values between 0 and 1 (Pavoine et al. 2009b).

Proxy of phylogenetic diversity

Since we did not have a complete phylogeny of species in the community, we used a Proxy of Phylogenetic Diversity (PPD) to estimate the phylogenetic pairwise distance between species. The PPD can be seen as a measure of the length of the path connecting two species traced through a Linnaean classification of the full set of species in the community (Clarke & Warwick 1998). We created a Euclidean pairwise distance matrix between parasite species by means of the taxa2dist function from vegan (Oksanen et al. 2019) applied to a classification table with parasite species at rows and taxa of such species at higher levels (genus, subfamily, family, suborder, order, class, phylum, in our case) at columns. We divided the resulting distance matrix by its maximum to bound the cell values between 0 and 1.

Correlation between functional traits and taxon-level distances

We assessed the correlation between the matrix of pairwise functional distances and the matrix of pairwise phylogenetic distances to study the relationship between the evolutionary history and ecological processes behind community assembly (Pavoine & Bonsall 2011). We tested such correlation by means of the Mantel test (mantel function in vegan).

5.3.3 Diversity analyses

Our samples were organised by three key factors: host species, locality and season (Table 5. 1). We evaluated the effect of the host species and the locality by splitting the diversity analyses into two case studies. This was due to *C. auratus* does not naturally occur in the lagoon. In Case 1, we assessed the influence of host species on the three facets of diversity. To that end, we analysed and compared TD, FD and PPD of the helminth parasite communities from the three host species, at SPS in two autumn surveys (2004 and 2005). In Case 2, we evaluated the effect of locality and season survey on the parasite communities. To that end, we analysed and compared TD, FD and PPD of the helminth parasite communities from *M. cephalus* at the three localities surveyed in spring and autumn 2005.

Influence of one factor on diversity

In the case studies considered, we measured diversity in two different ways. First, we used the Double Principal Coordinate Analysis (DPCoA) (Pavoine et al. 2004), that is a combined version of the Rao index of diversity (Rao 1982) and the Weighted Principal Coordinate Analysis (Gower & Legendre 1986). DPCoA allows comparing the partitioning of diversity at different levels of an organisational scale and the different facets of diversity. It is based on the

matrix of pairwise distances (functional or phylogenetic) between species in a sample and an abundance matrix of such species. When the scope of the analysis is TD, all cells of the distance matrix are set to 1. Hence, all species are considered equally and maximally distant, and Rao's index becomes equal to the Simpson's index of diversity, i.e. equals to the probability of any two individuals being of different species when randomly drawing from a community (Pavoine et al. 2004). Furthermore, the ordination of species according to the previous two matrices (i.e. pairwise distances and abundance) assembles species in a multivariate space, that is related with the decomposition of diversity in organisational levels (Pavoine et al. 2004). Since the DPCoA only allows studying one factor at a time, we analysed the databases by season survey to avoid crossed factors.

Under the DPCoA framework we measured α diversity with function dpcoa in ade4. In order to examine the relationship between α diversity (i.e. within a host individual) and the factors host species (Case 1) or locality (Case 2), we used function lm.rrpp of package RRPP (Collyer & Adams 2018a) that performs a linear model by residual randomisation and provides empirical sampling distributions for further ANOVAs. Following Collyer & Adams (2018b), univariate α values were log-transformed. Then, we performed ANOVAs (type I of sums of squares) using random distributions of the F-statistics (Collyer & Adams 2018b) for TD, FD and PPD, independently. When differences between samples from different host species or localities were significant, we ran posteriori pairwise comparisons of α TD, FD and PPD between host species or localities using function pairwise in RRPP.

We calculated β diversity at two different organisational levels under the context of the Rao index of diversity. β1 represents dissimilarity in parasite diversity among sampling units (hosts) within the same host species or locality. Whereas, β2 indicates dissimilarity in parasite diversity between host species or localities. In both cases, we calculated β diversities under the equivalent number approach (Ricotta & Szeidl 2009) using the third proposition of the Rao index of diversity in Pavoine et al. (2016) (EqRao function in adiv (Pavoine 2018)) since it was specifically developed for unbalanced samplings. Furthermore, we compared each of the TD, FD and PPD β1 and β2 diversities with 999 randomly simulated β1 and β2 values (rtestEqRao function in adiv) in order to establish whether the observed values significantly differ from those randomly simulated ($p < 0.05$). When significant, we compared observed and simulated results to determine whether the observed β1 or β2 were greater or lower than expected at random. This allows determining whether parasite communities from fish of the same species or locality (β1) or of different fish species or locality (β2) are more similar (the observed value is lower than simulated values) or more dissimilar (the observed value is greater than simulated

values) to each other than expected by chance. Finally, we used the standardised β1 and β2 given by EqRao function (observed β – mean of randomly simulated βs/ standard deviation of randomly simulated βs) to infer if the parasite species, traits or the phylogenetic proxy are overdispersed (negative standardised β) or clustered (positive standardised β) (Head et al. 2018) within a level of a factor (β1) or between levels of a factor (β2).

Influence of two crossed factors on diversity

To evaluate and disentangle the effect of crossed factors on diversity, we used crossed-DPCoA (Pavoine et al. 2013a). In both case studies, we analysed the effect of two crossed factors simultaneously: host species (Case 1) or locality (Case 2), and season. The crossed-DPCoA is grounded on the DPCoA but it analyses the effect of two crossed factors at the same time. Thus, it distinguishes the proportional contribution of the sampling unit, each factor individually and the effect of the interaction of both factors on the diversity of the community.

The crossed-DPCoA consists of three consecutive analyses. Following Pavoine et al.'s (2013a) terminology, each parasite community is associated with a component of the factor A (hosts species or locality) and a component of a factor B (season). The main version of the crossed-DPCoA plots in a DPCoA space the parasite species, the sampling units and the variables of the main factor A, without taking into account seasonal differences (factor B). Then, the first version of the crossed-DPCoA removes the amount of diversity among sampling units due to the sole effect of factor B, but retains combined effects of factors A and B (i.e. the interaction between factors A and B). Finally, the second version of the crossed-DPCoA eliminates any influence of the factor B on the factor A (including the interaction term). Thus, it provides diversity exclusively under the light of the main crossed-factor, factor A (host species or locality). We carried out the main, the first and the second versions of the crossed-DPCoA with functions crossdpcoa_maineffect, crossdpcoa_version1 and crossdpcoa_version2 in adiv, respectively.

5.4 Results

Parasite functional and phylogenetic-like pairwise distance matrices were correlated (Mantel test, $F = 0.77$, $p < 0.001$).

Case 1: Santa Pola Sea (SPS), autumn from 2004 and 2005 – *Chelon auratus*, *C. ramada* and *Mugil cephalus*

At the α level and in the first survey (autumn 2004), we found non-significant differences in terms of TD and FD of parasites hosted by the different fish species (Table 5. 2). However, the analysis of the PPD showed significant differences in α diversity among host species, because the parasite community of *C. ramada* was less diverse than that of *M. cephalus* (Figure 5. 1: autumn 2004; Table 5. 2; Supplementary Table 5 S 1a). In the second survey (autumn 2005), α diversity of the parasite community of *C. ramada* was significantly lower in terms of parasite species (lowest TD) and functional traits (lowest FD) than the parasite communities hosted by other two fish species (Figure 5. 1: autumn 2005, Supplementary Table 5 S 1b and c). As for PPD, the three host species displayed significantly different α diversity values with *C. ramada* having the lowest diversity and *M. cephalus* the highest (Figure 5. 1: autumn 2005; Table 5. 2; Supplementary Table 5 S 1d).

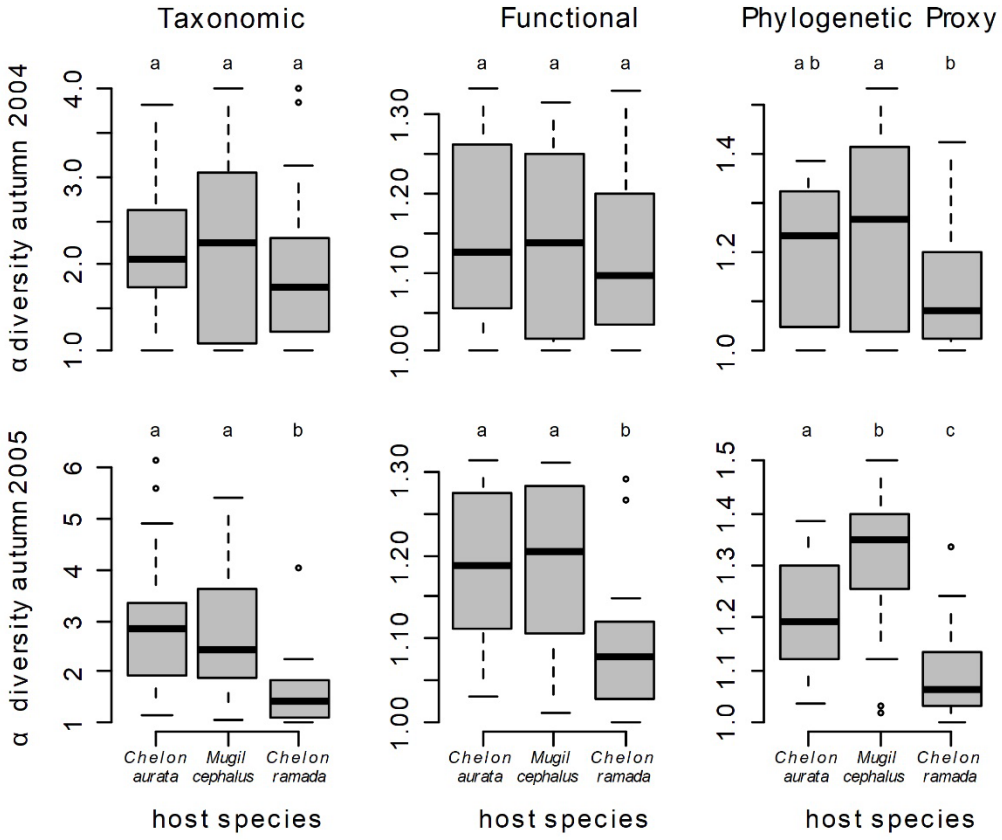

Figure 5. 1. Parasite α diversity in terms of taxonomy (TD), functional traits (FD) and a proxy of the phylogeny (PPD) for each host individual of each fish species. Different lowercase letters indicate significant differences between host species.

Table 5. 2. Results of Type I ANOVAs of α diversity.

	Case 1		Case 2	
	Autumn 2004	Autumn 2005	Spring 2005	Autumn 2005
TD	0.463^{NS}	0.001^{***}	0.13^{NS}	0.001^{***}
FD	0.681^{NS}	0.002^{**}	0.003^{**}	0.001^{***}
PPD	0.016^{*}	0.001^{***}	0.016^{*}	0.001^{***}

* < 0.05, ** <0.01, *** ≤ 0.001. TD, Taxonomic Diversity; FD, Functional Diversity; PPD, Proxy of Phylogenetic Diversity

At the β1 level, we found that the parasite communities of fish from the same sample (within host species) tended to be more similar than expected by chance only in "TD – autumn 2004" (Table 5. 3, Supplementary Figure 5 S 1a-c and Figure 5 S 2a-c). The negative values of the standardised β1 in both autumn surveys indicated that parasite species, traits and phylogeny tended to be overdispersed within host species (Table 5. 3). At the β2 level, the parasite communities from fish from different host species always differed significantly from randomness (Table 5. 3, Supplementary Figure 5 S 1d-f and Figure 5 S 2d-f). The positive values of the standardised β2s indeed indicated that the composition in parasite species, traits and phylogenies differed between host species in both autumn surveys (Table 5. 3).

Table 5. 3. Partition of diversity at two organisational levels of the host species factor. Statistical results of the partitioning of Taxonomic (TD), Functional (FD) and the Proxy of Phylogenetic (PPD) Diversity at two organisational levels (β1 [among host individuals within host species] and β2 [between host species]) in comparison to a distribution of 999 random replicates. Standardised observed values are given in parenthesis. A standardised β is negative when the community structure is overdispersed, and positive when the community structure is clustered.

	β1			β2		
	TD (std. ob.)	FD	PPD	TD	FD	PPD
autumn 2004	0.005* (-2.6)	0.09^{NS} (-1.7)	0.16^{NS} (-1.4)	0.001^{*} (13.9)	0.001^{*} (12.5)	0.001^{*} (12.9)
autumn 2005	0.17^{NS} (-0.3)	0.65^{NS} (-0.6)	0.48^{NS} (-0.8)	0.001^{*} (13.9)	0.001^{*} (11.6)	0.001^{*} (11.6)

*, p < 0.01; NS, non-significant results

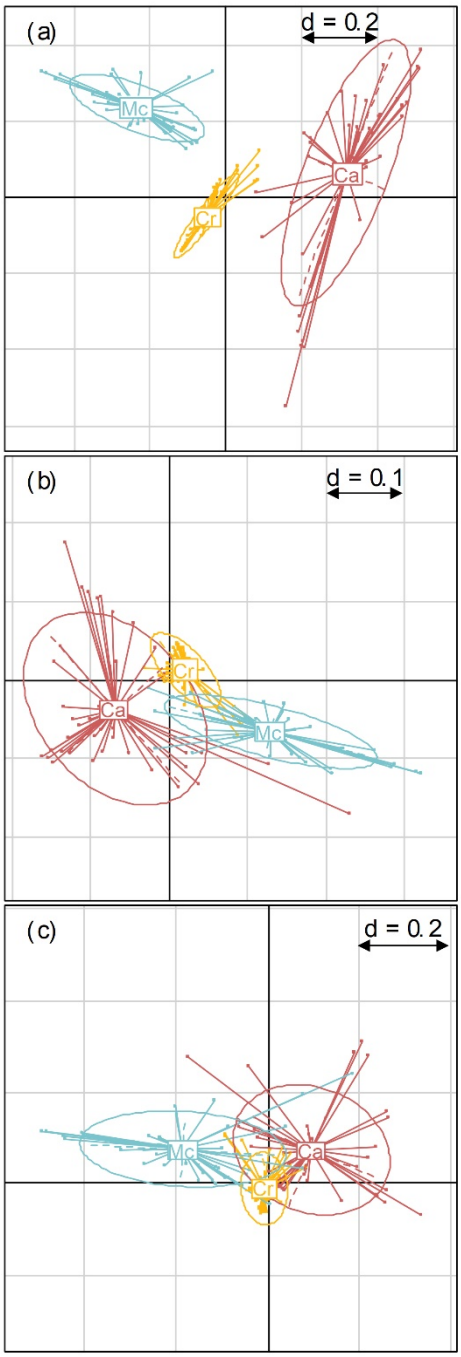

Figure 5. 2. Position of each host individual (dots) in the (a) parasite species space, (b) in the parasite trait space, and (c) in the parasite proxy of phylogeny space. Fish are grouped by host species. Abbreviations: Mc, Cr and Ca represent the centroid of each fish species, and stand for *Mugil cephalus*, *Chelon ramada* and *C. auratus*, respectively. These analyses were carried out with the second version of the crossed-DPCoA. The width and height of the ellipses are given by the variance of the coordinates of the individuals, and the covariance between the coordinates on the two axes gives the slope of the ellipse. "d" (top-right) indicates the length of side of the grey squares of the background grid.

When taking crossed-effects into account, diversity within each host individual constituted the highest proportion of parasite diversity in the whole community (49%, 50% and 55% for TD, FD and PPD respectively), followed by host species, the crossed effect of host species and seasons, and season (Table 5. 4a). The graphical representations of the second version of the crossed-DPCoA considering TD, FD and PPD (Figure 5. 2) were congruent. In all three cases, the first axis separated the parasite communities of the two coastal-related grey mullets (*C. ramada* and *M. cephalus*) from the marine-related *C. auratus*, although those of *C. ramada* were always in an intermediate position. The parasite communities of the three host species showed an overlap in the parasite functional trait and phylogenetic-like spaces, but host species was still a significant predictor for trait and phylogenetic diversity of the parasite communities (Figure 5. 2a).

Case 2: Ebro Delta Sea (EDS), Santa Pola Sea (SPS) and Santa Pola Lagoon (SPL) – spring and autumn from 2005 – *Mugil cephalus*

The statistical analyses of α TD, FD and PPD did not show congruent results. In spring, localities did not differ in the composition of the parasite species (TD in Table 5. 2) but did in FD and PPD. This was due to SPL being significantly less diverse than the other two localities in terms of FD and than EDS in terms of PPD (Table 5. 2; Supplementary Table 5 S 2a and b; Figure 5. 3: spring 2005). In

the autumn survey (Figure 5. 3: autumn 2005), SPL had significantly lower diversity than the other two localities in terms of TD (Supplementary Table 5 S 2c). In terms of FD, the three localities differed significantly (Supplementary Table 5 S 2d). For PPD, SPS had significantly higher diversity than the other two localities (Supplementary Table 5 S 2e).

Table 5. 4. Percentage of diversity associated with each factor. Diversity of parasite communities (a) of three host species and autumn 2004 and 2005 (Case 1); (b) and from three localities and spring and autumn 2005 (Case 2).

(a)	TD	FD	PPD
Host individual	49	49.9	55.02
Host species	31.7	24.8	18.33
Season	1.7	1.5	1.12
Host species × season	17.6	23.8	26.53
(b)			
Host individual	57.9	53.7	52.3
Locality	5.8	11.6	12.4
Season	1.3	0.6	0.9
Locality × Season	35	34.1	34.4

TD, Taxonomic Diversity; FD, Functional Diversity; PPD, Proxy of Phylogenetic Diversity

At β1 level, the dissimilarity in diversity of the parasite communities from hosts within the same locality tended to be lower than expected by chance only for TD in both season surveys (Table 5. 5, Supplementary Figure 5 S 3a-c and Figure 5 S 4a-c). The standardised observations with negative values indicated that parasite species, traits and the phylogenetic proxy were overdispersed within hosts from the same locality, except for "PPD - autumn" (Table 5. 5). At β2 level, differences in parasite diversity between localities always differed significantly from randomness (Table 5. 5) and were always greater than expected (Supplementary Figure 5 S 3d-f and Figure 5 S 4d-f). The positive values of the standardised β2 reflected that parasite species, traits and phylogeny were clustered at this level of the organisational scale.

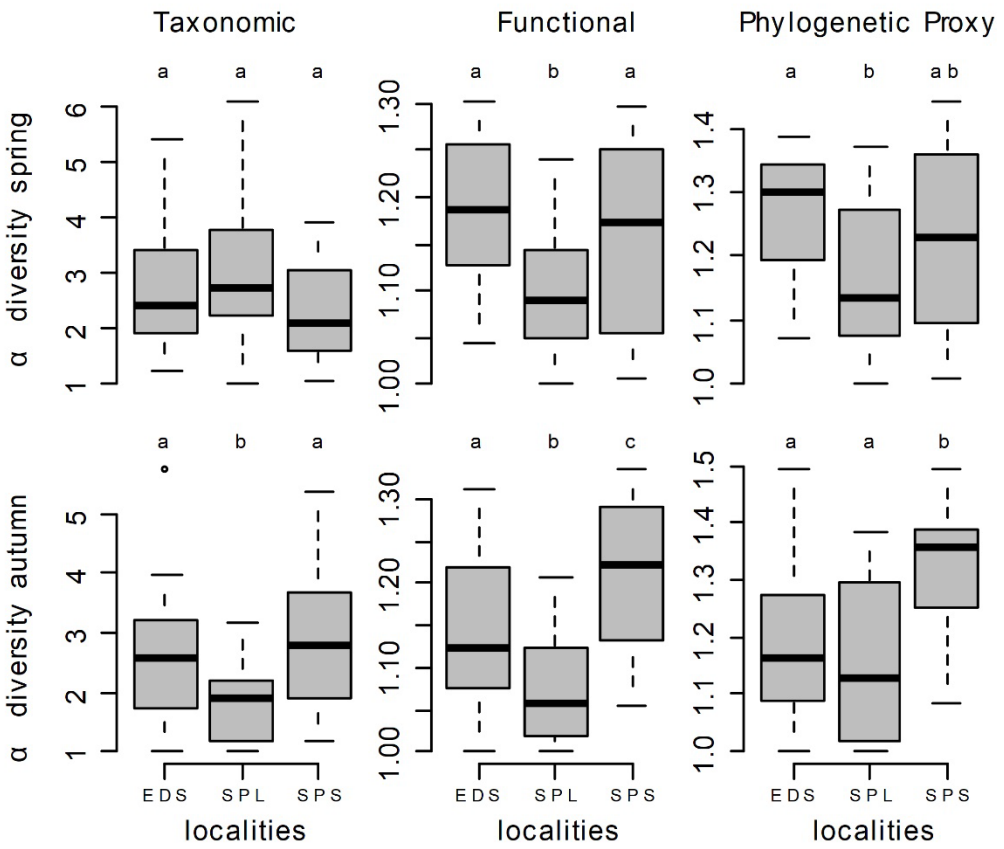

Figure 5. 3. Parasite α diversity in terms of taxonomy (TD), functional traits (FD) and a proxy of the phylogeny (PPD) for each locality. Different lowercase letters indicate significant differences between localities. Abbreviations: EDS, Ebro Delta Sea; SPS, Santa Pola Sea; SPL, Santa Pola Lagoon.

Table 5. 5. Partition of diversity at two organisational levels of the locality factor. Statistical results of the partitioning of Taxonomic (TD), Functional (FD) and Proxy of Phylogenetic (PPD) Diversity at two organisational levels (β1 [among host individuals within locality] and β2 [between localities]) in comparison to a distribution of 999 random replicates. Standardised observed values are given in parenthesis. A standardised β is negative when the community structure is overdispersed, and positive when the community structure is clustered.

	β1			β2		
	TD (std. ob.)	FD	PPD	TD	FD	PPD
spring 2005	0.01* (-2.9)	0.14NS (-1.5)	0.51NS (-0.6)	0.001* (4.4)	0.001* (10.2)	0.001* (6.9)
autumn 2005	0.01* (-3.9)	0.17NS (-1.5)	0.84NS (0.2)	0.004* (3.1)	0.001* (6.6)	0.001* (5.7)

*, $p < 0.01$; NS, non-significant results

When we considered the crossed factors simultaneously, diversity within each host individual constituted the highest proportion of the parasite diversity in the whole community

(57% for TD, 54% for FD and 52% for PPD), followed by the crossed effect of locality and season, the locality and the season, for the three facets of diversity (Table 5. 4b). The graphical representation of the second version of the crossed-DPCoA (Figure 5. 4), displayed similar patterns for TD, FD and PPD. The first axis slightly separated the diversity of parasite communities of the two marine localities (SPS and EDS) from the lagoon locality (SPL). Furthermore, both marine localities (EDS and SPS) overlapped substantially in the species (TD), trait (FD) and phylogenetic-like (PPD) spaces (Figure 5. 4).

5.5 Discussion

We examined the mechanisms driving the helminth parasite assemblages of grey mullets. Four main findings can be extracted from our results. First, the diversity of these parasite communities shows at least two opposed patterns (i.e. overdispersion and clustering). These patterns are found at different levels (β1 and β2, respectively) of the two organisational scales (i.e. host species or locality). Second, the diversity of the two organisational scales is influenced by several variables. Third, the three facets of diversity (i.e. TD, FD and PPD) did not always show congruent results among them, which is unsurprising and follows the general trend reported for diverse groups of organisms (e.g. Devictor et al. 2010, Hevia et al. 2016). Thus, conclusions might be biased if any of the facets of diversity is overlooked (Jarzyna & Jetz 2016). Four, the phylogenetic-like signal on functional traits suggested that functional traits are, at least, constrained by the phylogeny. Nonetheless, even when traits have strong phylogenetic signals, there may be differences between patterns of phylogenetic diversity and patterns of functional diversity (Pavoine et al. 2013b). Therefore, PD (or in our case PPD) is often a poor surrogate of FD.

At the lowest organisational level (α diversity), the most notable case of incongruences between the three facets of diversity was the spring survey at SPL (Figure 5. 3), which was the richest in terms of number of species (highest TD) mainly because of trematodes. However, SPL displayed the lowest mean for α FD and α PPD probably because trematode species are redundant (*sensu* Carmona et al. 2016) in terms of FD and PPD. At the α level as well, we found that host individual accounts for most of the diversity for the three facets of diversity (~50% of the total diversity, crossed-DPCoA results), regardless of the case study considered. This suggests that the parasite community found at each host individual (α diversity) is to a large extent a random subset of a larger pool of parasite species within a host species or locality (Poulin & Morand 2004, Chave 2013).

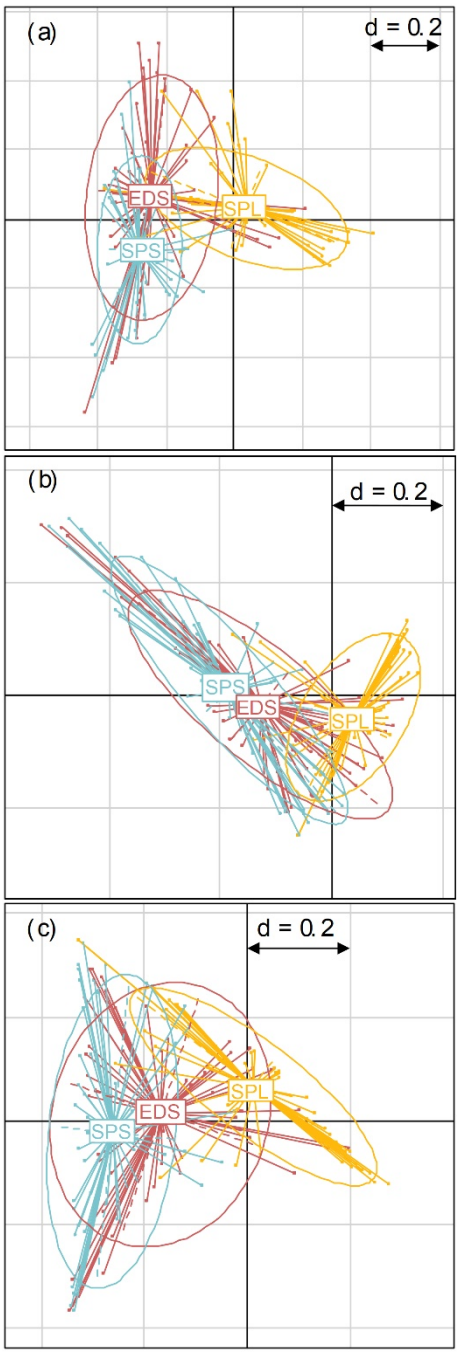

Figure 5. 4. Position of each host individual (dots) in (a) the parasite species space, (b) in the parasite trait space and (c) in the parasite phylogenetic-like space. Fish were grouped by localities. Abbreviations: EDS, SPL and SPS represent the centroid of each locality, and stand for Ebro Delta Sea, Santa Pola Lagoon and Santa Pola Sea, respectively. These analyses were carried out with the second version of the crossed-DPCoA. The width and height of the ellipses are given by the variance of the coordinates of the individuals, and the covariance between the coordinates on the two axes gives the slope of the ellipse. "d" (top-right) indicates the length of side of the grey squares of the background grid.

In the case of β1 diversity (within a host species or locality), differences between host individuals of the same species were less important than differences between host individuals of the same locality to determine the variation of the parasite communities (Table 5. 4). In other words, host species is more determinant of the parasite community than locality. In fact, the parasite community of grey mullets, especially monogenans of genus *Ligophorus* (Dactylogyridae), tend to be host-species specific while they are not so geographically constrained within the Mediterranean Sea (e.g. Sarabeev et al. 2013).

At a given place and for a given host species, TD at β1 level indicates low diversity among host individuals. This may indicate that, although the parasite community of a host individual is composed by a random drawing of the total diversity of the community, some species are more abundant than others, leading to many species shared and a few species unshared with other host individuals. Nonetheless, parasite FD and PPD were randomly distributed among host individuals (Table 5. 3, Table 5. 4). A similar result was found by Krasnov et al. (2005). These authors proposed that when the number of parasite species infecting a host species becomes saturated, a random assemblage in PPD suggests that all the parasite species are ecologically interchangeable and contribute equally to the saturation. In our case, random assemblages of FD and PPD would indicate that parasite species are functional and phylogenetic

interchangeable entities. Furthermore, the span of the boxes (Figure 5. 1, Figure 5. 3) and the size of the ellipses (Figure 5. 2, Figure 5. 4) reflect the heterogeneity in infection among host individuals from the same species or locality, which could result from the almost universal observation of parasite aggregation among host individuals (Poulin 2007a). It is difficult to interpret the causes of parasite aggregation in host individuals in our system because we do not have enough data about inequality in environmental pressures among host individuals (Thieltges & Reise 2007), host genetic background (Poulin 2007a), host traits (Timi & Poulin 2003) or parasite characteristics (such as dispersal ability) (Poulin 2007b).

At the β2 level (between host species or localities), the positive standardised observations indicated that species, traits and the proxy of the phylogeny were clustered, i.e. the species, trait, and phylogenetic compositions of parasite communities depend on the species host and the locality. Both environmental filtering and competition can be responsible for these patterns (Cadotte & Tucker 2017). Moreover, intrahost speciation has also been pointed out as a cause of clustering in parasites (Krasnov et al. 2014). Parasite species competition is difficult to demonstrate (Mideo 2009). However, the filtering of parasite species has received much more attention, particularly after the framework developed by Combes (2001). Due to the strong association between parasite and host species (Figure 5. 2), we consider that environmental (host) filtering is an important driver in these communities. As for intrahost speciation, it has been suggested for some *Ligophorus* spp. in our system (Blasco-Costa et al. 2012). Consequently, intrahost speciation could be an additional mechanism accounting for the clustering of such species. As for geographical differences in parasite assemblages, the overlap in the three spaces of diversity of the two marine localities was considerable (Figure 5. 4). This suggests that habitat condition (marine vs lagoon) is perhaps a stronger driver than geographical distance of the parasite communities. The environmental characteristics of the lagoon together with the high site fidelity of *M. cephalus* to lagoons (Chang et al. 2004) might eventually determine the slightly differentiated parasite community from SPL (Figure 5. 4). These results are indicative of environmental filtering in the parasite diversity. Our results are similar to those found by Levy et al. (2019) for the parasite community of the Argentine silverside *Odontesthes argentinensis*. In terms of TD, significant differences in the parasite communities of *O. argentinensis* from close marine localities (about 16-35 km apart) were accounted for by oceanographic properties of the area and the high site fidelity of the fish populations (Levy et al. 2019).

Finally, the season survey had a moderate effect on the parasite diversity of the host species and localities (crossed-DPCoA results). However, the capacity to differentiate parasite

communities from different localities might be seasonal-dependent. Since freshwater effluents widely vary in EDS seasonally, and, thus, the parasite diversity in EDS and SPL localities could be more similar in some seasons than in others (Míguez-Lozano et al. 2012).

To sum up, our study supports the idea that the assembly rules driving parasite communities depend on the level of the analysis and the facet of diversity considered. Particularly, the host-individual level seems to hold a random subset of parasite species of a larger pool of species, traits and phylogeny of parasites. At intermediate levels, similar parasite species are limited to coexist, whereas at higher levels, the environment filters the parasite diversity, since a joint influence of host phylogenetic origin (*Chelon* vs *Mugil*) and environmental preferences (marine-related vs coastal-related) might drive the parasite TD, FD and PPD. Although this was less clear for the last two facets of diversity. Finally, the habitat conditions of the locality seem to be more determinant of the TD, FD and PPD than geographic distance. The fact that diversity in these parasitic communities is subjected to random and deterministic processes simultaneously, but at different organisational levels, bring together Clements and Gleason ideas. These can be seen as two polar cases along a single deterministic-stochastic continuum of community organisation outcomes (Götzenberg et al. 2012, Liautaud et al. 2019). Clearly, parasitologists should pay attention to patterns of diversity at different facets and organisational levels.

5.6 References

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5.7 Supplementary Information

Table 5 S 1. Pairwise comparisons of α diversity. Parasite Taxonomic Diversity (TD), Functional Diversity (FD) and Phylogenetic Proxy of Diversity (PPD) at the fish individual (i.e. diversity at the sampling site or α diversity). Fish are of three species (*Chelon auratus*, *Mugil cephalus* and *Chelon ramada*), from the same locality (Santa Pola Lagoon) and two seasons (autumn 2004 and 2005) (Case 1). Only results with at least one significant comparison ($p < 0.05$) are given.

(a) Autumn 2004, PPD	*Chelon auratus*	*Mugil cephalus*	*Chelon ramada*
Chelon auratus	1		
Mugil cephalus	0.38	1	
Chelon ramada	0.18	0.00	1

(b) Autumn 2005, TD	*Chelon auratus*	*Mugil cephalus*	*Chelon ramada*
Chelon auratus	1		
Mugil cephalus	0.55	1	
Chelon ramada	0.00	0.00	1

(c) Autumn 2005, FD	*Chelon auratus*	*Mugil cephalus*	*Chelon ramada*
Chelon auratus	1		
Mugil cephalus	0.70	1	
Chelon ramada	0.00	0.00	1

(d) Autumn 2005, PPD	*Chelon auratus*	*Mugil cephalus*	*Chelon ramada*
Chelon auratus	1		
Mugil cephalus	0.00	1	
Chelon ramada	0.00	0.00	1

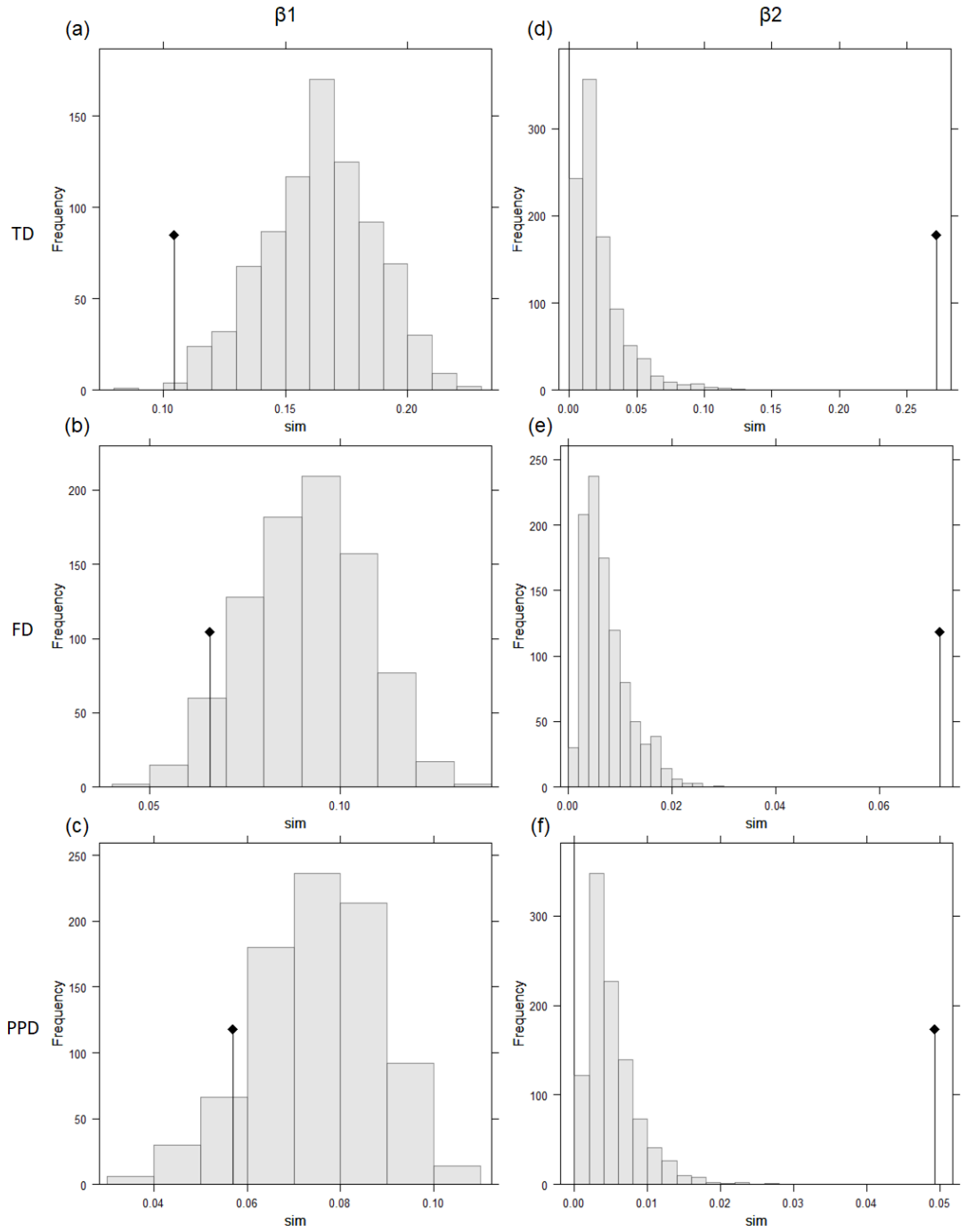


Figure 5 S 1. Observed and simulated (sim) β diversity values (Case 1: autumn 2004). (a, b, c) β1 diversity or extent of dissimilarity in the diversity of parasite communities among host individuals within each host species (*Chelon auratus*, *Mugil cephalus* and *Chelon ramada*). (d, e, f) β2 diversity or extent of dissimilarity in the diversity of parasite communities between host species. Diversity was measured in terms of (a, d) Taxonomic Diversity (TD), (b, e) Functional Diversity (FD) and (c, f) the Proxy of Phylogenetic Diversity (PPD). Samples are from Santa Pola Lagoon and autumn 2004 (Case 1). Observed β values (black diamond on the top of the black vertical line) and distribution of the simulated (x-axis: sim) β values (grey bars).

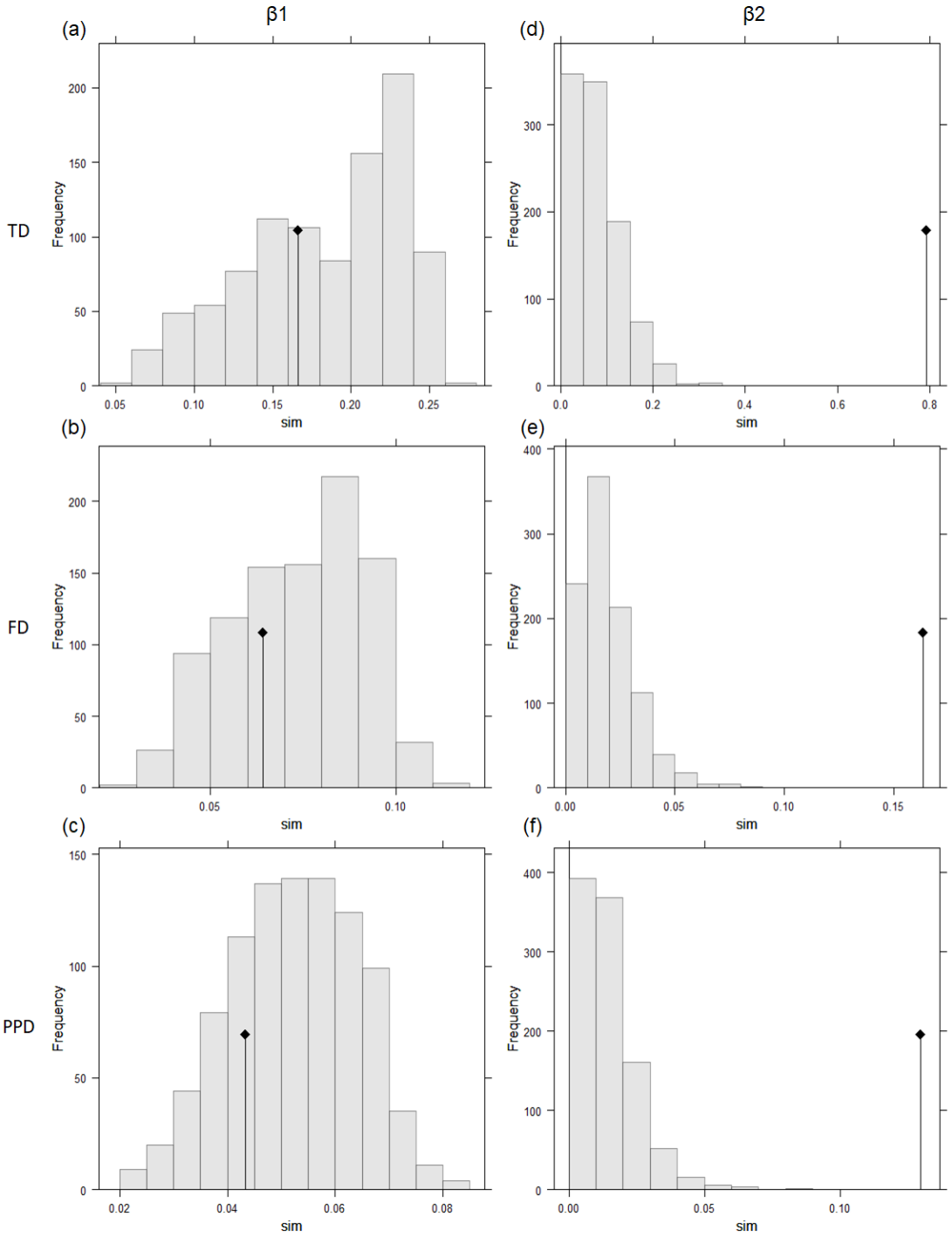


Figure 5 S 2. Observed and simulated (sim) β diversity values (Case 1: autumn 2005). (a, b, c) β1 diversity or extent of dissimilarity in the diversity of parasite communities among host individuals within each host species (*Chelon auratus*, *Mugil cephalus* and *Chelon ramada*). (d, e, f) β2 diversity or extent of dissimilarity in the diversity of parasite communities between host species. Diversity was measured in terms of (a, d) Taxonomic Diversity (TD), (b, e) Functional Diversity (FD) and (c, f) the Proxy of Phylogenetic Diversity (PPD). Samples are from Santa Pola Lagoon and autumn 2005 (Case 1). Observed β values (black diamond on the top of the black vertical line) and distribution of the simulated (x-axis: sim) β values (grey bars).

Table 5 S 2. Pairwise comparisons of α diversity. Parasite Taxonomic Diversity (TD), Functional Diversity (FD) and Phylogenetic Proxy of Diversity (PPD) at the fish individual (i.e. diversity at the sampling site or α diversity). Fish are of three localities (Ebro Delta Sea, Santa Pola Lagoon and Santa Pola Sea), from the same species (*Mugil cephalus*) and two seasons (spring 2005 and autumn 2005) (Case 2). Significance level $p < 0.05$.

(a) Spring 2005, FD	Ebro Delta Sea	Santa Pola Lagoon	Santa Pola Sea
Ebro Delta Sea	1		
Santa Pola Lagoon	0.00	1	
Santa Pola Sea	0.16	0.04	1

(b) Spring 2005, PPD	Ebro Delta Sea	Santa Pola Lagoon	Santa Pola Sea
Ebro Delta Sea	1		
Santa Pola Lagoon	0.00	1	
Santa Pola Sea	0.25	0.19	1

(c) Autumn 2005, TD	Ebro Delta Sea	Santa Pola Lagoon	Santa Pola Sea
Ebro Delta Sea	1		
Santa Pola Lagoon	0.00	1	
Santa Pola Sea	0.33	0.00	1

(d) Autumn 2005, FD	Ebro Delta Sea	Santa Pola Lagoon	Santa Pola Sea
Ebro Delta Sea	1		
Santa Pola Lagoon	0.00	1	
Santa Pola Sea	0.02	0.00	1

(e) Autumn 2005, PPD	Ebro Delta Sea	Santa Pola Lagoon	Santa Pola Sea
Ebro Delta Sea	1		
Santa Pola Lagoon	0.23	1	
Santa Pola Sea	0.00	0.00	1

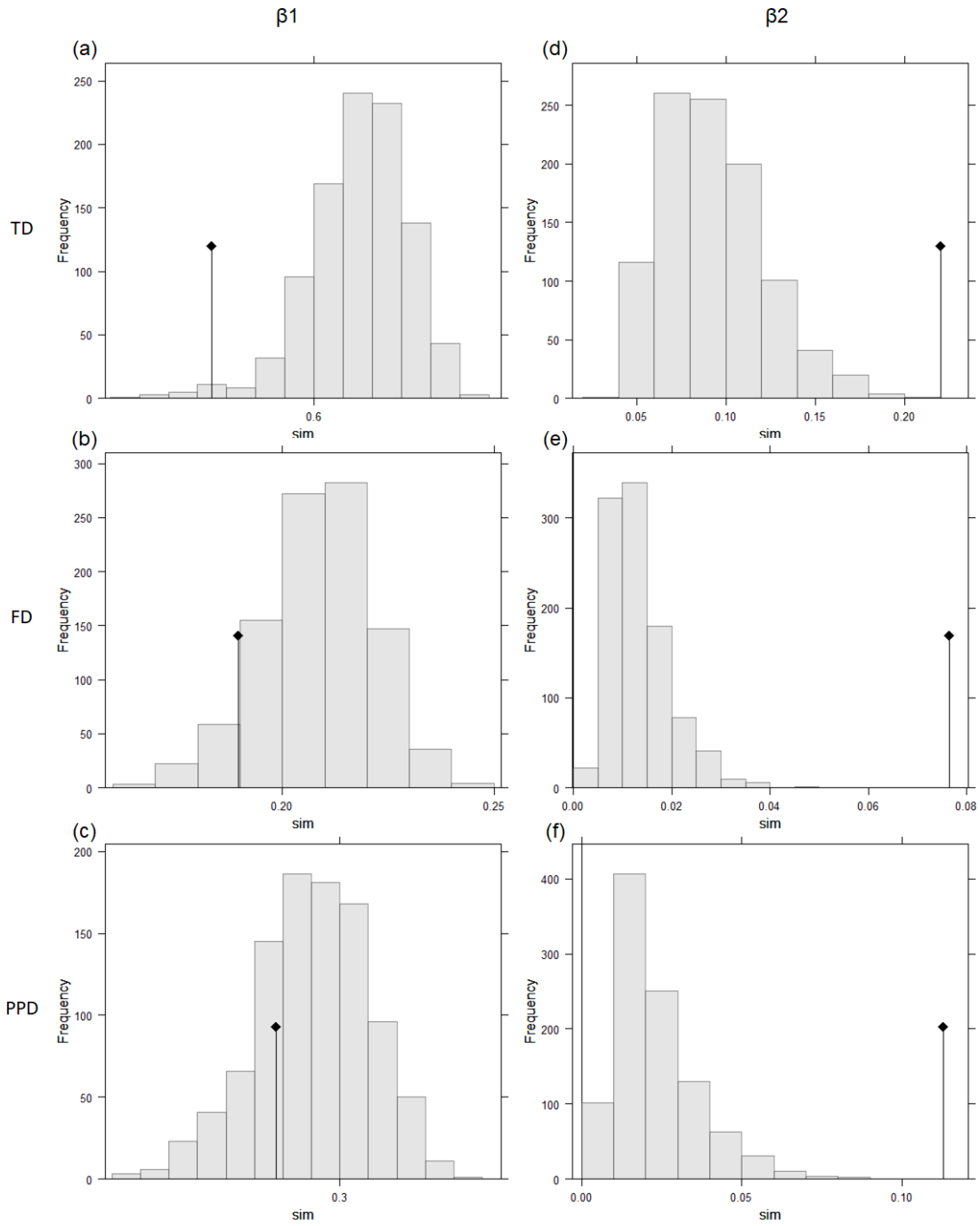


Figure 5 S 3. Observed and simulated (sim) β diversity values (Case 2: spring 2004). (a, b, c) β1 diversity or extent of dissimilarity in the diversity of parasite communities among host individuals within each locality (Ebro Delta Sea, Santa Pola Lagoon and Santa Pola Sea). (d, e, f) β2 diversity or extent of dissimilarity in the diversity of parasite communities between localities. Diversity was measured in terms of (a, d) Taxonomic Diversity (TD), (b, e) Functional Diversity (FD) and (c, f) the Proxy of Phylogenetic Diversity (PPD). Samples are of the host species *Mugil cephalus* and from spring 2005 (Case 2). Observed β values (black diamond on the top of the black vertical line) and distribution of the simulated (x-axis: sim) β values (grey bars).

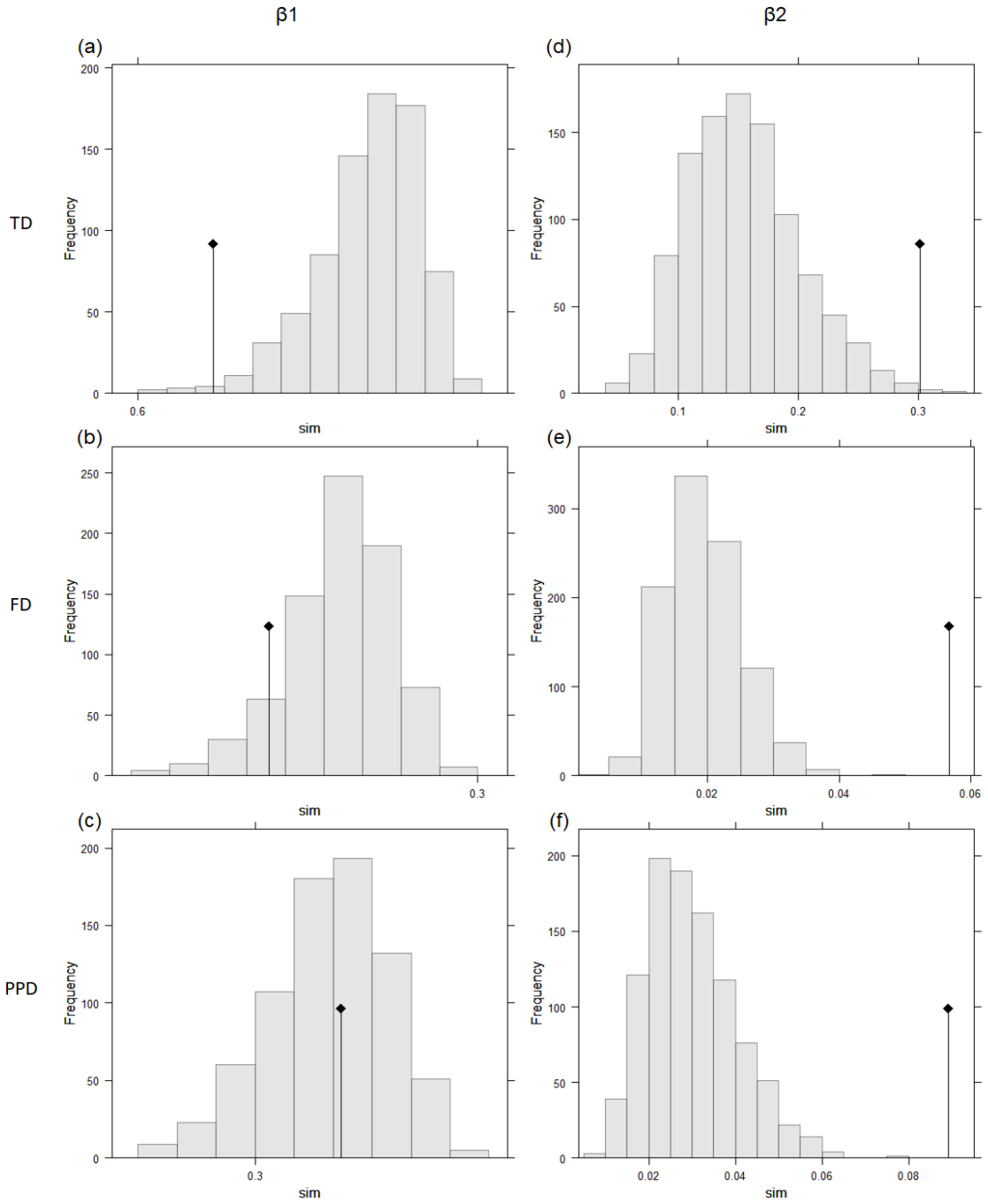
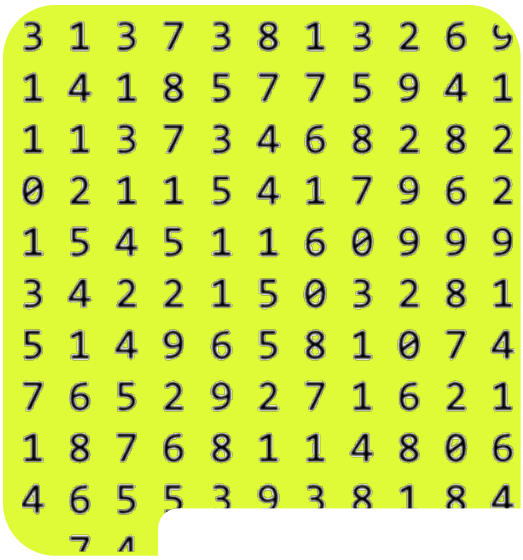


Figure 5 S 4. Observed and simulated (sim) β diversity values (Case 2: autumn 2005). (a, b, c) β1 diversity or extent of dissimilarity in the diversity of parasite communities among host individuals within each locality (Ebro Delta Sea, Santa Pola Lagoon and Santa Pola Sea). (d, e, f) β2 diversity or extent of dissimilarity in the diversity of parasite communities between localities. Diversity was measured in terms of (a, d) Taxonomic Diversity (TD), (b, e) Functional Diversity (FD) and (c, f) the Proxy of Phylogenetic Diversity (PPD). Samples are of the host species *Mugil cephalus* and from autumn 2005 (Case 2). Observed β values (black diamond on the top of the black vertical line) and distribution of the simulated (x-axis: sim) β values (grey bars).



6. Native and invasive hosts play different roles in host-parasite networks

6. Native and invasive hosts play different roles in host-parasite networks

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6.1 Abstract

Parasites are often key players in biological invasions since they can mediate the impact of host invasions or can themselves become invasive species. However, the nature and extent of parasite-mediated invasions are often difficult to delineate. Here, we used individual-based, weighted bipartite networks to study the roles (degrees of interactions of individuals in a modular network according to their within- and among-module connections) played by native and invasive host individuals to their parasite communities. We studied two phylogenetically and ecologically close fish species, *Mugil cephalus* s.l. and *Planiliza haematocheilus* (Teleostei: Mugilidae). *Planiliza haematocheilus* is native to the Sea of Japan and invasive in the Sea of Azov whereas, *M. cephalus* s.l. is native to both seas. Based on the common evolutionary history that drives native host–parasite networks, we hypothesised that 1) native networks have higher modularity than invaded ones; and 2) invasive hosts in the invaded area play a peripheral role to structure parasite communities. We analysed the whole parasite community and subsets based on transmission strategy and host specificity of the parasite species to establish whether modularity and host roles are related to these features in the native and invaded areas. All networks were found to be modular. However, modularity tended to be higher in networks of the native area rather than those of the invaded area. Host individuals of both fish species played similar roles in the native area, whereas invasive hosts played a peripheral role in the networks of the invaded area. We propose that long-term monitoring of the roles of invasive hosts in parasite communities can be a useful proxy for estimating the maturity of the establishment of the invasive hosts in an ecosystem.

6.2 Introduction

Biological invasions are human-mediated introductions of species outside their original distribution, which manage to establish viable populations throughout space and time (Richardson et al. 2000). Invasive species represent a major threat to ecosystems, as they do not allow enough time to elapse for gradual evolutionary adjustments of the native species to their presence (Poulin 2017). When species are introduced into a new range, different scenarios can alter ecosystem functioning (Lymbery et al. 2014). Among those, biological invasions are of concern because of their potential to disrupt host–parasite dynamics (Chalkowski et al. 2018). As a result of the invasion (Figure 6. 1): invasive hosts can lose their parasites (enemy release); parasites can be introduced as free-living stages; invasive hosts can introduce parasites from their native range (or from an intermediate location, Figure 6. 1b) and transmit parasites to native species (spillover, co-invasion) or, contrary, do not transmit these parasites (co-introduction). Likewise, the invasive hosts can acquire parasites from native species (acquisition), favouring an increase in the abundance of the native parasite species and, thus, increasing the likelihood of native hosts becoming infected (spillback); or can act as an ecological sink, because they are not fully competent hosts when they become infected with a parasite from the native species (dilution effect) (Kelly et al. 2009, Lymbery et al. 2014, Goedknegt et al. 2016, Chalkowski et al. 2018) (Figure 6. 1).

Under these scenarios, native and invasive hosts and their native and/or acquired parasite communities can interact in different ways with subsequent outcomes for the ecosystem (Chalkowski et al. 2018). The study of host–parasite associations in an invaded community has been addressed by different types of analyses. For example, Sarabeev et al. (2017a) found support for the enemy release hypothesis, i.e. the invasive species would be benefited by a reduction of natural enemies, such as parasites, in the invaded area (Torchin & Lafferty 2009), by comparing the abundance and aggregation of the parasite community in native and invasive areas. Likewise, Poulin & Mouillot (2003) found that the amount of parasite species in invasive hosts over a short (ecological) period of time results in parasite assemblages becoming more taxonomically diverse than those developed over much longer (evolutionary) time periods in the native range of the host species. This fact highlights that ecological drivers are at least as important as evolutionary processes to model parasite communities (Poulin & Mouillot 2003).

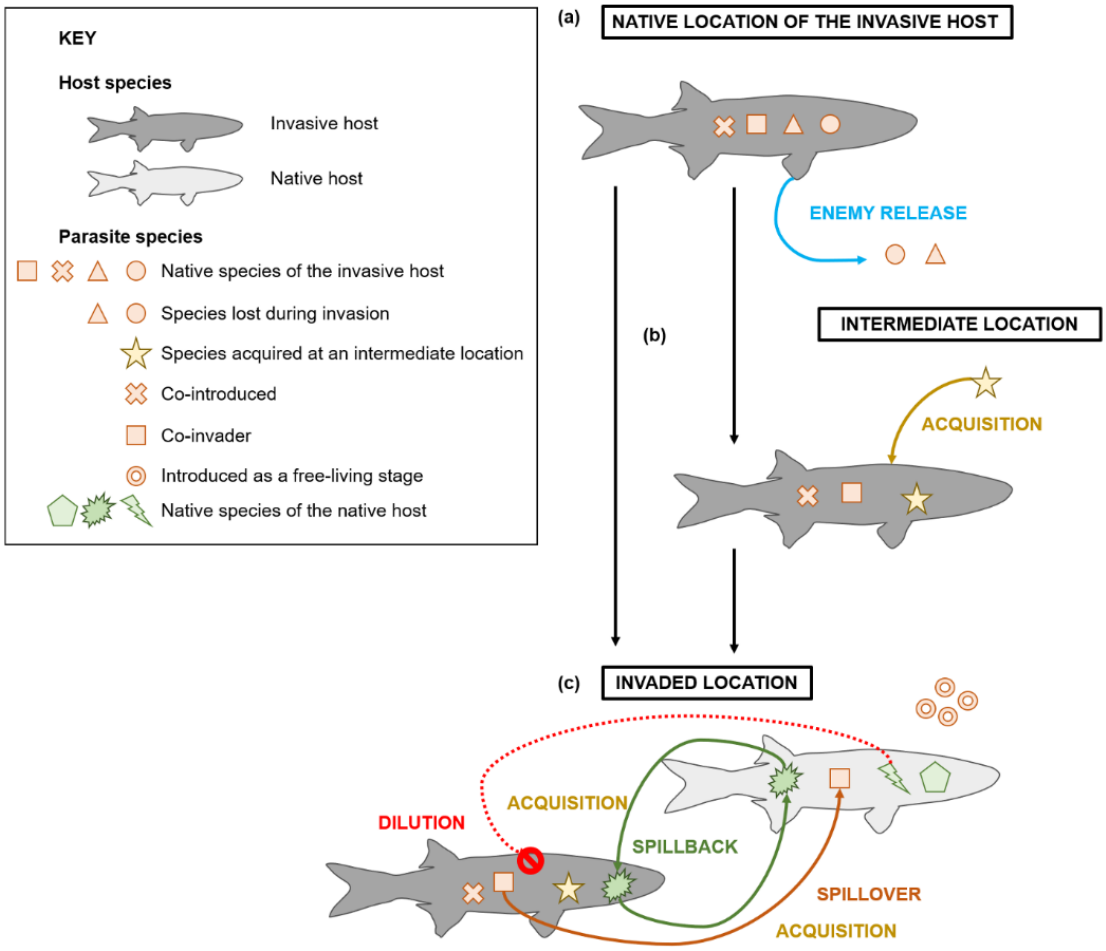

Figure 6. 1. Processes and key concepts to define parasite species during invasions. (a) Invasive host at its native area with its own parasite community. (b) Potentially, the invasive host can lose part of its parasite community (enemy release) and be colonised by new species (acquisition) during the invasion. (c) The invasive host arrives at the invaded location with some of its parasite species (co-introduced). It can acquire new parasite species from the native host. As a result, the probability of the native host to be infected increases (spillback). If the native host acquires co-introduced parasites species these become co-invaders (spillover). The invasive host can be a sink for native parasites if it gets infected, but it is not a competent host (dilution effect). Invasive parasites can arrive at the invaded location as free-living stages.

Interactions between hosts and parasites, or any other sets of individuals from two different taxa or ecological guilds (e.g. plant–pollinator), can be explored with bipartite network analysis. The analysis of such biological networks is particularly relevant for parasite ecology since it can illuminate the way in which host individuals and parasites are associated in a community (Poulin 2010). Bipartite networks are usually characterised by non-random associations between individuals or taxa (Fortuna et al. 2010), and one of the patterns that describes this non-randomness is modularity (Newman & Girvan 2004). In modular networks, subsets (i.e. modules) of individuals are expected to interact more frequently among

themselves than with individuals from other modules. Thus, modularity measures how well interacting pairs can be separated into different modules, and higher values of modularity indicate better segregation of modules (Newman & Girvan 2004). This may imply barriers to parasite dispersion between hosts from different modules that, for example, differ in behavioural or diet preferences (Pilosof et al. 2015) or are phylogenetically distant (Bellay et al. 2011, Krasnov et al. 2012, Poulin et al. 2013). In consequence, module drivers, such as taxonomic affiliation, have been proposed as predictors of the performance of invasive species in a network through its position in a module (Poulin et al. 2013). Beyond the ubiquity of modularity as a network pattern (Fortuna et al. 2010), we can classify the functional role of each individual in a modular network according to its number of both within-module (z-score) and among-module (c-score) links into four role categories (Guimerà & Amaral 2005, Olesen et al. 2007):

1. Module hubs, or individuals linked to many individuals within their own module (high z, low c).
2. Connectors, or individuals linking several modules (low z, high c).
3. Network hubs, or individuals acting as both connectors and module hubs (high z, high c).
4. Peripherals, or individuals that have a few links within its own module and to other modules (low z, low c).

Thus, we can graphically represent the position of each host individual by values of its cz-scores (see Figure 1D in Olesen et al. 2007) and expect that individuals with the same role play similar functions in determining the structure of the parasite community. In fact, these cz-scores have been used to explain the specificity of host–endoparasite networks (Bellay et al. 2011, 2013) or to determine the role of native and invasive plants and pollinators (Traveset et al. 2013).

Network analysis has arguably been under-exploited in studies of host–parasite invasions (Médoc et al. 2017), although it has been more widely applied to understand invasions of other biological systems. For example, Traveset et al. (2013) found that invaders made the Galápagos Archipelago resistant to species loss but vulnerable to disease spread. Regarding host–parasite invasions, one of the few examples is the study of Amundsen et al. (2013) in which the authors evaluated how the introduction of two fish species, followed by the co-introduction of five parasite species and four predatory bird species, altered the topology of a native food web.

To fill the gap in the understanding of host–parasite interactions during invasions, we study the host individual–parasite species associations of two, native and invasive, host species by bipartite network analysis. Furthermore, we characterise networks of the native and the invaded distributions of both host species. To our knowledge, this represents the first study that evaluates and compares real (i.e. not simulated computationally) native and invaded networks. Furthermore, the position of an individual in a host–parasite network gives us an idea of its relative importance in the flow of parasites (Godfrey 2013). So, we downscale analyses to host-individual level to control for intraspecific host variation. This is necessary because parasite distribution across hosts can be patchy depending on the individual capacity of each host to prevent infection, and it will ultimately determine the ability of a parasite species to invade a new area (Morand & Deter 2009). Furthermore, the success of an invasive parasite can be greatly facilitated by the high abundance of the suitable host or even the number of invasive hosts can also determine amplification or dilution effects of native parasites (Telfer & Bown 2012). Finally, we will also implement role analysis (modularity and cz analyses) to understand the structure of native and invaded host–parasite communities, and the ecological impact of invasive hosts and parasites in an existing (native) community.

The grey mullets (Teleostei: Mugilidae) and their helminth parasites represent excellent systems to study and compare the role variation of individuals of a host species depending on its distribution (native or invasive) (Sarabeev et al. 2017a). They also provide a benchmark to control for such variation. Here we study the roles played by individuals of *Planiliza haematocheilus* and *Mugil cephalus* s.l. for their parasite communities in the native area for both hosts (Sea of Japan) and in an area where *P. haematocheilus* is invasive and *M. cephalus* is native (Sea of Azov). Since 1972, *P. haematocheilus* was repeatedly introduced into the Black Sea and the Sea of Azov for commercial purposes. In the early 80s, it established a reproductive population in these seas (Sabodash & Semenenko 1998, Occhipinti-Ambrogi & Savini 2003). The arrival of *P. haematocheilus* at its new habitat entailed a deep structural change in its parasite community: it lost native parasite species with complex life cycles, acquired new ones from the invaded area, and co-introduced some of its ectoparasite species (simple life cycle) to the invaded area (Sarabeev et al. 2017a). Although *M. cephalus* is now considered to represent a complex of sibling species (Whitfield et al. 2012), we assume that *M. cephalus* entities from the Sea of Azov and Japan are phylogenetically and ecologically equivalent (i.e. equally close) in their relationships with *P. haematocheilus* (Sarabeev et al. 2017a) and in the function performed in ecosystems.

We specifically employ modularity and cz analyses to determine the role of individuals of both host species for the whole parasite community and for parasites exploiting certain transmission strategies. Based on previous studies (Sarabeev 2015, Sarabeev et al. 2017a, 2017b, 2018, 2019), we first expect that modularity will be higher in native than in invaded networks. Native generalist parasites will parasitise the invasive host, and the acquisition of such parasites should connect existing distinct modules and decrease modularity. In contrast, the modularity signal should be similar in native and invaded communities when the analysis of networks includes highly host-specific parasites (i.e. carried species, Figure 6. 1) as these species should enhance modular structure. Second, we hypothesise that hosts of both species will play similar roles in the native area whereas they will display different roles in the invaded area. In the latter, the invasive hosts will mainly perform a peripheral role (low c and z scores) since they do not share a common evolutionary history with local parasite species (most of them are acquired, Sarabeev 2015) and will have low relevance in maintaining within- and among-module cohesion. Third, the role of both host species could also vary regarding the parasite transmission strategy considered. We expect that *P. haematocheilus* individuals will play a peripheral role for passively/trophically transmitted parasites in the invaded sea since all of them have been acquired from this sea. In contrast, we expect hosts of both species to play similar roles for their actively transmitted parasites in the Sea of Azov for two reasons. First, some of the actively transmitted parasites are host-generalists (able to infect a new host species by its own means). So, they will parasitise native and invasive host species equally. Second, the remainder of the actively transmitted parasites are highly host-specific and exclusively parasitise one host species (those of *P. haematocheilus* were carried from the Sea of Japan). So, they share a well-established evolutionary history with their hosts.

6.3 Material and Methods

Data

Our study is based on a database of fish and helminth parasites previously collected and identified as described in Sarabeev (2015) by standardised sampling methods across sites, seasons and years. We considered 872 fish individuals from 11 localities in the Azov-Black (hereafter Azov) and Japan seas, during three seasons (spring, summer and autumn) and seven years (1998, 1999, 2004, 2005, 2009, 2011, 2013) (Supplementary material Appendix 1 Table 6 S1. 1). We aggregated data from different samplings because the analyses of short periods of time possibly misrepresent the real dynamic of the network structure at a macroecological scale (Poulin 2010). These two fish species differ in their migration periods and paths for wintering

and spawning (Sarabeev et al. 2017b). As a consequence, fish from both seas were not always collected at the same localities (Sarabeev 2015) (Supplementary material Appendix 1 Table 6 S1. 1). In total, our database includes 52 helminth parasite species of Acanthocephala, Nematoda and Platyhelminthes in adult and larval stages. Six of these species were co-introduced monogeneans (Figure 6. 1) from the Sea of Japan into the Sea of Azov, so they occur in both seas.

By means of bipartite network analyses, we asked about the roles of the host individuals for (see summary of databases in Table 6. 1):

1. The whole helminth parasite community.
2. Actively transmitted parasites (Monogenea and metacercaria of Trematoda), i.e. species with larval stages that actively swim to reach the fish.
3. Passively/trophically transmitted parasites (adults of Trematoda and Acanthocephala and larva and adults of Nematoda), i.e. transmitted via the food web.
4. Ectoparasites (Monogenea). This is the only group with species introduced into the Sea of Azov. With the exception of one species, these monogeneans are highly host-specific and are not able to infect both host species (Sarabeev 2015). Note also that this group is a subset of 2) above.

Besides, different life stages of parasites were analysed as different nodes because they belonged to different species. In other words, parasite individuals of a species were always found in the same developmental stage in the analysed hosts.

For each of these four subsets, we constructed infection networks as incidence matrices where rows represented fish individual hosts and columns represented parasite species for each location (native versus invasive). Each cell contained the abundance of a parasite species in each host individual (i.e. edges values were the number of individuals of a particular parasite species in a single infected host). Across our two locations, we therefore ended up with eight different networks to analyse.

Table 6. 1. Sample size of the eight studied networks. The number of modules found in each modularity analysis is given in brackets.

	Sea of Azov	Sea of Japan
Whole parasite community	612 (fish hosts) x 31 (parasite species) (modules: 9)	260 x 27 (5)
Actively transmitted community	565 x 16 (8)	251 x 16 (4)
Passively/ Trophically transmitted community	462 x 15 (8)	240 x 11 (5)
Ectoparasites (Monogenea)	525 x 10 (5)	241 x 13 (5)

Network analyses

Modularity

Modularity analyses were run for each of the eight networks under study (Table 6. 1). We used the Beckett (2016) algorithm because it considers quantitative information (i.e. weighted networks). This algorithm was implemented with function computeModules from package bipartite (Dormann 2011) in R (R Core Team, 2018). The algorithm assigns fish individuals and parasite species to modules to compute a modularity value (Q) that is higher when links (i.e. interactions) within modules are more prevalent and/or stronger than links between modules. To account for modularity dependence on network size, we transformed the observed Q value into a standardised score (z-score *sensu* Dormann & Strauss 2014; we did not call it z-score to avoid possible confusion between z-scores in Dormann & Strauss (2014) and Olesen et al. (2007)) (Eq. 6. 1):

$$standardised\ Q = \frac{Q_{observed} - \bar{Q}_{null}}{\sigma Q_{null}} \quad \text{(Eq. 6. 1)}$$

To test the significance of our Q values, we compared them with those of 1000 bipartite networks generated randomly with the function nullmodel from bipartite. We assumed the null hypothesis that the eventual organisation of host–parasite interactions into modules, or symmetry of the strength of the interactions, is driven by relative abundance of species in a sample, thus interactions are random between individuals. To validate our results of modularity, we carried out analyses in two different ways that work with quantitative link information. First, we tested modularity with the less constrained method described by Vázquez et al. (2007), that randomises the total number of host–parasite interactions observed in the original interaction matrix, constrains the connectance, but not the marginal totals. So, the number of observed infections is the same as in the original interaction matrix. The method relies on the reciprocal relative frequency of interactions (s) of one actor (e.g. a parasite species,

i) over the another (e.g. host individual, *j*). The difference between the two reciprocal coefficients of s, $d_{ij} = s_{ij} - s_{ji}$, measures the symmetry of the strength of an interaction. Then, if we focus on a parasite species, *i*, A_i is the sum of all d_{ij} divided by its number of links (k_i). Under the abundance–symmetry null hypothesis, we expect a positive correlation between species abundance (N_i) and A_i. Second, we tested modularity with the swap.web algorithm (Dormann et al. 2009). In addition to connectance as in the previous method, it also constrains marginal totals that are taken from the original interaction matrix. The procedure starts with a Patefield-generated matrix. Then, it randomly selects 2×2 submatrices without zeros and subtracts the minimum values of the diagonal from the diagonal (thereby it generates an empty cell) and adds this value to the values on the minor diagonal. The marginal totals are maintained while the number of links is reduced. This procedure is repeated until the number of links (i.e. connectance) is equal to that of the real network (Dormann et al. 2009).

Finally, we bootstrapped with replacement the eight networks to compare and assess the overlap of the standardised *Qs* across networks. The number of individuals of each species in the bootstrapped matrices was fixed.

c and z scores

The c and z scores were calculated with function cz values from bipartite. We performed these analyses for each of the eight networks (Table 6. 1). Following Guimerà & Amaral (2005) and Olesen et al. (2007), we classified host fish as "peripherals" ($z \leq 2.5$ and $c \leq 0.62$), "connectors" ($z \leq 2.5$ and $c > 0.62$), "module hubs" ($z > 2.5$ and $c \leq 0.62$) and "network hubs" ($z > 2.5$ and $c > 0.62$).

Statistical analyses

We used Fisher's exact tests (fisher.test function from R package stats) to assess whether individuals of both fish species were similarly distributed among the four role categories in each of the eight networks (Supplementary material Appendix 2 Table 6 S2. 1-Table 6 S2. 8). When significant differences were revealed, we tested whether the number of peripherals of *P. haematocheilus* or *M. cephalus* was higher or lower than expected by chance in comparison to the observed proportion of peripherals of *M. cephalus* and *P. haematocheilus*, respectively. Since we hypothesised that individuals of *P. haematocheilus* would tend to play peripheral roles (i.e. less connected with other members of the network) in the parasite community, we predict more peripheral individuals of *P. haematocheilus* in the invaded area than expected by chance. Similarly, since we expected that both species would play similar roles in the parasite community in the

native area, the observed proportion of peripheral individuals of both species should be similar to the proportion expected by chance. To test this, we simulated 10 000 replicates of the number of peripheral individuals observed in each of the original cz analyses, independently. The number of individuals of each species in each replicate was set as the number of peripherals of each species observed in the cz analysis and the proportion of peripheral individuals of each species in each replicate were calculated. Finally, we established whether the observed proportion of peripherals of each species in our sample fell within the 95% confidence interval of the simulated proportions.

Furthermore, we performed season-specific modularity and cz analyses to evaluate the existence of a seasonal effect on species roles. Due to the reduced size of season-specific networks, we only tested the seasonal effect on the whole network (database (1)). As for the global analyses, we tested whether individuals of both fish species were similarly distributed within each of the four role categories for each season and sea, independently, by means of Fisher's exact test. Then, we assessed if the number of peripherals of *P. haematocheilus* and *M. cephalus* differed from the expected numbers, independently, by simulating 10 000 replicates as described above.

6.4 Results

The eight networks (Table 6. 1) were all significantly modular ($p < 0.05$), regardless of the null model used. The number of modules found for each network is reported in Table 6. 1 and ranged from 9 to 4. The standardised Q scores of the whole and the trophically transmitted parasite communities were higher in networks from the Sea of Japan than those from the Sea of Azov (first hypothesis in the Introduction). Although, the networks that involved monogeneans (actively transmitted and ectoparasite community) had higher standardised Q scores (i.e. higher modularity) in the invaded area than in the native one, their confidence intervals (bootstrap results) overlapped. This suggests that their modularities are not significantly different between the two areas at least for these two networks (Figure 6. 2: actively transmitted and ectoparasites).

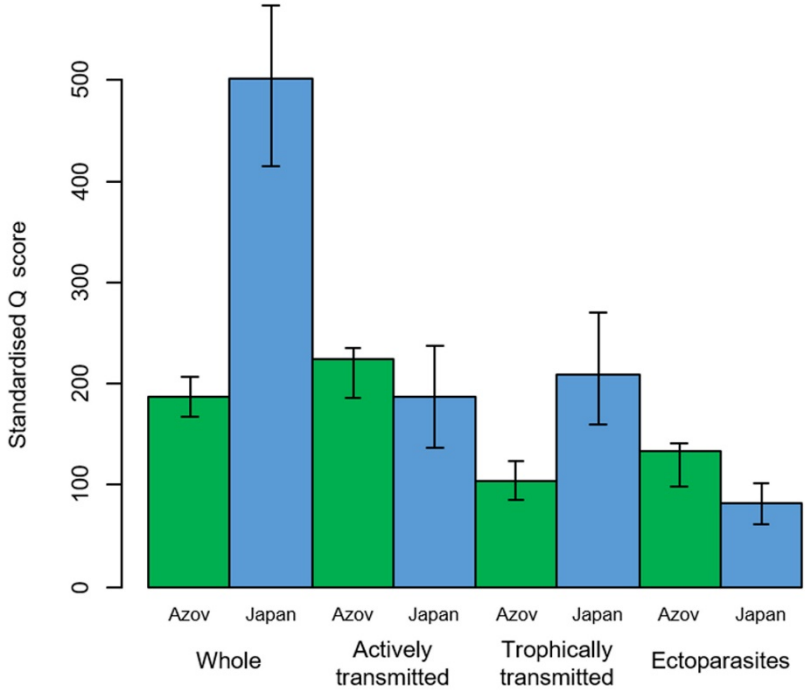

Figure 6. 2. Standardised modularity (*Q*) scores. The error bars indicate the standardised *Q* scores derived from 1000 bootstrapped matrices.

The cz analyses revealed that individuals of both fish species were distributed among the four role categories. However, individuals most frequently played peripheral roles, and only a few individuals were hubs (Figure 6. 3). Regarding our second and third hypotheses, we did not find significant differences between the role played by individuals of the two fish species in the Sea of Japan (native area). We also found no significant differences in analyses involving ectoparasites (actively transmitted and ectoparasite communities), regardless of the area studied (Figure 6. 4, Table 6. 2). When significant differences were found, the proportion of peripherals of *Planiliza haematocheilus* in the Sea of Azov (invaded area) was always larger than the proportion of peripherals of *Mugil cephalus* (Figure 6. 4).

The seasonal distribution of both fish species among the four roles was consistent with the results obtained from the combined analyses, except for spring at the Sea of Azov. In this case, we found no significant differences in the roles played by both host species (Supplementary material Appendix 3 Table 6 S3. 1-Table 6 S3. 5 and Figure 6 S3. 1-Figure 6 S3. 5).

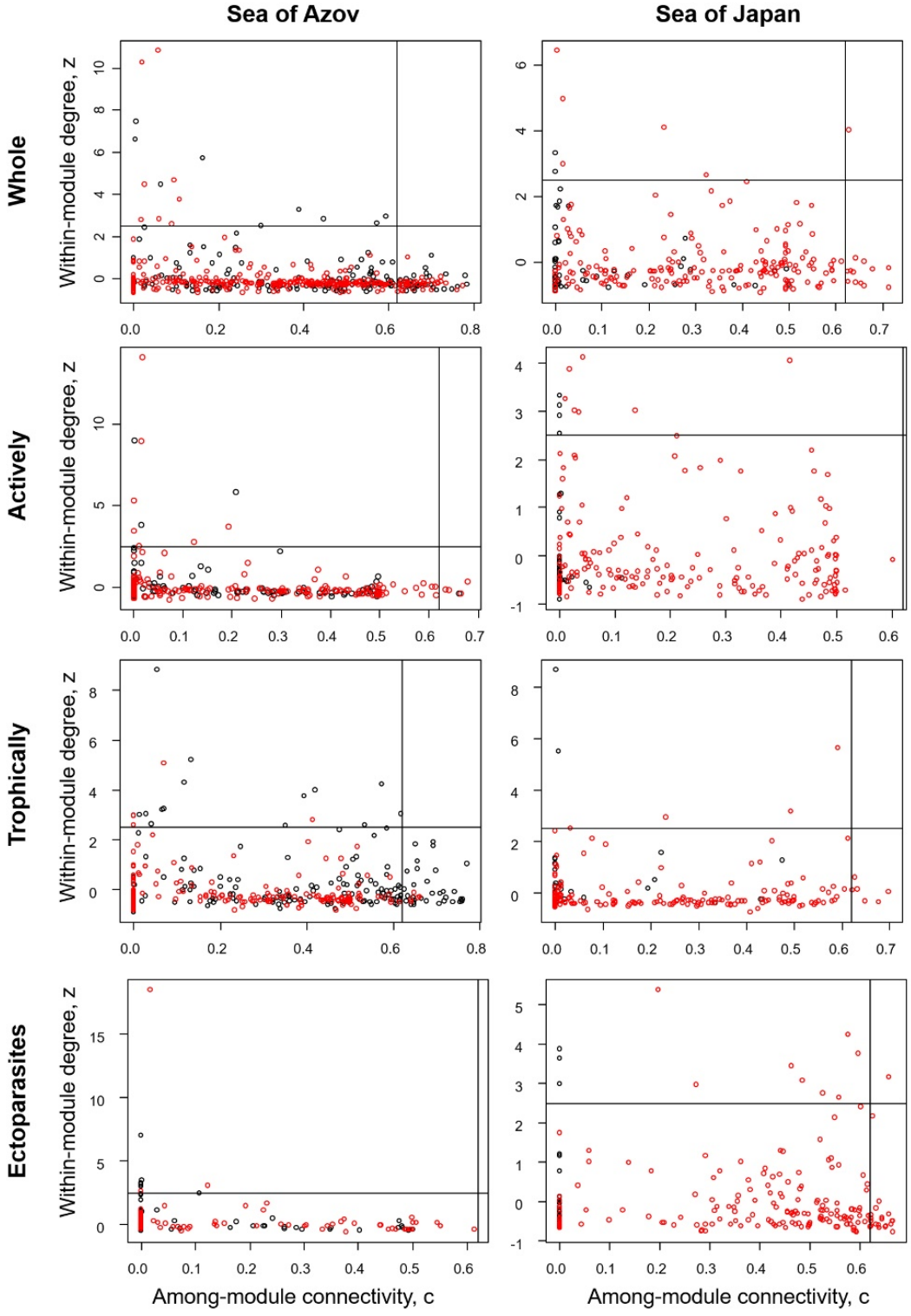

Figure 6. 3. Distribution of *Mugil cephalus* (black) and *Planiliza haematocheilus* (red) individuals from the Sea of Azov and the Sea of Japan among the four network roles considered by Olesen et al. (2007): Peripherals: $z \leq 2.5$ and $c \leq 0.62$; connectors: $z \leq 2.5$ and $c > 0.62$; Module hub: $z > 2.5$ and $c \leq 0.62$; Network hubs: $z > 2.5$ and $c > 0.62$.

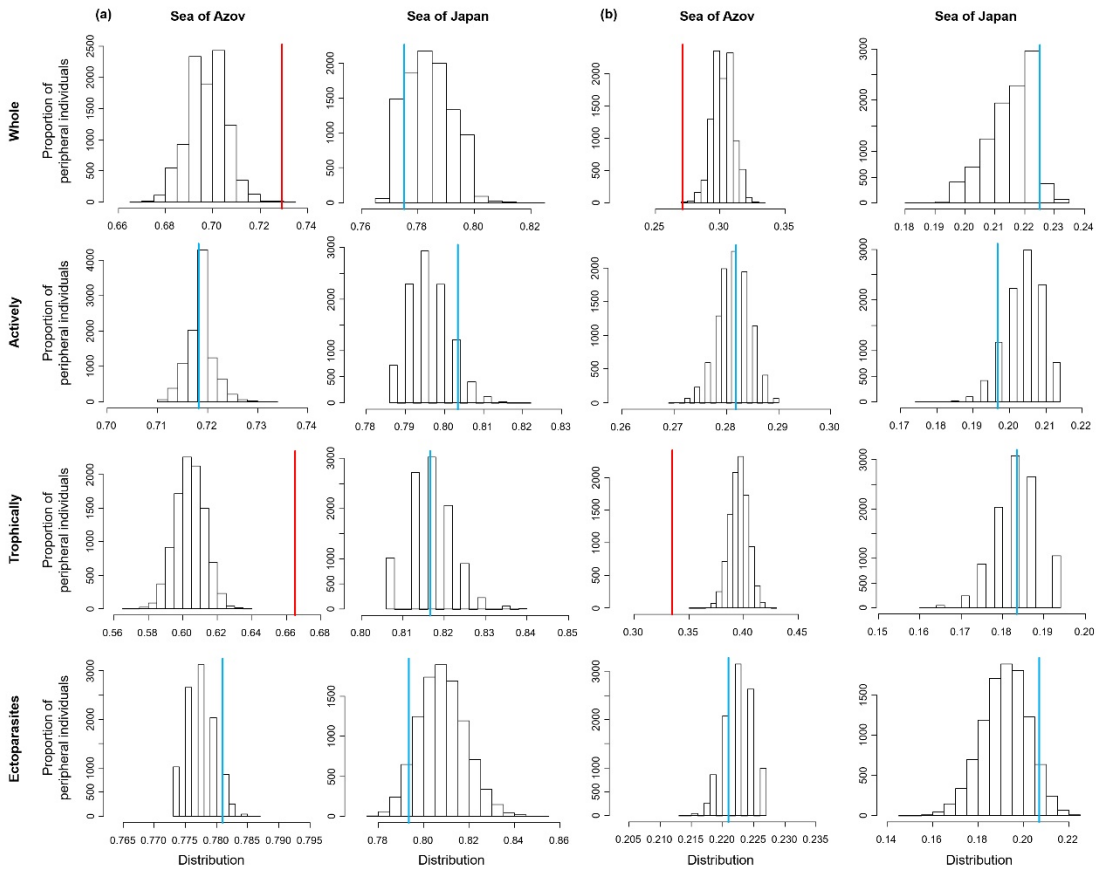

Figure 6. 4. Observed proportion of host individuals as peripherals (red and blue lines) and expected proportions in an ensemble of 10 000 random replicates (white bars) from the Sea of Azov and the Sea of Japan. (a) *Planiliza haematocheilus*; and (b) *Mugil cephalus*. Red lines indicate that the observed proportions significantly differ from the expected by the null models, whereas blue lines indicate non-significant results.

Table 6. 2. Results of the Fisher's Exact Test for differences in the proportion of individuals of the two host species among the four role categories (i.e. peripheral, connector, module hub, network hub).

Parasite community	Sea of Azov	Sea of Japan
Whole	*	NS
Actively transmitted	NS	NS
Passively/Trophically transmitted	*	NS
Ectoparasites (Monogenea)	NS	NS

NS: non-significant, * p value < 0.001

6.5 Discussion

We have compared field data (i.e. not simulated computationally) on invaded and native networks, which provides direct insight into the post-invasion changes in host–parasite associations. A study of this nature has been repeatedly called for to unveil well-grounded macroecological patterns and to avoid biases in the conclusions, such as overestimates of enemy release (Roy & Lawson Handley 2012). To our knowledge, such a study had not been implemented yet to date. Our case study provides a clear example on how to compare quantitatively the roles of invaders in the native and invaded areas.

A second innovative aspect of the present study is that we have downscaled network analyses from species to individuals. Most previous studies that work with bipartite networks have been carried out at species level. However, different authors have recently proposed to implement network analyses at the individual level in order to determine the properties that emerge at this scale (Dupont et al. 2014, Tur et al. 2015). This is important because, in a species-based network analysis, individuals of *Mugil cephalus* and *Planiliza haematocheilus* would strictly belong to one module or another (Tur et al. 2015). However, in a study like the one presented here, modules of the whole parasite communities included individuals of both host species, regardless of the area considered. This allows the identification of individuals of different species that overlap in some traits or niche preferences that make them to hold a similar role in the parasite community (Dallas et al. 2019). In other words, the heterogeneous partitioning in the use of resources by a single population would be missed at a higher-level analysis (Tur et al. 2015) and this distinction at individual level can be especially important during the host–parasite invasion process. For example, parasite distributions across hosts are usually highly aggregated, in which most host individuals harbour few or no parasites, whereas a few hosts harbour the majority of the parasite population (Poulin 2013). Even in an invasion, the individual ability of hosts to avoid parasites can be determined by such ability of the conspecifics that also arrived at the invaded locality (Ugelvig & Cremer 2012), which might lead to different roles played by host individuals in their parasite communities. Then, if we pool host individuals together, we focus on parasite mean abundance in a host species and will miss within-species variation which, eventually, carries information about host individual role in the transmission of the parasites (Telfer & Bown 2012). Furthermore, the subsetting of the networks into parasite infracommunities (i.e. at the host individual level) affords evaluating the change in the performance of the fish individual depending on the parasite characteristics. This can be especially relevant for researchers who want to predict changes in host–parasite dynamics.

Our modularity analyses revealed that networks are composed of subsets of host individuals that interact more frequently with certain parasite species than others. Our predictions of modularity (i.e. higher modular signal in native networks than in invaded networks) were partly supported by the results. As for the roles played by *M. cephalus* and *P. haematocheilus*, the results also supported our hypothesis. Individuals of both host species had similar roles in the native area (Table 6. 2). Finally, the results did not provide evidence for a strong seasonal effect on the roles of both host species regardless of the native or invaded area condition. Although all eight networks were modular, modularity was higher in the native area than in the invaded area for the whole parasite community and the passively/trophically transmitted parasites (Figure 6. 2). The higher modularity in these two native communities may indicate that associations between host and parasites are well grounded. In contrast, modularity may not be so well defined in the invaded area because accidental associations would blur the structure of the community. Furthermore, the network subsetting allowed us to unveil mechanisms that would be neglected otherwise. Analyses of the actively transmitted parasites and ectoparasites (both of them involved ectoparasites carried from the native area to the invaded one), displayed similar results in native and invaded communities. In addition to a previous study showing that *P. haematocheilus* co-introduced part of its ectoparasite fauna (Sarabeev 2015), our results suggest that it also maintained its community structure with the co-introduction. This is especially true for the monogeneans *Ligophorus* spp., as they were not able to colonise host species from the invaded area (Sarabeev 2015), which probably results from their high host specificity (Sarabeev et al. 2013).

This contention also gains strength from results of the role analyses (i.e. peripheral, connector, module hub and network hub host categories established by cz-scores) (Figure 6. 4). When actively transmitted (partially including ectoparasite) and ectoparasite communities were considered, individuals of *M. cephalus* and *P. haematocheilus* played similar roles in both the native and invaded areas. In contrast, individuals of *P. haematocheilus* mostly played a peripheral role in the invaded sea when considering the trophically transmitted parasite community. This community is mainly formed by parasites of *M. cephalus* and other sympatric grey mullets (Sarabeev 2015), which implies that *P. haematocheilus* partially shares similar prey items in the trophic network to the native grey mullet *M. cephalus*. Thus, our results suggest a lack of a shared evolutionary and ecological history between invasive hosts and native parasites, which conforms with the enemy release hypothesis (Torchin & Lafferty 2009). In addition to the native versus invasive status of the hosts, we acknowledge that the ecological properties of the regions could also generate differences between the parasite composition of the fish species

and, consequently, be a confounding factor. However, if the effect of the invasion is important, we will detect changes in the roles of the fish species as time passes. In this scenario, more individuals of the introduced species could play connector or hub roles and change the structure of the community over time (Traveset et al. 2013), which concurs with the colonisation time hypothesis, i.e. the longer an invader is established, the more native parasites it should have acquired (Gendron et al. 2012). Eventually, *P. haematocheilus* might adopt a more central role in this community (Médoc et al. 2017) and the benefit of parasite release would be finally suppressed (Gendron et al. 2012). Therefore, long-term monitoring of the distribution of invasive individuals for the acquired parasites between the four categories of the c and z scores (Olesen et al. 2007) should be encouraged because it can be used as a proxy of maturity of the establishment of the invasive species in a community.

We consider that this role approach can stimulate future research despite being limited to modular networks (Guimerà & Amaral 2005). For example, it represents a means to assess the capacity of invasive individuals to act as ecosystem disruptors by determining their roles in transmitting parasites in the new community. Furthermore, it is well known that host–parasite associations are driven by host traits and/or phylogenetic determinants (Kamiya et al. 2014). In consequence, future studies could take into account traits/taxonomic position of the most connected native hosts to predict the effect of invasive hosts to maintain or spread diseases across communities. Finally, on the parasite side, their impact on invasion processes depends on their life-history traits that can influence host invasion by aiding or limiting expansion (Roy & Lawson Handley 2012). Future research could hence be aimed at explaining or predicting the roles of parasites in terms of traits. For instance, do connector species/individuals have the same traits and peripherals never possess them? Also, we could test whether the enemy/parasite release hypothesis can still be verified in terms of trait diversity of enemies rather than species diversity of enemies. In sum, role analyses similar to those performed here would illuminate the mechanisms by which host–parasite interactions change during the biological invasion process.

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Data availability statement

Data available from the Harvard Dataverse Repository: <https://dataverse.harvard.edu/> (Llopis-Belenguer et al. 2019).

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Author contributions

CLB, IBC, JAB and DBS conceived the ideas and designed methodology. VS collected the data. CLB arranged the databases and analysed the data. CLB led the writing and IBC, JAB, VS and DBS contributed to the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

6.7 Supplementary Information

Supplementary Material Appendix 1

Table 6 S1. 1. Fish sample sizes by host species, seasons and localities. (a) Sea of Azov; (b) Sea of Japan.

(a)		*Mugil cephalus*	*Planiliza haematocheilus*
Spring	Kerch Strait	32	28
	Molochny Estuary	0	25
	Obitochny Bay	0	22
	Sivash Lake	15	0
Summer	Kerch Strait	60	71
	Molochny Estuary	0	18
	Obitochny Bay	0	26
	Sivash Lake	0	84
	Utluksky Estuary	0	29
Autumn	Balaklava Bay	30	0
	Kerch Strait	0	31
	Molochny Estuary	0	14
	Sivash Lake	48	40
	Taganrog Bay, Mariupol	0	27
	Utluksky Estuary	0	12
(b)			
Spring	Artemovka Delta	0	24
	Kiyevka Bay	25	0
	Razdol'naya Delta	0	23
Summer	Artemovka Delta	0	6
	Kiyevka Bay	31	0
	Razdol'naya Delta	0	34
Autumn	Posiet Bay	0	59
	Razdol'naya Delta	0	58

Supplementary Material Appendix 2

Table 6 S2. 1. Number of individuals of each species per role. Sea of Azov, whole parasite community, cz weighted analysis.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	377	42	8	0
Mugil cephalus	140	36	9	0

Table 6 S2. 2. Number of individuals of each species per role. Sea of Azov, actively transmitted parasite community, cz weighted analysis.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	395	4	7	0
Mugil cephalus	155	1	3	0

Table 6 S2. 3. Number of individuals of each species per role. Sea of Azov, trophically transmitted parasite community, cz weighted analysis.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	270	5	4	0
Mugil cephalus	136	32	15	0

Table 6 S2. 4. Number of individuals of each species per role. Sea of Azov, ectoparasite community, cz weighted analysis.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	403	0	5	0
Mugil cephalus	114	0	3	0

Table 6 S2. 5. Number of individuals of each species per role. Sea of Japan, whole parasite community, cz weighted analysis.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	186	12	5	1
Mugil cephalus	54	0	2	0

Table 6 S2. 6. Number of individuals of each species per role. Sea of Japan, actively transmitted parasite community, cz weighted analysis. Note: the original web contained 251 host individuals. However, only 250 hosts are included in this table because one host formed its own module. This host did not have z score (within-module links). Consequently, it could not be assigned to a role category.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	192	0	7	0
Mugil cephalus	47	0	4	0

Table 6 S2. 7. Number of individuals of each species per role. Sea of Japan, trophically transmitted parasite community, cz weighted analysis.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	187	6	3	0
Mugil cephalus	42	0	2	0

Table 6 S2. 8. Number of individuals of each species per role. Sea of Japan, ectoparasite community, cz weighted analysis. Note: the original web contained 241 host individuals. However, only 240 hosts are included in this table because one host formed its own module. This host did not have z score (within-module links). Consequently, it could not be assigned to a role category.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	165	20	8	1
Mugil cephalus	43	0	3	0

Supplementary Material Appendix 3

We performed season-specific modularity and cz analyses of whole weighted databases to know if it exists a seasonal effect on species roles. As for the global analyses, we tested whether individuals of both fish species were similarly distributed within each of the four role categories for each season and sea, independently, by means of Fisher's Exact Test. Then, we tested by 10,000 replications: (a) if the number of peripherals of *P. haematocheilus* (Ph) differed from the number expected by chance in comparison to the observed number of peripherals of *M. cephalus* (Mc); (b) if the number of peripherals of *M. cephalus* differed from the number expected by chance in comparison to the observed number of peripherals of *P. haematocheilus*.

Sea of Azov – Spring

- Matrix size: 122 (individual hosts: 75 Ph; 47Mc) x 30 (parasite spp)
- Fisher test: p value = 0.1277. Individuals of both fish species are similarly distributed within each of the four role categories.

Table 6 S3. 1. Number of individuals of each species per role.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	66	6	3	0
Mugil cephalus	46	0	1	0

- 10,000 replications:

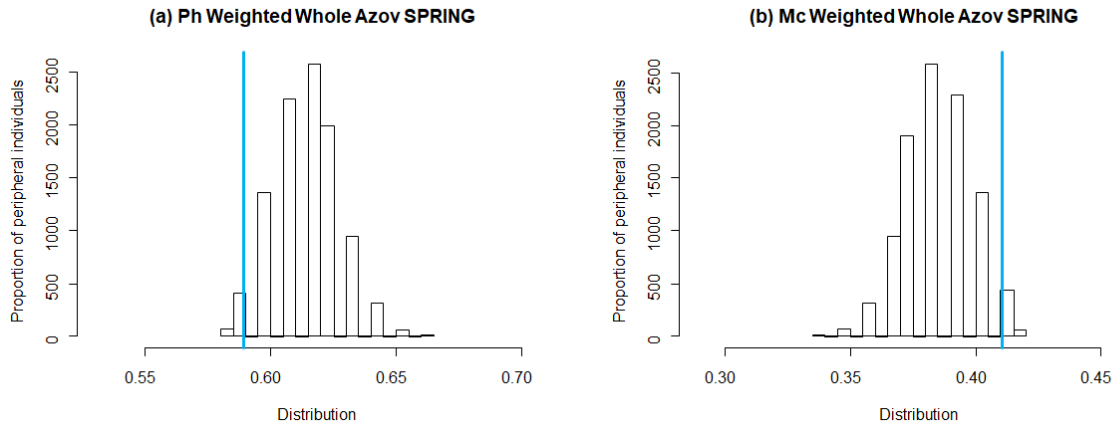

Figure 6 S3. 1. Observed proportion of peripheral individuals of (a) *Planiliza haematocheilus* (Ph) and (b) *Mugil cephalus* (Mc) as peripherals (blue lines, non-significant differences) and proportions found in 10,000 replications (white bars).

Sea of Azov - Summer

- Matrix size: 288 (individual hosts: 228 Ph; 60 Mc) x 29 (parasite spp)
- Fisher test: p value = 3.393e-09. Individuals of both fish species are NOT similarly distributed within the four role categories.

Table 6 S3. 2. Number of individuals of each species per role. Note: the original web contained 288 host individuals. However, only 286 hosts are included in this table because two hosts formed their own modules. These hosts did not have z scores (within-module links). Consequently, they could not be assigned to a role category.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	206	11	9	0
Mugil cephalus	37	21	1	1

- 10,000 replications:

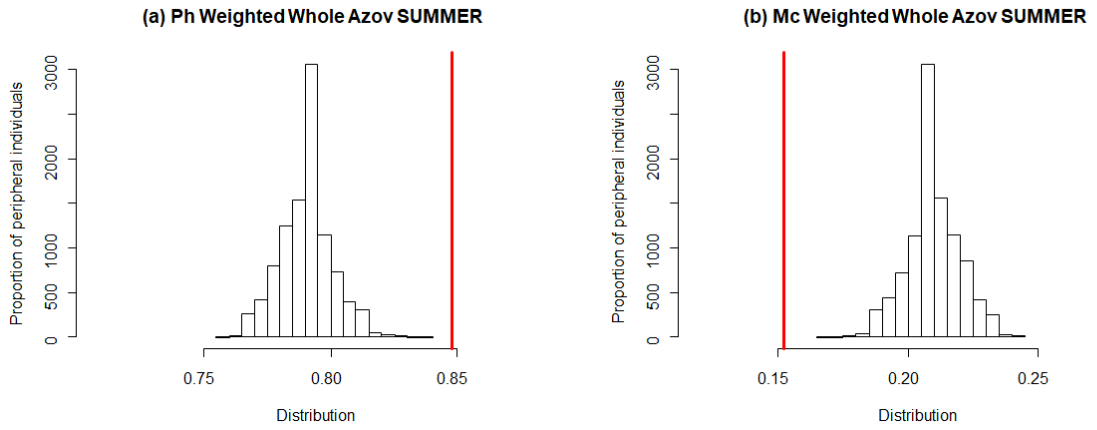

Figure 6 S3. 2. Observed proportion of peripheral individuals of (a) *Planiliza haematocheilus* (Ph) and (b) *Mugil cephalus* (Mc) as peripherals (red lines, significant differences) and proportions found in 10,000 replications (white bars).

There are more individuals of *P. haematocheilus* (invader) playing a peripheral role than expected by chance.

Sea of Azov – Autumn

- Matrix size: 202 (individual hosts: 124 Ph; 78 Mc) x 30 (parasite spp)
- Fisher test: p value = 0.008613. Individuals of both fish species are NOT similarly distributed within each of the four role categories.

Table 6 S3. 3. Number of individuals of each species per role.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	110	10	4	0
Mugil cephalus	56	16	6	0

- 10,000 replications:

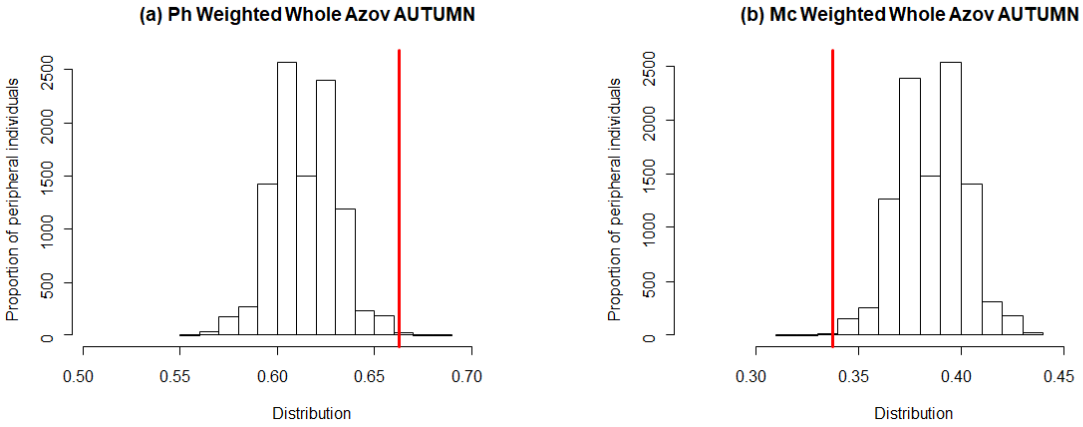

Figure 6 S3. 3. Observed proportion of peripheral individuals of (a) *Planiliza haematocheilus* (Ph) and (b) *Mugil cephalus* (Mc) as peripherals (red lines, significant differences) and proportions found in 10,000 replications (white bars).

There are more individuals of *P. haematocheilus* (invader) playing a peripheral role than expected by chance.

Sea of Japan – Spring

- Matrix size: 72 (individual hosts: 47 Ph; 25 Mc) x 24 (parasite spp).
- Fisher test: p value = 0.4781. Individuals of both fish species are similarly distributed within each of the four role categories.

Table 6 S3. 4. Number of individuals of each species per role.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	40	6	1	0
Mugil cephalus	23	1	1	0

- 10,000 replications:

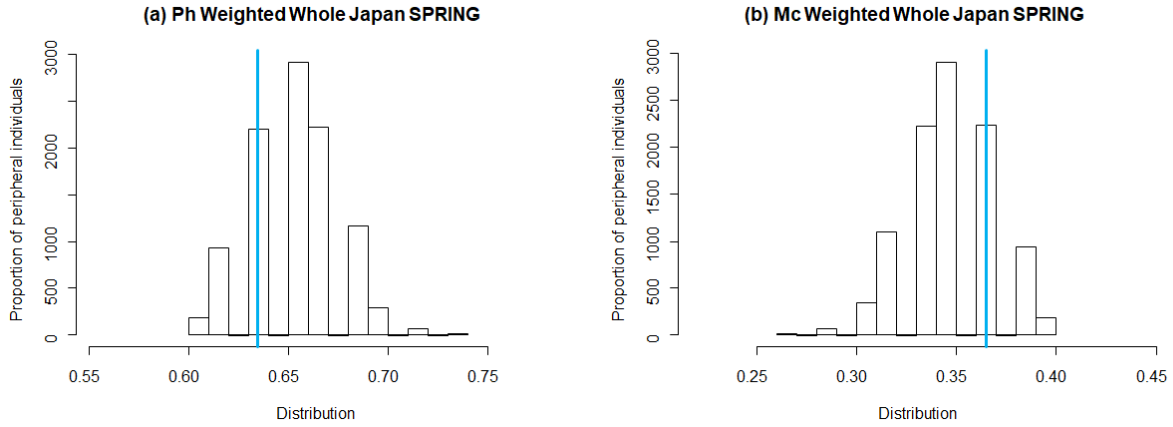

Figure 6 S3. 4. Observed proportion of peripheral individuals of (a) *Planiliza haematocheilus* (Ph) and (b) *Mugil cephalus* (Mc) as peripherals (blue lines, non-significant differences) and proportions found in 10,000 replications (white bars).

Sea of Japan – Summer

- Matrix size: 71 (individual hosts: 40 Ph; 31 Mc) x 25 (parasite spp)
- Fisher test: p value = 0.1277. Individuals of both fish species are similarly distributed in the four categories.

Table 6 S3. 5. Number of individuals of each species per role.

	Peripheral	Connector	Module hub	Network hub
Planiliza haematocheilus	33	5	2	0
Mugil cephalus	30	0	1	0

- 10,000 replications:

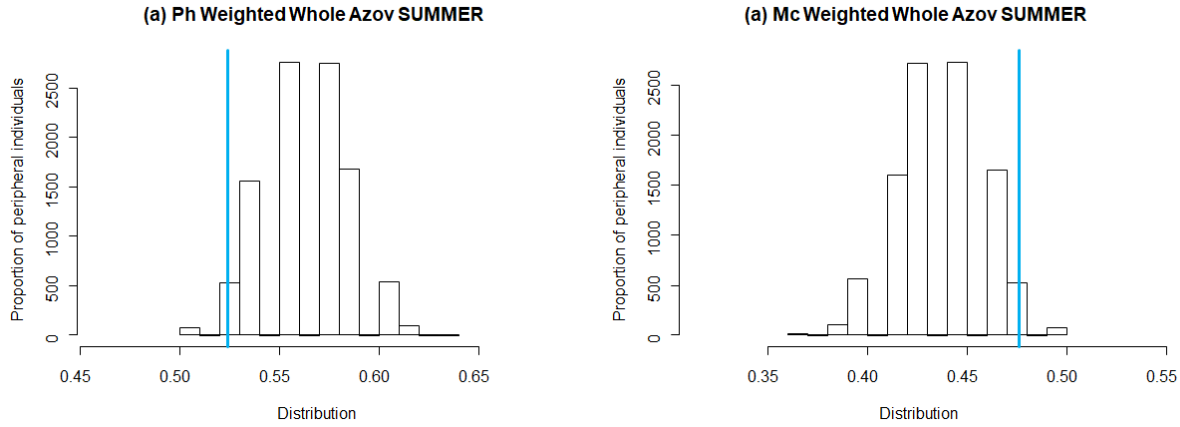

Figure 6 S3. 5. Observed proportion of peripheral individuals of (a) *Planiliza haematocheilus* (Ph) and (b) *Mugil cephalus* (Mc) as peripherals (blue lines, non-significant differences) and proportions found in 10,000 replications (white bars).

Sea of Japan – Autumn

We could not do these analyses for autumn because this sample only contains individuals of *Planiliza haematocheilus*.

Conclusions

Conclusions

This thesis is devoted to the study of parasite communities from an ecological perspective, with special emphasis on the helminth parasite communities from grey mullets (Teleostei: Mugilidae). Cutting-edge methodologies of community ecology have been applied and adapted for the study of parasite communities. These methodologies involve both an approach grounded on the Rao's index of diversity and the bipartite network analysis. The contributions of this investigation are timing, because they side with the current aim in the field of community ecology at revealing the processes driving the assemblage of diversity.

This dissertation provides several theoretical considerations and novel findings for the study and understanding of parasite communities, which I discussed in the previous chapters. Hence, only the main conclusions will be highlighted in this section.

In Chapter 3, my co-authors and I developed and validated the accuracy of traditional methods and methods based on Clay Modelling and Image Analysis to estimate the mass of small individual parasites. The methods based on Clay Modelling and Image Analysis provided the best approximation to the direct measurement of individual mass. Whereas, the traditionally employed Geometric Approximation showed the lowest accuracy and results significantly differed from the direct measurement. So, we strongly recommend abandoning its use. The disparate morphology and diverse phylogenetic origin of the model species demonstrated that these methods can be useful to quantify the mass of the huge diversity of invertebrates. Particularly, for the scope of the present doctoral thesis the Image Analysis approach was useful to estimate the functional trait mass (Chapter 4) of the samples processed in Chapter 5.

In Chapter 4, we built a theoretical framework to define functional traits of parasites based on current ecological considerations. Furthermore, we identified seven functional traits virtually measurable from any metazoan parasitic individual and able to deal with any ecological question. We expect that this framework will help to unveil ecological and evolutionary questions in parasitology. Furthermore, it will enhance comparisons among studies and even inspire further extension to non-metazoan parasites. Moreover, it will allow comparing parasite and host diversity on common terms, thereby paving the way for ecologists to broadly include parasites in community ecology.

In Chapter 5, we disentangled the rules that manage structure of the diversity of parasite communities from grey mullets in the Western Mediterranean. We found that these rules are dependent on the level of the analysis and the facet of diversity considered. By and large, host phylogenetic origin (*Chelon* vs *Mugil*) and host environmental preferences (marine-

related vs coastal-related) determine the parasite community of a host individual. Whereas, the habitat conditions and geographical location of the localities are not so determinant of parasite communities. We concluded that the parasite communities cannot be fully understood if any of the facets of diversity is neglected in a study.

In Chapter 6, taking advantage of the bipartite network analysis and an exclusive host-parasite system, we evaluated the role played by native and invasive grey mullet host individuals to their parasite communities. My co-authors and I found that individuals of both host species played a similar role in the area where both host species are native. However, in the area where one host species is native and the other is invasive, the invasive host individuals played a peripheral role to the parasite communities, except when their co-introduced parasites were considered. This fact suggests that, together with the co-introduction, the structure of the host-parasite interactions was also maintained. We propose that long-term monitoring of the roles of invasive hosts in parasite communities can be a useful proxy for estimating the maturity of the establishment of the invasive hosts in an ecosystem.

Overall, this thesis shows how parasite communities can be studied under the light of the current community ecology theory. It gives novel tools to parasitologists to improve our understanding of the role of parasites in ecosystems and we expect it can encourage ecologists to consider parasites in their studies to get a complete resolution of ecosystem processes.

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Appendix

Appendix

Beyond counting species: A new way to look at biodiversity

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Abstract
In modern ecology, the traditional diversity indices (usually of richness, abundance, and species evenness) have been highly revealing and useful for monitoring community and ecosystem processes. However, around two decades ago, a pioneering research team noticed that these indices did not completely resolve their open questions. Thus, they suggested changing the way biodiversity was measured. At its base, this new methodology considers the distance between species (in phylogenetic or functional terms) before subsequently applying the appropriate biodiversity indices. Including phylogenetic and functional elements in the evaluation of diversity allows us to approach the concept of biodiversity in a more comprehensive way.

Keywords
Classical diversity indices, functional diversity, phylogenetic diversity, functional traits, genetic distances.

Introduction

Biodiversity, or biological diversity, is a concept that refers to the variety of life present on Earth as a result of thousands of millions of years of evolution. What probably first comes to mind when we hear this word are idealised scenarios: tropical rain forests full of green, woody, and lush trees, populated by exotic mammals and birds. We might even include some flashy insects in our mental picture, like colourful butterflies. Or perhaps we imagine a coral reef with fish swimming around and combining into impossible colours. However, the "evolutionary" dimension of the term makes us suspect that there must be something else to this scenario.

Life on Earth comprises many plant and animal groups, but also contains many eukaryotic groups (organisms whose cells have a nucleus), and even more prokaryotic groups (organisms whose cells do not have a nucleus). All these forms of life are protagonists in the

ecosystem processes, and these processes have made understanding environmental biodiversity a constant concern for humans. Firstly, to find uses for them or to extract their resources; secondly, to protect them for reasons beyond mere utilitarianism, which thus brings a more eudaemonic dimension to this conservation; and lastly, in recent decades, to evaluate and mitigate the impact of climate-change related disturbances to our planet's life. Biodiversity encompasses variability at three different levels: "between ecosystems"; between the taxonomic units (hereon in species)[1] inhabiting the ecosystems ("interspecific"); and among each species, in other words, "intraspecific" (Glowka et al. 1994). Therefore, one of the fundamental pillars to managing biodiversity is to reliably quantify it while taking these organisation levels into account.

The spatio-temporal decomposition of biodiversity

Historically, ecologists soon became aware that biological variability can present different patterns depending on the scale at which it is analysed. Whittaker (1960) was the first to describe the different spatial components in which biodiversity can be measured; he proposed dealing with the study of biodiversity along several hierarchical spatial scales (Figure i). Thus, he defined gamma diversity (γ) as the diversity of species within a region or ecosystem. In contrast, the lowest hierarchical scale, corresponding to the sampling point, was called alpha diversity (α). Lastly, he defined beta diversity (β) – which establishes the dissimilarity between two comparable modules, normally within the same hierarchical level – as the dissimilarity in biodiversity between several sampling points.

Because of its usefulness and extensive use, the beta component was later redefined. Thus, as shown in Figure i, it was distinguished between beta diversity 1 (β1) – the dissimilarity between sampling points within the same community – and beta diversity 2 (β2) – the dissimilarity between the communities in a region (Excoffier et al. 1992). The most recent studies in the field have proposed that biodiversity can be decomposed over time, in an analogous way to spatial decomposition. In this way, the hierarchical scale could be extended to nested time modules, such as years, decades, and centuries. A logical consequence of this design is the possibility of combining the spatio-temporal aspects of biodiversity, but the approach still needs to be thoroughly developed (Pavoine & Bonsall 2011).

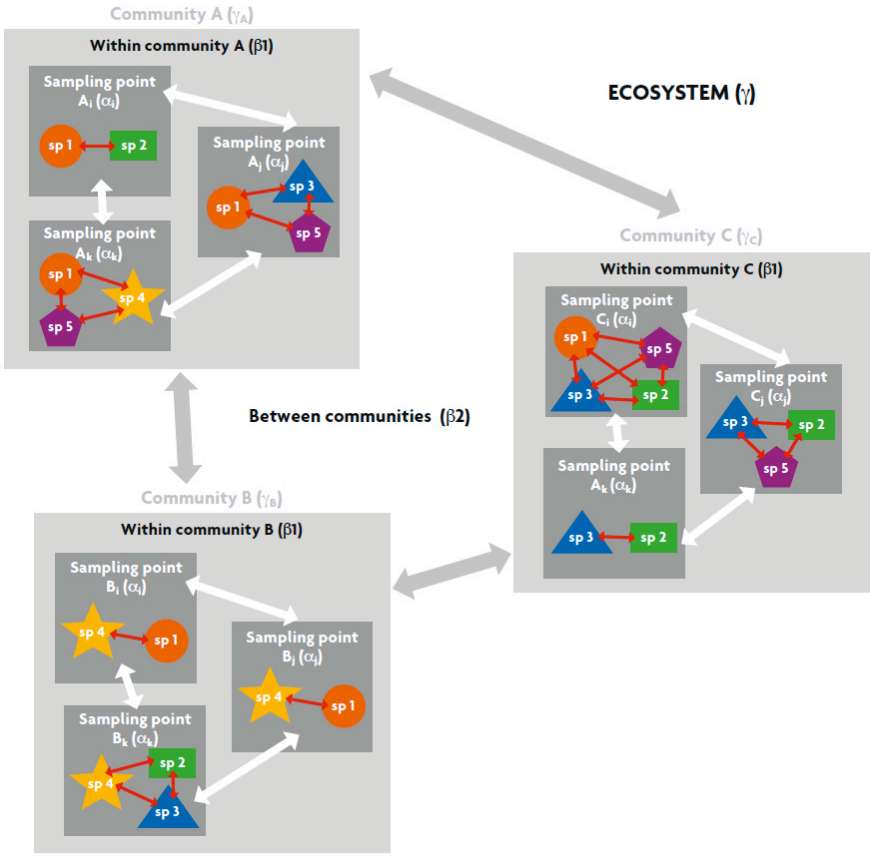

Figure i. Biodiversity can be measured by considering different scales, among them spatial scales. The diagram shows an example of the different spatial components in which biodiversity can be measured, considering an ecosystem comprising three communities (A, B, C). In each community, the species (sp.) present have been registered at three sampling points (i, j, and k). Gamma diversity is the diversity of species in the ecosystem (γ) or community (γA, γB, γC). The diversity at the sampling point is called alpha diversity (α). Beta diversity 1 (β1) is the dissimilarity between sampling points within the same community and beta diversity 2 (β2) is the dissimilarity between the communities in an ecosystem. White and grey arrows refer to the differences measured by the biodiversity indices between the sampling points or communities required to calculate the beta 1 and 2 components, respectively. Red arrows indicate the possibility of calculating the distance between species (in terms of genetic or functional distances) before measuring biodiversity.

How do we measure biodiversity?

Regardless of the spatio-temporal scale (α, β, γ), the traditional indices that quantify and characterise diversity have primarily been based on evaluating variability at the "interspecific" level because it is much easier to observe and quantify there. Conversely, the "intraspecific" and "ecosystem" diversities have enjoyed much less attention. Traditional biodiversity indices mainly quantify the richness, abundance, and evenness of species in a sample. Thus, for a given sample defined in a spatial or time scale, the richness of species indicates the number of species that are present, the abundance quantifies the number of individuals of each species and, lastly, the evenness connects richness and abundance to establish the degree to which the individuals

are distributed among all the species in the sample. Thanks to these traditional indices, we were able to easily and intuitively characterise biodiversity at any spatial scale (α and γ) and even compare modules at the same hierarchical level (β).

Nonetheless, these indices do not offer all the nuances required to obtain a complete idea of biodiversity, because they neglect two of its dimensions: phylogenetics and function. For instance, if we use traditional indices with two communities (community *i* and community *j*, as we can see in Figure ii A) with the same abundance for all the species present, and species richness of $Si = 5$ and $Sj = 3$, the evenness will be $Ei = 100\ \%$ and $Ej = 100\ \%$.

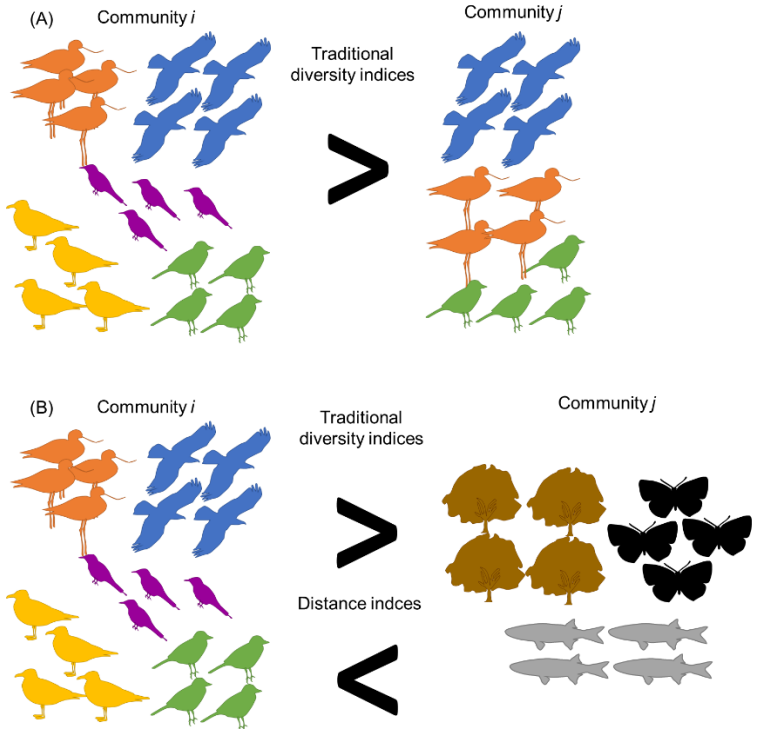

Figure ii. A and B show two communities with the same abundance for all the species present, but with higher species richness in community *i* than in community *j* in both cases. However, species have been replaced by other phylogenetically or functionally distant species in community *j* of the figure B. According to traditional indices, community *i* is more diverse than community *j* both in (A) and (B). While according to distance indices (phylogenetic diversity and functional diversity), community *j* is more diverse than community *i* in (B).

However, as shown in Figure ii B, when we replace the species in community *j* with others that are different phylogenetically (genetically) or functionally (other species that occupy different niches and provide very different services in the ecosystem), the traditional biodiversity indices will still provide the same relative results. In other words, community *i* will be more diverse than community *j*, without considering how different the species in the communities are from each other. This example clearly shows a fundamental limitation in the

traditional biodiversity indices. Therefore, in recent decades, many ecologists have been trying to define a new mathematical framework to describe the phylogenetic and functional differences in the species in a community.

Among the earliest approaches, Faith (1992) tried to evaluate and consider the phylogenetic distance between species. He argued that, before measuring the biodiversity in a given sample, the phylogenetic distance between the species that make up such a sample should be calibrated. Thus, we could know the evolutionary uniqueness of each lineage (whether they had diverged earlier or later from each other). Thus, he proposed measuring the phylogenetic relationship between the species in a sample as a matrix that compared pairs of species. This matrix is extracted from the distance between each pair of species in a phylogenetic tree. In this way, by studying phylogenetic diversity, we can refer to any analysis that bases its measurement of biodiversity on the phylogenetic distance between species.

Later, Petchey and Gaston (2002) proposed a protocol that was similar to Faith's (1992), but in this case the relationships between species were not constructed using phylogenetic sequences, but rather, functional traits[2]. Functional traits are the units used to measure functional diversity in a group of species and allow us to evaluate the consequences of a wide variety of ecological questions, e.g. the impact of climate change on diversity or ecological succession after the restoration of a habitat. In this case, the functional complementarity between the species in our sample would be measured to build a matrix showing the distance between each pair of species using data about their functional traits. Cross-referencing this matrix with the appropriate biodiversity index, we can understand how functionally diverse our sample is.

Finally, if we re-examine Figure ii B, but before measuring the biodiversity of each sample we calibrate the distance between the species in our communities in terms of their phylogenetic or functional-diversity distance, we will reach a completely different conclusion about which one is the most diverse community.

What do these studies provide?

In recent studies we can find remarkable claims; for instance, that a flamboyant coral reef is less biodiverse than an austere mountain ecosystem (Figure iii). While the former was favoured by radiation or emergence of new species, each of these are very close in phylogenetical terms and almost functionally identical. Therefore, if we add or remove a species from the ecosystem, its phylogenetic or functional diversity values will remain almost the same. Conversely, the number of species in the mountain ecosystem is lower, but these species are very distant in

phylogenetic and functional terms, so losing one of them would lead to a dramatic decrease in its phylogenetic or functional diversity values. With this we do not mean that a coral ecosystem is less deserving of conservation than a high mountain one. However, we do want to convey that the correct consideration of the functional and phylogenetic aspects of biological communities can help us to understand the biodiversity structure of the planet better, and so these elements should be taken in to account when establishing specific conservation measures.

Figure iii. According to the traditional diversity indices, the fish community in a coral reef (on the right) would be more biodiverse than an entire mountain ecosystem (on the left) because it has a larger number of species. However, these species are phylogenetically (evolutionarily) and functionally (ecologically) similar, so they are redundant in terms of phylogenetic and functional diversity. Conversely, in the mountain ecosystem, even though there are fewer species, each of them is phylogenetically and functionally different from each other.

Next stop?

Carrying out these studies is more complex than with the traditional approaches because they require two challenges to be dealt with. The first is the identification of significant and non-redundant functional traits to quantify functional diversity; the second is the availability of information about the kinship between species for use in the quantification of phylogenetic diversity. Interestingly, however, unlike traditional biodiversity indices, the concepts of phylogenetic and functional diversity are directly applicable at the individual level. Although less explored, this approach allows us to extend our studies to cover intraspecific diversity, which means that this aspect can be integrated into biodiversity studies. For instance, measuring intraspecific phylogenetic diversity could be essential to understanding phylogeographic patterns and to recognising subspecies so that biological conservation plans can be properly implemented (Excoffier 2008).

In addition, phylogenetic and functional diversity indices have been useful in the characterisation of a many terrestrial and aquatic ecosystems, using everything from herbaceous or woody plants to insects and vertebrates as models. Nevertheless, many fields in which these analyses can be applied are still likely to remain. For example, because parasites depend on other organisms, they have traits that make them useful in the revelation of hidden ecosystem processes. Moreover, parasites are omnipresent in ecosystems; some have complex life cycles, so they are useful for tracing food-web pathways and for discovering spatio-temporal patterns (Poulin & Morand 2000). Despite this, very few authors have tried to study biodiversity in parasite communities from the phylogenetic or functional point of view, although some recent studies indicate that parasitic organisms fulfil regulation, protection, and stability functions in ecosystems. Moreover, because of the nested structure[3] of parasitic communities, studying parasite-host systems provides powerful comparative instruments which can offer generalisable conclusions about other biological communities.

Conclusion

It is currently difficult to imagine a study trying to explain or predict the processes that take place in ecosystems not using phylogenetic or functional data from the taxonomic units considered in the sample. However, we would like to point out that studies based on comparing results obtained using different biodiversity indices, as well as those performed at different hierarchical scales, can reveal evolutionary, biogeographical, or radiation processes that would otherwise go unnoticed. Therefore, we invite interested researchers to use this new conceptual framework in their studies.

Notes

[1]In the text we simplify the description of taxonomic units, equating them merely to the description of biological species. However, we must note that biodiversity indices, both traditional ones and those based on phylogenetic or functional differences, can be applied to any level of the taxonomic classification of organisms, even to viruses (Shi et al. 2016), which in some ways escape the normal definition of a biological organism.

[2]According to Carmona et al. (2016), a functional trait is any morphological, physiological, phenological, or behavioural trait that can be measured at the individual level and which affects survival or reproduction.

[3]The analysis of nested groups (for instance, parasite-host associations) can reveal non-random ecological patterns and are useful exploratory tools that can be used to suggest which mechanisms might structure a given community (González & Poulin 2005).

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