

Galemys pyrenaicus
Pirinioetako muturluzearen
ekologia espazial eta trofikoa:
kontserbaziorako funtsezko giltzarriak



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eman ta zabal zazu



Universidad
del País Vasco

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Etxekoei

Saia gaitezen eskuzabaltasuna eta altruismoa irakasten,
berekoi jaio baikara
(Richard Dawkins, Gene berekoia)

Galemys pyrenaicus
**Pirinioetako muturluzearen
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giltzarriak**

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Irratia

Amarauna (Euskadi Irratia), 2014/08/16.

Ekosfera (Euskadi Irratia), 2017/05/04.

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Laburpena

Pirinioetako muturluzea *Galemys pyrenaicus* (É. Geoffroy Saint-Hilaire, 1811) iturburuko erreketan bizi den eta nagusiki jarduera gautarra duen ugaztun intsektiboro erdi-urtarra da, galzorian dagoena. Endemikoa da Iberiar Penintsularen iparraldean eta Pirinioetan. Nahiz eta bere populazioen oraingo egoera ez ondo ezagutu, azken hamarkadetan bere banaketa-eremua %50 baino gehiago murriztu dela estimatu da, eta beraz, espezie Zaurgarri gisa sailkatuta dago Naturaren Kontserbaziorako Nazioarteko Batasunaren (IUCN) zerrenda gorrian. Beherakada honen atzean dauden arrazoiak ez daude argi, Euskal Herrian bezala saneamendu eta ur-arazketa zerbitzuei esker kutsadura gutxitzea lortu den herrialdeetan ere ematen ari delako. Espeziearen beherakada beste faktore potentzial batzuek eragiten dutela iradokitzen du horrek, eta haien artean habitat fisikoaren andeaketa nagusitzen da. Muturluzearen ekologiaren inguruko zenbait alderdi ongi ez ezagutzeak, ordea, kontserbaziorako neurrien diseinua zailtzen du. Hortaz, ikerketa eta kudeaketa premiazko gaiak dira muturluzearen kontserbaziorako.

Tesi honetan Pirinioetako muturluzearen ekologia espaziala ikertu da bi erreka desberdinetan: bata kontserbazio-egoera bikainean (Elama), eta bestea jarduera hidroelektrikorako ur-erazketa sistemen eraginpean egonik (Leitzarán). Helburua muturluzeak bere bizi-eremuen baitan egiten duen bazka-habitaten hautespena zehaztea zen, beren habitataren egokitasuna baldintzatzen duten faktoreak identifikatu ahal izateko. Irrati-telemetriak, Pirinioetako muturluzeak ur-bare eta putzuen aldean urlasterrak positiboki hautatzen zituela erakutsi zuen, bereziki ur-erazketen eraginpean zegoen errekan. Irrati-telemetriak, ordea, ezin zuen bereizi muturluzearen habitat-hautespena urlasterretako bazka-eskuragarritasun hobearengatik edo bestelako arrazoiren batengatik gertatzen ote zen. Hortaz, aldi berean Pirinioetako muturluzearen ekologia trofikoa ikertu nuen. Horretarako, DNA metabarcoding bidez dieta aztertzeo hasle desberdinen eraginkortasuna aztertu nuen aurrena, hasle bakoitzarekin zein euren konbinazioz lortzen zen estaldura taxonomikoa aztertuz. Gillet eta Zealeren hasleen artekoa izan zen, harrapakinen gama taxonomiko ahalik eta zabalena identifikatzeaz gain, muturluzea bera ere identifikatzeko konbinazio eraginkorra. Orduan, muturluzearen dieta eta harrapakin hautespena ikertu nituen, aldez aurretik aipaturiko bi erreka desberdinetan kaka-lekuetan bildutako gorotz laginak aztertuz. Kontsumitutako harrapakinak identifikatzeko 94 gorotz aztertu nituen DNA metabarcoding bidez erreka bakoitzean. Aldi berean, harrapakin eskuragarritasuna ere deskribatu nuen, erreka bakoitzeko habitat mota bakoitzean bildutako 10 Surber laginetatik. Harrapakin eskuragarritasuna eta dieta ezberdinak izan ziren erreken artean, muturluzea selektiboagoak izanik egoera ekologiko hobereana zuen errekan. Orokorrean, Simuliidae

eta Psychodidae (Diptera), Baetidae eta Heptageniidae (Ephemeroptera), Odontoceridae (Trichoptera) eta Gammaridae (Crustacea) hautatu zituen positiboki muturluzeak; Planorbidae (Gastropoda), Goeridae (Trichoptera), Perlidae (Plecoptera) eta Athericidae (Diptera) negatiboki hautatu zituen. Bazka eskuragarritasuna ez zen desberdina izan habitaten artean, eta hortaz ez zuen muturluzearen urlasterrekiko zaletasuna azaldu. Hori dela eta, Pirinioetako muturluzearen dieta hautespena zerk bultzatzen duen identifikatzeko berriro analizatu nituen datuak, oraingoan harrapakin espezieetan baino, haien ezaugarri —*trait*— biologikoetan zentratuz. Horrez gainera, muturluzearen habitat hautespena harrapakin *traitek*iko zaletasunen bidez azaltzerik ote zegoen ikertu, eta hobetsitako *traitak* bi ibaien artean desberdinak ote ziren aztertu nuen. Harrapakin *traiten* hautespena aztertzeko, muturluzeen harrapakin hautespenean eragin zezaketen 9 *trait* erabili ziren; horietako 4 (sakonerarekiko zaletasuna, korrontearen abiadurarekiko zaletasuna, substratuarekiko zaletasuna eta ur-fluxuarekiko esposizioa) segur aski habitat motaren (urlasterra, ur-barea edo putzua) eraginpean daude, eta beste 5 (substratuarekiko mugikortasun/lotura mota, arintasuna, agregazio joera, gorputzaren tamaina potentzial maximoa eta gorputzaren malgutasuna) habitatarekiko independenteak dira. Muturluzeek positiboki hautatutako harrapakin kategoriak hauek izan ziren: ur-zutabeen eta gune epibentikoan bizi diren harrapakinak, substratu gisa blokeak dituzten ur bizkorretan bizi direnak, ur-fluxuarekiko esposizioa dutenak, 2-8 mm-ko tamaina potentzialekoak, arintasun handikoak, taldekatuta bizi direnak, eta igerilariak zein substratuari lotzeko mekanismoak dituztenak. Bestetik, negatiboki hautatutako harrapakin kategoriak hauek izan ziren: ur-abiadura moteletan bizi direnak, substratu finen barrukoak, ur-fluxuarekiko gordeta bizi direnak, 2 mm baino tamaina potentzial txikiagoa dutenak, agregazio joerarik ez dutenak, eta herrestariak. Hobetsitako harrapakin *trait*-kategoria guztiak (gorputz malgutasun handia izan ezik) pixka bat ugariagoak izan ziren urlasterretan, baina aldeak txikiak ziren eta erreken artean aldakorak, eta hortaz litekeena da beste arrazoi batzuek azaltzea animalia honen urlasterrekiko zaletasuna —muturluzeen flotagarritasun naturalak, esaterako—.

Tesi honek, oro har, Pirinioetako muturluzearen habitat-erabileraren eta dietaren espezializazio handia berresten du. Hortaz gain, muturluzearentzako ur garbiak nahikoa ez direla ere azpimarratzen du, muturluzearen kontserbaziorako urlasterrak eta erreken konektagarritasuna funtsezko faktoreak izanik.

1. Kapitulua



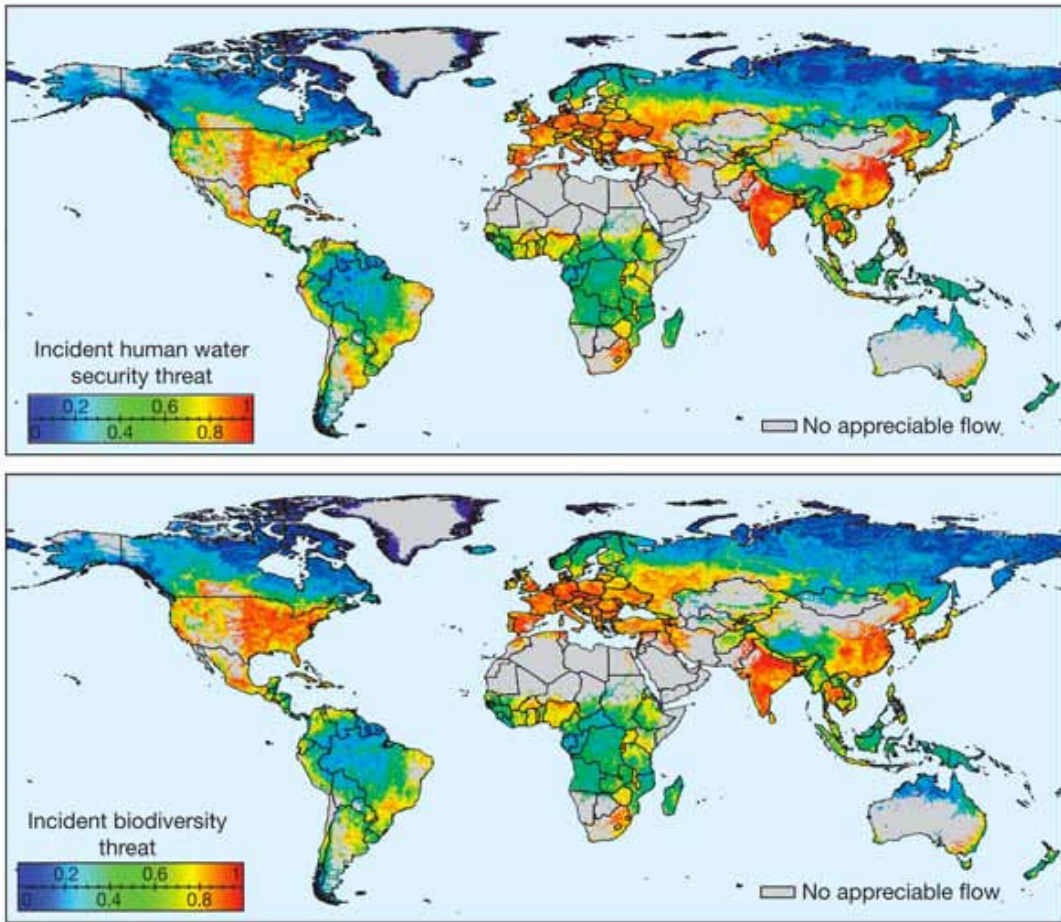
Sarrera orokorra

Mundu-mailako “biodibertsitatearen krisia” eta ur gezatako ekosistemak

Gaur egungo giza jardueraren ondorioz Lurra gure planetak pairatzen duen biodibertsitatearen galera handia “biodibertsitatearen krisi” global gisa definitu da (ikus Abell, 2002), eta zenbait autoreren arabera seigarren iraungitze masiboaren aurrean gaude (Barnosky eta kideak, 2011; Leakey eta Lewin, 1997; Myers, 1990). Lurraren erabilera, kudeaketa-praktikak, kutsadura eta giza demografiaren aldaketak dira, besteak beste, galera horren eragileak (Root eta kideak, 2003; Rosenzweig eta kideak, 2008). Giza-eraginezko klima aldaketak are gehiago okertzen du joera hori (Walther eta kideak, 2002).

Euren azalera kontutan hartuz, ur gezatako ekosistemek biodibertsitatearen zati oso handia gordetzen dute (Dudgeon eta kideak, 2006). Ekosistema horiek, eta bereziki iturburuko errekek, biodibertsitatearen galera handiagoa jasaten ari dira mundu osoan zehar erasotuen dauden ekosistema lurtarrak baino (Richardson, 2019). Reid eta kideek (2019) barnealdeko uren mehatxu nagusien artean hidroelektrikaren hedapena, klima aldaketa, gaixotasun infekziosoak eta estresatzaile metagarriak sartu zituzten; mehatxu ertainen artean, aldiz, e-salerosketa, inbasioak, algen loraketa kaltegarriak, argia eta soinua, ur-gezen gazitzea eta kaltzioaren beherakada aipatu zituzten, eta eragin ez horren garbiko mehatxuen artean, kutsatzaileak.

Bide askeko ibaietan bizidunen sakabanaketa ibilguan gora zein behera eman daitekeelako mantentzeko daiteke biodibertsitatea. Baina zatitutako eta erregulatutako ibaietan sakabanaketa hori mugatuta egon daiteke. Ibilguko egitura fisikoek, ibaiko emariaren aldaketek, edota oztopo efektua eragiten duten uraren kalitatearen aldaketek arriskuan jar dezakete ibaien konektagarritasuna (Grill eta kideak, 2019). Nilsson eta kideek (2005) ikertutako ibai-sistema handien erdia baino gehiago presen eraginpean daude, eta Europa da aldaketarik gabeko ibai-sistema handien proportzio baxuena duen kontinentea. Vörösmarty eta kideek (2010), gizakien eta biodibertsitatearen ikuspuntuak batera kontutan harturik, estresatzaile desberdinek ur-gezatako baliabideetan inpaktu larriak dituztela erakutsi zuten (1. Irudia). Autore hauen arabera, 2000. urtean munduko populazioaren ia %80 gizakiontzako ur-ziurgabetasunaren mehatxua zuten edo biodibertsitate-mehatxuak 75-eko pertzentila gainditzen zuten guneeetan bizi zen, eta munduko ibaien emarien eta ur horrek eskainitako uretako habitaten %65-ak mehatxu ertaina edo altua zuen. Munduko ibaien zati txiki bat besterik ez zen mantentzen aldaketarik gabe. Herrialde aberatsek uraren teknologian egindako inbertsio masiboek estresatzaile maila altuak konpentsatzea



1. Irudia. Gizakiontzako ur-ziurgabetasunaren mehatxuen (“*Incident human water security threat*”) eta biodibertsitatearen mehatxuen (“*Incident biodiversity threat*”) mundu-mailako geografia. Mapak erakusten dituen gizakiontzako ur-ziurgabetasunaren eta biodibertsitatearen inpaktu pandemikoak, haien artean oso koherenteak izan arren, ez dira erabat berdinak. Vörösmarty eta kideen (2010) lanetik moldatutako irudia.

ahalbidetzen dute, baina horien atzean dauden kausak konpondu gabe; aberastasun gutxiago duten herrialdeek, aldiz, zaugarri izaten jarraitzen dute.

Iragarritako ingurumenaren aldaketa globalak eragindako biodibertsitate galerak, ekosistemen funtzionamendua eta hauek gizartea ondasun eta zerbitzuez hornitzeko zuten gaitasuna aldatuko ditu (Cardinale eta kideak, 2012), gizakiontzan zuzenean eraginez. Ur-gezatako biodibertsitateak ondasun (adb. janaria, arrainak) eta zerbitzu (adb. ur gabia eta hondakinen asimilazioa) baliotsu ugariz hornitzen ditu gizarteak, horietako batzuk ordezkaezinak izanik (Covich eta kideak, 2004). Barnealdeko urek, hain zuzen ere, baliabide natural baliotsua eraten dute ekonomiari, kulturari, estetikari, zientziari eta hezkuntzari dagokienean, eta horien kontserbazioa eta kudeaketa erabakigarriak dira

herrialde eta gobernu guztien interesentzako (Dudgeon eta kideak, 2006). Adibidez, Mekong ibaiaren bailaran, biztanleak arrantzaren eta arrozaren nekazaritzaren menpe daude, eta bi jarduera hauek ibaiko uraren kalitatearen eta kantitatearen menpekoak dira. Akuikultura komertzialerako arrain esplotazio handiek eta presa hidroelektriko handien eraikuntzak, ordea, arrainen stockak murriztu, migrazioa eragotzi, eta nekazaritzan eragiten dute, lehorte eta uholde artifizialak eraginez (Coates eta kideak, 2005; Hortle, 2009). Aldakuntza horiek bertako biztanlegoaren sostengua mehatxatzen dute, bizimodua aurrera ateratzeko baliabide naturalen beharra dutelako. Ur-gezatako perlek ere gainbehera izan dute. Historikoki, ur-gezatako muskuiluak oso ugariak izan dira mundu osoan zehar, baina gaur egun galtzeko arrisku handiena duten organismo taldeen artean daude (Moorkens eta kideak, 2017). Giza-jarduerak espezie askoren banaketaren gainbehera eragin du, besteak beste arrainena (adb. ibai-aingira *Anguilla anguilla*; Jacoby eta Gollock, 2014), anfibioena (adb. baso-igel pirinioarra *Rana pyrenaica*; Bosch eta kideak, 2009), eta dortoka, kokodrilo zein izurde batzuen gisako bestelako megafaunarena (He eta kideak, 2019). Gai kezkarri bat zera da: espezie bat galdu orduko, bertako gizarteak berataz ahanzteko joera duela eta, hortaz, ez direla kontziente ibaiak euren egoera naturaletik zenbat desbideratu diren (Soga eta Gaston, 2018). Joera horri “*shifting baseline*” edo “abiapuntu aldakor” izena eman izan zaio (McHarg, 1969). Gaizkata arrunta (*Acipenser sturio*) eta Europar kastorea (*Castor fiber*), esaterako, ahaztutako espezieen adibide onak dira. Arduragabekeria horren parte bat zerbitzuen arteko trukeak eskala espazial eta tenporal desberdinean ematen direlako gertatzen da: ekosistemak sinplifikatzearen irabaziak tokikoak izaten dira, baina kostuak aldiz beste kokaleku batetan dauden pertsoneri edo etorkizuneko belaunaldiei transmititzen zaizkie (Cardinale eta kideak, 2012).

Iturburuko errekek ur-gezatako ekosistema sentikorrenen artean egonik, ekosistema paregabeak dira, euren ingurura oso konektatuta daudenak: urte-sasoika moderatutako tenperaturak izaten dituzte; areriorik gabeko espazioak izaten dira (ez delako harrapari handirik izaten bertan, edo oso gutxi egoten direlako); eta erlatiboki espezie askori babeslekua eskaintzen dioten ingurune fisiko egokiak izaten dira (Richardson, 2019). Ibai-ekosistemen egitura dendritikoak ondorio interesgarriak ditu biotan, guztiz urtarrak diren izakien mugimenduan eragiten duelako, eta gutxiago anfibioetan. Ur-gezatako animalien banaketa ibaien sarera mugatuta dago (Hughes, 2007) eta iturburuko errekek isolatuta egoten dira, batetik bestera mugitzeko ibaian behera jaitsi eta berriro igo egin behar izaten baita (Fagan, 2002). Populazioen tamaina txikia eta gene-fluxu mugatuari lotutako isolamenduak, populazioen arteko bereizpen genetiko altua eta endemismoak ugariak izatea dakar (Sterling eta kideak, 2012). Populazio oso ezberdinez osatutako izakien adibide ditugu Ipar Ameriketako ibai-karramarroak (Taylor eta kideak, 2007) eta ur-gezatako muskuiluak (Inoue eta kideak, 2014). Gaur egun asko dira mehatxatuta dauden

ur-gezatako espezieak, eta adierazleek populazioen gainbehera azkarra eta iraungipen arrisku handia iradokitzen dute (Reid eta kideak, 2019).

Dudgeon eta kideek (2006) gogorarazi zutenez, gizakiok urarekiko ditugun eskakizunen joerak aldaketarik gabe mantentzen badira, eta espezieen galerak gaur egungo erritmoan jarraitzen badu, ur-gezatan geratzen den biodibertsitate gehiena kontserbatzeko aukerak galdu egingo dira. Beraz, ur-gezatako biodibertsitatearekiko eta ekosistemen kudeaketarekiko jarrera aldaketa handia behar da. Reid eta kideek (2019) zenbait erraminta eta teknika aurkeztu zituzten ur-gezaren kontserbaziorako. Ur-gezatako komunitateetan, ordea, espezie gehienak paregabeak dira (Sheldon, 1988), eta ez dira ongi ezagutzen, ez beren historia naturalak (ekologia, biologia, eta abar.), ez eta euren gainbeheraren arrazoiak. Ur-gezatako espezieak kontserbatzeko lehenengo pausoa degradazioa eragiten duten kausak geldiaraztea da, besteak beste kutsadura, habitaten aldaketa eta gain-ustiaketa. Nahiz eta espezie askoren mehatxuen inguruko ezagutza eskasa izan, naturaren kontserbaziorako lehenengo pausoa ikerketa gehiago egitea dela esateak espezie horiek iraungitzea dakar, denbora nahikorik ez dagoelako galtzeko arrisku handiena duten espezieak iraungi aurretik garrantzitsua den informazio gutzia lortzeko (ikus Croxall eta kideak, 2012).

Pirinioetako muturluzea

Ezaugarri morfologikoak

Pirinioetako muturluzea *Galemys pyrenaicus* (É. Geoffroy Saint-Hilaire, 1811) (2. Irudia) satorren familiako (Talpidae) intsektiboro erdi-urtarra da. Bere gorputza 11.5-13.5 cm luze da, isatsa 10-16 cm luze, eta heldu baten masa 44-76 g da (Nores, 2012). Bere ahaideak diren satorrak — *Talpa* sp. — ez bezala, muturluzea igeriketarako moldatua dago, atze-hankak aurre-hankak baino handiagoak izanik (Palmerim eta Hoffmann, 1983). Hondoari ongi eustea ahalbidetzen dioten atzapar sendoak ditu, eta igeriketa errazteko mintz interdigitala du hatzen artean, oinarrian. Aurre-hankak txikiak dira eta ez daude zulaketarako moldatuta. Atze-hankak aldiz handiagoak dira, ezkataz estaliak, eta kanpoaldean luzeak, sendoak eta konpaktuak diren ileen ilara bat dute. Isatsa, ia biluzia, ezkataz eta ile gogor gutxi batzuek estalirik dago, ebaketa zilindrikoa du punta zapalduan izan ezik, eta igeriketean lema gisa erabilia izan daiteke. Isatsaren oinarrian musketa-likidua jariatzen duen guruin bat izaten du, segur aski euren lurraldeak markatzeko erabiltzen duena (Palmerim eta Hoffmann, 1983; Richard eta Viillard, 1969). Muturra da Pirinioetako muturluzearen

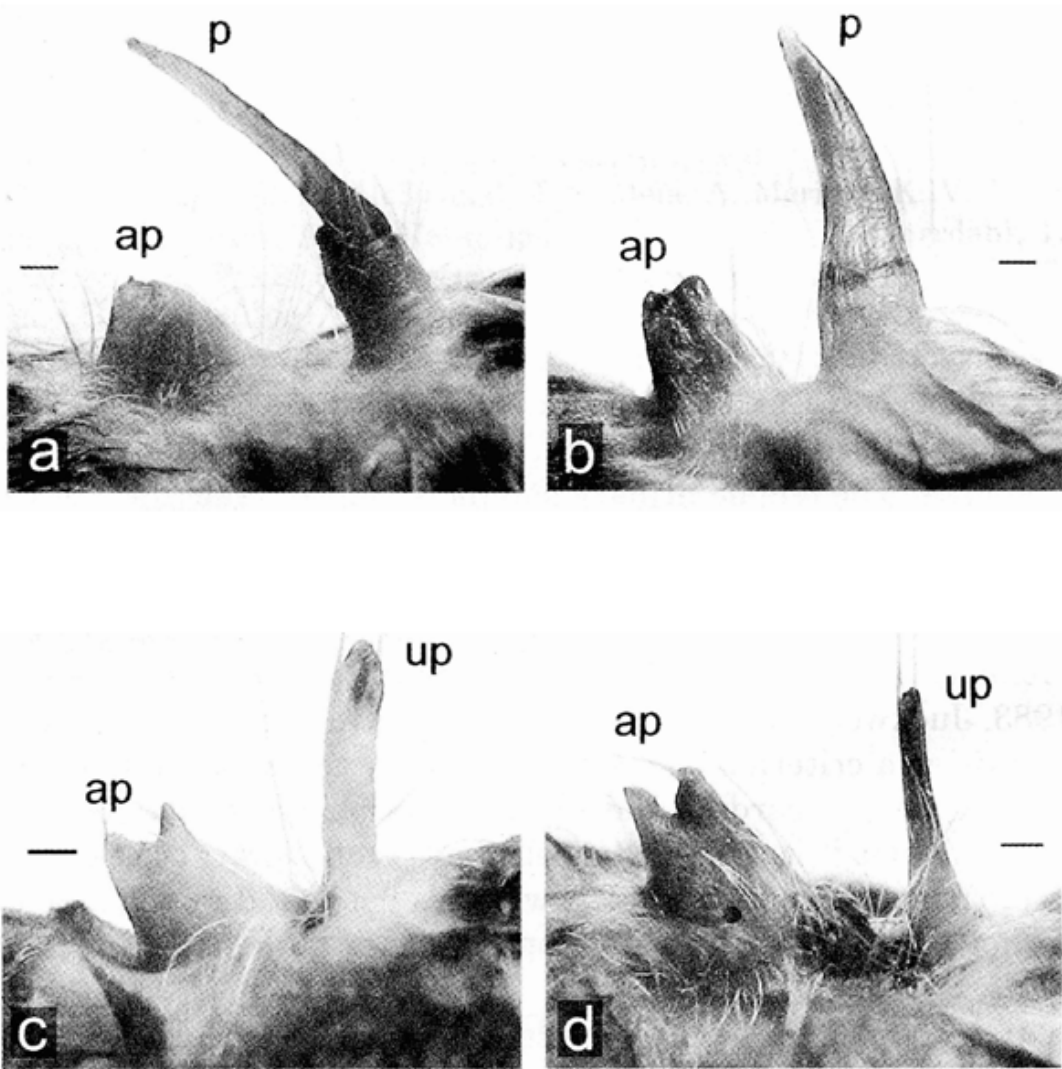


2. Irudia. Pirinioetako muturluzea. Argazkia: Amaieur Esnaola.

ezaugarri bereizgarriena: ilerik gabeko tronpa bat da, eta bere amaieraren alde dortsalean sudur-zuloak irekitzen dira. Beren aurpegi luzatuak eusten ditu goiko intzisibo nabarmenak (4. Irudia), eta beren hortz-formula 3.1.4.3/3.1.4.3 da (Palmerim eta Hoffmann, 1983). Satorraren antzera, Pirinioetako muturluzeak oso begi txikiak eta kanpotik ikusezinak diren belarriak ditu, barrunbe zilindriko batetara murriztuak izan direnak.

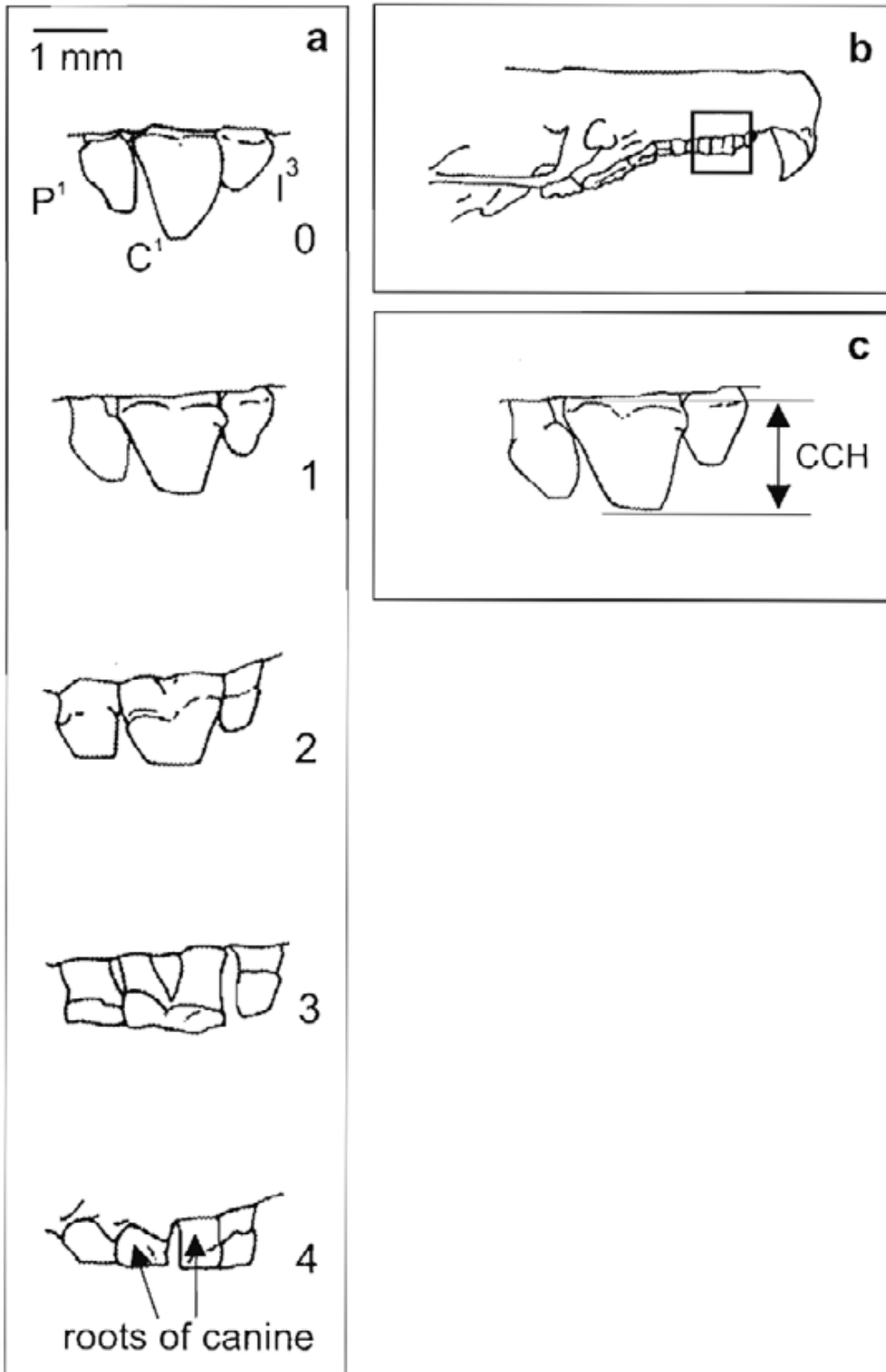
Muturluzearen bibrisak, satorrarenak baino luze eta lodiagoak, muturraren bi aldeetan daude kokatuta eta txikitu egiten dira tamainaz alde distalerantz jo ahala (Richard, 1981). Ilazkia eta babes-ileak gorputzean zehar banatuta daude. Muturluzeek bi babes-ile mota dituzte, *leithaar* izenez ezagun diren babes-ile zuzenak eta *grannen* deritzaien zorrotz-okerrak (Poduschka eta Richard, 1985). Azken horiek errazak dira identifikatzen, muinik ez dutelako, euren erdi-alde distala barnealderantz tolesturik dutelako eta lantza-mutur baten formako gune zapal apikala aurkezten dutelako. Ezaugarri horiei esker, ileek muturluzearen gorotzen identifikaziorako garrantzi berezia dute, orrazketaren ondorioz ustekabean irensten dituztelako (Stone, 1987b).

Muturluzearen gorputzak, marroixka izan arren, urpean brontze eta zilarrezko distira metalikoak islatzen ditu, bere ile-mota bereziei esker: *grannen*-en espatula formako guneak animalia biziaren zein hilaren ilajeen distira eta



3. Irudia. Pirinioetako muturluzearen kanpoko sexu-organoen alboko ikuspegia: (a) ar gaztea (urte 1 baino gutxiagokoa), (b) ar heldua (2 urte baino gehiagokoa), (c) eme gaztea (urte 1 baino gutxiagokoa), eta (d) eme heldua (2 urte baino gehiagokoa). Uzki-papila (ap), zakila (p) eta gernu-papila (up) ageri dira irudian. Arretan guriak agerian uzteko, prepuzioa kendu zen. Eskala-barra: 1 mm. González-Esteban eta kideen (2003) lanetik berreskuratutako irudia.

iridiszentzia ikuskorrak eragiten ditu (Puissegur, 1935). Tronparen eta gorputz-adarren azala belzkara da eta zilar koloreko ileak ditu. Alde dortsala bentrala baino argiagoa da. Haragi-kolore argia du isatsak, eta ile zuriska edo horixkak ditu. Kolorea, tamaina eta masarekiko dimorfismo sexuala, dagoenean, ez da nabaria. Hare gehiago, ugal-sisteman hautematea ere zaila da, bi sexuek zakil-itsurako organoa baitute. Muturluzeen sexuaren identifikazioan arku pelbiko oso fina da lagungarri (Peyre, 1957): eme helduetan 5 mm inguruko hutsune biguna hauteman daiteke, eta ar helduetan berriz arkua beti itxita egoten da. Ernalketa



4. Irudia. Hartz-higatzea Pirinioetako muturluzean. (a) Goiko letaginaren forma (ezpain-aldea) ezarritako bost higatze klaseetarako; goiko 1. aurreko hagina (P¹), goiko letagina (C¹) eta goiko 3. intzisiboa (I³) irudikatu dira. (b) Muturluze baten garezurraren aurpegi-aldea (alboz); koadroak (a)-n azaldutako goiko barailaren zatia erakusten du. (c) Indizean erabilitako goiko letaginaren koroaren altuera (HCC). González-Esteban eta kideen (2002) lanetik berreskuratutako irudia.

garaian, baginaren irekidura irekita eta pigmenturik gabe izateari esker bereiz daitezke emeak (Richard, 1986). Dena den, González-Esteban eta kideek (2003b) urte-sasoirekiko independentea den sexuak identifikatzeko metodo berria aurkeztu zuten: kanpoko sexu-organoak presio arin batez zapalduz hauek kanpora aterarazten dira, eta arretan kono itxurako guruina ikusten den artean, emeek gernu-papila luze eta fina soilik erakusten dute (3. Irudia).

Muturluzearen adina ezin da masatik ondorioztatu eta inork ez du inoiz muturluze jaioberririk ikusi. Richardek (1976) eta González-Esteban eta kideek (2002) adinaren zehaztapenerako kriterio batzuk ezarri zituzten. Alde batetik, kriterio kuantitatibo bat proposatu zuten, luzera kondilobasalarekiko (LCB) eta goiko letaginaren koroaren altueraren (HCC) arteko balio erlatiboan oinarritutako indizea (D) erabiliz, non $D = (CCH/LCB) \times 100$. Hala eta guztiz ere, kriterio semikuantitatiboa da gehien erabiltzen dena, non goiko letaginaren higatzean oinarriturik ondorengo klaseak zehazten dituzten (4. Irudia): 0) 1. urtean, osorik edo pixka bat kamustuta soilik dagoen letagina ageri da; 1) 2. urtean, kamustutako gailurra duen letaginetan, higadurak koroaren laurdena hartzen du; 2) 2.-3. urteetan, higadurak koroaren erdia hartzen du; 3) 3.-5. urteetan, higadura maximora heltzen da eta gutxi gorabehera koroaren hiru laurdenak hartzen ditu; 4) 3.-6. urteetan, letaginen koroaren erabateko higadura gertatzen da, hortzen bi erroak bereizita ikus daitezke, eta 2. eta 3. intzisiboen oso antzekoak dira tamainari eta formari dagokionean.

Zentzumenen mundua

Muturluzeak itsuak dira ia, baina bazka ikusmenaz kokatzeko gai ez diruditen arren, argiaren intentsitatearen aldaketak hautemateko gai direla uste da (Richard, 1973). Muturluzeek soinu bitxi batzuk igor ditzakete (garrasi ozen gisa deskribatuak) manipulatuak direnean edo espezie bereko beste kideekin topo egiten dutenean, nahiz eta normalean isilik gelditzen diren (Richard, 1973). “Danbor-jotze” arraro bat ere egiten dutela esan izan da, uraren azalera aurre-hankekin joz (Richard, 1973). Nahiz eta Niethammerrek (1970) hori “bazkal ostean eskuak garbitzeko” jokabide gisa interpretatu, Richardek (1981) bibrazioen bidez uraren azaleko oztopoak detektatzeko egiten zutela proposatu zuen. Badirudi muturluzeek oso mekanohartzaille garatuak dituztela, bibrisa luze eta konplexuekin eta Eimerren organoekin (Bauchot eta kideak, 1973), zeinak muturluzeen muturra oso sentikorra izatea eragiten duten. Ugaztun urtar gehienetan bezala, bibrisak ugariak, luzeak eta sendoak dira, euren erroetan mugiezinak, eta inerbazioetan aberatsak (Richard, 1985b). Muturraren hiru laurdena baina gehiago bibrisen nerbio eferenteez estalirik dago, eta hauek gertu dauden objektuekiko kontaktua zein igarotzen den uraren mugimendua hautematen dute. Bai bibrisak eta baita Eimerren organoak ere arrainen albo-

lerroarekin konparatu izan dira (Richard, 1973; Sarko eta kideak, 2007). Eimerren organoak epidermisetik irtendako nerbio ehunaren inguruan pilatutako zelulen egitura dute (Richard, 1981, 1985b), talpidoen mekanohartzaile eksklusiboak dira, eta animaliairen muturraren amaieran mm² gutxitan 100.000 baina gehiago izan daitezke. Kontaktuairekiko eta animaliairen mugimenduaren ondoriozko uraren presioaren aldaketekiko oso sentikorak dira, eta uretan oztopoen detekzioa ahalbidetzen dute. Tronpak urpeko oztopoekin talka egitea oztopatzeko edota bazka topatzeko balio duela ikusi izan da (Richard, 1973, 1985b).

Kimiohartzaileei dagokienean, mingaineko dastamen organoen eskasiak (Richard, 1986) muturluzeek murgildutako beren harrapakinak usaimenaz detektatzen dituztela iradokitzen du (Richard eta Micheau, 1975; Richard, 1986). Jacobsonen organoa ere muturluzearen material likidoekiko kimiohartzaile garrantzitsua dela iradoki izan da (Richard, 1985b). Jacobsonen organoa barnealdetik zimurtuta dagoen hobiska sinple bat da, ahoaren inguruko uretan disolbatuta dauden molekula usaintsuak modu pasiboan jasotzen dituen usaimen-mukosa bat duena (Richard, 1986). Organo bikoitz honen ahoko irekidurak zailak dira topatzen, bi balbulaz ezkutaturik daudelako. Horiek goiko intzisiboen atzean daude kokatuta, ahosabai bigunean, ahotik sudur-barrunbera edukiak itzultzea ekidinez, eta usaimenaz aztertze hartu den urari pasatzen utziz. Badirudi beheko intzisiboek balbula horiek irekitzen laguntzen dutela, ahosabai biguna estutuz (Richard, 1986). Usaimen-organo paregabe hau normalean ez da ugaztun helduetan agertzen, baina intsektiboroetan mantendu egiten da, eta bereziki garatua dago Talpidoetan. Muturluzeak burbuilak botatzen ditu urpean sudur-zuloetatik (Richard, 1986), beraz, baliteke muturluzeek ere beren harrapakinak urpean usainduko dituztela, aire-burbuilak botaz eta arnastuz, Errusiako muturluzean (*Desmana mochata*, Ivlev eta kideak, 2013) eta antzeko ekologia duten *Sorex palustris* (Catania eta kideak, 2008) eta *Condylura cristata* (Catania, 2006) bezalako beste ugaztun batzuetan deskribatu izan den bezala.

Ugalketaren biologia

Frantziako Pirinioetako 500 banako baina gehiagoren azterketa histologikoa egin ostean, Peyrek (1962) azaldu zuen muturluzeen araldia eta erditze-garaia urtearen lehen erdian kontzentratzen dela, eta emeek erditu osteko araldia dutela, urteko gehienez hiru kumaldi izanik. Kumaldiak 1-5 kumekoak ziatez dira, 4-ko modarekin (Peyre, 1956). Zoritzarrez, naturan ez da inoiz muturluzeen kumerik edo kumedun habiarik ikusi. Berriki egindako ikerketa batek (Escoda eta kideak, 2019) iradokitakoaren arabera, muturluzeak ez dira urte bat izan arte ugaltzen, ez dira monogamoak —emeak ar desberdinekin ernaltzen baitira ugalketa desberdinetan—, eta kumeez emeak soilik arduratzen dira.

Bizi-eremuak eta mugimenduak

Markatze-berratzemate tekniketan oinarrituz, Richard eta Viallardek (1969) muturluzeen bizi-eremua definitzeko lehenengo saiakera egin zuten. Bizi-eremua funtsean errekan zeharreko zati linear baten gisan definitu zuten, eta bere luzera hamarnaka metrotatik gehienez 200 metrotarainokoa izan zitekeela ikusi. Arrek eta emeek bizi-eremua konpartitu dezaketela esan zuten. Gatibutasunean mantendutako animaliak ere ikertu zituzten eta, nagusiki gautarrak izan arren, eguerdian bazkatzeko jarduera-une bat zutela azpimarratu zuten. Palmerim eta Hoffmannen (1983) ustez, muturluzeek “buruz ikasitako” ezagumendua dute beren bizi-eremuaren inguruan, izan ere edozein objekturen lekualdaketak animalia nahasten zuela behatu baitzuten, eta muturluzeek euren lurraldeak babesten zituztela ere esan zuten, sexu berekoen artean ere oldarkorrek izanik.

Nahiz eta Richardek (1985a) gatibutasunean mantendutako muturluzeak udazken eta neguan udaberrian eta udaran baino aktiboagoak zirela esan (segur aski ugalketa garaiarekin lotuta), irrati-telemetria lanak hasi arte ezin izan zen landako muturluzeen jarduera ikertu. Era horretako lehenengo ikerketan, Stone eta Gormanek (1985) banako batzuk bakartiak izan arren helduek normalean neurri handi batean eskusiboak izaten diren bizi-eremuak kontrako sexuak banakoekin konpartitzen zituztela esan zuten. Emeen bizi-eremuak (301 ± 6.8 m; $n = 7$) beren bikotekide arren eremu handiagoen (429 ± 10.0 m; $n = 7$) baitakoak izaten ziren eta bizilagunak ziren arren arteko eremuak neurri handi batean eskusiboak izanik ere, gainjartze maila bat erakusten zuten. Elkarren segidan bizi ziren emeen, zein eme eta arren artean, ez zen gainjartzerik ikusi. Bestalde, gazteak 249-300 m-ko luzerako eremu erlatiboki txikitik bizi ziren. Elkarren segidan bizi zirenen jarduera sinkronizatuta zegoela zirudien, izan ere bikote bakoitzeko kideak habiatik errekan gora mugitzen baitziren jarduera-aldi batean, eta errekan behera hurrengoan, 24 orduetan euren eremu guztia estaliz. Honela, bizilagunen arteko liskarrak denborazko isolamenduaz saihesten zirela iradoki zuten.

Stonek (1985) muturluzeek euren bizi-eremuekiko zuten leialtasun sendoa berretsi zuen, eta beren populazioak gune berean bizi diren muturluze ar eta eme heldu banaz osatutako talde sedentarioez eta gazteak zein heldu nomadak izan daitezkeen banako iragankorrez osatuta zeudela esan zuen. Muturluze iragankorren (bakartien) deskribapena nahasgarria izan daiteke, izan ere baliteke beren bizi-eremuan harrapatu ez diren beste banako batzuk egotea. Stonek eme eta gazteek, ar helduek ez bezala, habien inguruan euren eremuen amaieretan baino denboraren proportzio handiagoa pasatzen zutela gehitu zuen. Arren mugimenduen erregulartasuna lurraldearen mugapenerako metodo garrantzitsua zela gehitu zuen —usain seinaleak etengabe berrituz,

gorotzez zein isats-azpiko sebo-guruinaz—, era berean, bizilagunen zein animalia iragankorren etorreraren kontrako uxatzaile gisa jarduten zuelarik. Stoneren arabera, muturluze guztiek (ar batek izan ezik) habia nagusi bakarra erabiltzen zuten, banako bakoitzarentzako eskusiboa zena. Stonek (1987b) gazteek bizilagunak ziren muturluzeekin edo beste gazte batzuekin neurri handi batean eskusiboak ziren zein guztiz gainjarriak ziren eremuak erabiltzen zituztela esan zuen. Muturluzeen antolaketa espaziala lurraldetasun forma bat dela iradoki zuen, izan ere espezie bereko banakoek oso jarrera oldarkorra baitute eta euren eremuko gunek bakoitzarekin ezagutza altua lortzen baitute, egunero ohiturazko mugimenduen patroia bat jarraituz. Habietako grabagailu automatikoak erabiliz ikerketa batek (Stone, 1987a) muturluzeei bi babesleku mantentzen zituztela plazaratu zuen, bata atsedenerako aldi laburretarako (10-15 min) era irregularrean aldi baterako erabiltzen dena, eta bestea, aldi luzeagotarako erabiltzen den habia nagusia. Lehenago deskribatu den bezala, banakoek bi jarduera-aldi desberdin erakusten dituzte 24 orduko aldi bakoitzean, egunean zeharreko aldi labur batekin eta gauean zeharreko aldi luzeago batekin. Patroia hori harrapakinen jarduera altuena den momentuan aktibo egotearekin lotuta egon zitekeela iradoki zuen.

Chora eta Quaresmak (2001) Portugalen eginiko lehenengo irratitelemetria lana argitaratu zuten, zeinak muturluzearen mugimenduetan jarduera hidroelektrikorako sistema txikiak zuten eragina ebaluatu zuen. Muturluzeei presa baxuak gaituztela esan zuten. Melero eta kideek (2012) Katalunian muturluzeen bizi-eremua batazbeste 523 ± 50.85 m-koa zela, sexuen arteko desberdintasunak esangarriak ez zirela, eta espeziea guztiz lurraldekoa ez dela esan zuten. Bestalde, espezie bereko banakoen artean erasorik ematen ez zela behatu zuten, elkarrekintza sozialak ohizkoak izanik, eta sexua edo adina edozein zelarik ere banakoek euren artean habiak konpartitzen zituztela esan zuten. Atsedenerako lekuen sarrera urperatuta edo erdi-urperatuta egoten zen normalean. Melero eta kideek (2014) muturluzea nagusiki gautarra zela eta bere jarduera-patroia udaberrian bimodala eta udazkenean trimodal-tetramodala izan zitekeela erakutsi zuten. Egunean zeharreko mugimenduak zuzenak ziren beti, aurrerako eta atzerako mugimendurik gabeak. Aurrerako behaketen aldean, autorek jarrera oldarkorren seinalerik gabeko banakoen arteko topaketak ikusi zituzten. Hortaz, Melero eta kideek muturluzeei euren jarduera edo jokabide espaziala espezie bereko banakoak saihesteko aldatzen ez dutela iradoki zuten.

Oztopoak eta isolamendua

Escoda eta kideek (2017), Errioxako DNA laginak erabiliz, bizilagunak ziren populazioen arteko harreman genetiko txikia aurkeztu zuten, muturluzeei ibaien artean gutxitan mugitzen direla adieraziz. Bestalde, emaitzek artoen arteko

banalerroak lehorreko mugimenduen bidez zeharkatu zitzaketela iradoki zuten. Escoda eta kideek (2019) pedigrietan oinarrituriko ikerketa bat erabili zuten Zamorako DNA laginekin oztopo desberdinen (hots, arro arteko banalerroen eta presen) eragina ebaluatzeko. Belaunaldiko sakabanatze-distantzia 4 km-koa zela eta emeen arteko filopatria handiagoa zela ohartu ziren. Eskualdeko oztoporik garrantzitsuena ibaien arroen arteko banalerroa zela ondorioztatu zuten, presa handi batez eta sakabanatzean eragin txikiagoa zuten presa txikiagoez jarraituz. Zatiketak eta konektagarritasun mugatuak endogamiaren beheraldia eta, azkenik, muturluze populazioen tokiko iraungitzeak eragin ditzaketela ondorioztatu zuten autoreek.

Habitata

Muturluzearen habitataren lehenengo deskribapen ezagunak (Trutat, 1891) ibai txikiak, erroten kanalak eta zelai zingiratsuak nahiago zituela eman zuen aditzera. Cabrerak (1914) muturluzeak erreka eta ibai handien ondoan bizi direla eta ibai-ertzeko arrakala eta barrunbeetan edo ur-arratoiek irekitako zuloetan babesten direla azaldu zuen. Peyrek (1956), espezie honen habitataren lehen deskribapen osoan, Pirinioetako muturluzea edozein ibilgu motatan bizi dela azaldu zuen, mendiko erreka txikietatik ibai handietara, baita erroten kanaletan ere, baldin eta bi baldintza betetzen badituzte: lehena, ur erregularra, hotza eta oxigenatua, faunan oso aberatsa, funtsean intsektuen larbez eta krustazeoz osatua, eta bigarrena, babesleku natural edo artifizialen presentzia. Peyrek muturluzearen jokabidea ere deskribatu zuen, bere jarduera handia eta ohitura gautarrak nabarmenduz, nahiz eta egunean zehar bazka bilatzeko txango azkarrak egin ditzakeen, bereziki ugalketa-garaian. Hark azaldu zuen muturluzeek oso igeri gutxi egiten dutela ur azalean, eta horren ordeztu murgilaldi luzeak egiten dituztela, non hondoon zehar tronparekin harri txikiak altxatzen edo harean aztarrika egiten ibiltzen direla dirudien.

Azken hamarkadetan modelo desberdinak egin izan dira muturluzearen presentzia baldintzatzen duten faktoreak argitze aldera. Barbosa eta kideek (2009) Espainia, Portugal eta Iberiar Penintsulako muturluzearen banaketarentzako lehenengo modelo aurkeztu zuten. Ondoren, Morueta-Holme eta kideek (2010) muturluzearen Espainiako banaketa modelizatu zuten klima aldaketak horren gainean nola eragiten duen ikusteko. Morueta-Holme eta kideek esan zuten gaur egungo klima, eta bereziki uraren balantzea eta udarako tenperatura direla espeziearen banaketaren mugatzaile nagusiak, nahiz eta ziur aski eskala handiagoan sakabanatzeak ere banaketa izugarri mugatuko duen. Horren ostean Frantzia egindako modeloetan oinarrituriko ikerketa berri batean, ordea, Williams-Tripp eta kideek (2012) prezipitazioari eta ez tenperaturari loturiko faktoreen garrantzia nabarmendu zuten. Hiru

urte beranduago, Frantziar Pirinioetako datuak aztertuz, Charbonnel eta kideek (2015) emariaren aldaketek, substratuaren heterogeneotasunak eta babeslekuen presentziak muturluzeen gorotzen detektagarritasunean eragina izan zezaketela iradoki zuten, muturluzeen habitaten okupazioa eragiten zuen faktore nagusia emaria zen bitartean. Haiek emari altua ornogabeen ugaritasunarekin lotuta dagoela, zeinak Pirinioetako muturluzearen harrapakin nagusiak diren, eta emariaren garrantziak urlasterrekiko zaletasuna iradokitzen duela eztabaidatu zuten.

Tesi hau hasi baina lehenago argitaratutako modeloetan oinarrituriko ikerketa guztiek bereizmen espazial eskasa zuten eta muturluzeen gorotzak detektatzea posible zen erreka-tarteetako datuetan soilik oinarritzen ziren. Ikerketa horien emaitzek erreka-tarte bakoitzeko substratuen heterogeneotasunaren eragina izan dezakete eta ez dute mikrohabitat mailako muturluzeen zaletasun espaziala ez eta espazioaren erabileraren intentsitatea bereizten uzten. Aurretiaz egindako irati-telemetry lanei dagokienean, haiek alderdi sozialetan jartzen zuten arreta habitataren erabileran jarri beharrean, eta beraz ez zuten muturluzeen tokiko baldintzen deskribapena egitea ahalbidetzen. Tesi hau hasi zenetik modeloetan oinarrituriko lan gehiago argitaratu dira: lehenengo, Biffi eta kideek (2016) Frantziar Pirinioetako hiru eskualde hidrologikoetan muturluzeez okupatutako ibai-tarteetan tokiko eskalan neurtutako habitat faktoreen inguruko informazioa eskaini zuten, Charbonnel eta kideek (2016) Frantziar Pirinioetako muturluzeen banaketa historikoa eta gaur egungoa konparatu zituzten bitartean; ondoren Quaglietta eta kideek (2018) bi garaien arteko iraungitze tasa neurtu zuten Portugalen; eta duela gutxi Biffi eta kideek (2019) habitata ezaugarritu dute tokiko eskalan Frantziar Pirinioetan. Hala ere, ikerketa horietako bakarrak ere ez du muturluzeen bizi-eremuen baitako mikrohabitat desberdinen erabileraren intentsitatea aztertu. Ikerketa hauek 2. eta 6. Kapituluetan eztabaidatu dira.

Bazka-ekologia

Zuzeneko behaketetan oinarrituriko lehenengo ikerketek (Cabrera, 1914) muturluzea nagusiki intsektuez bazkatzen zela esan zuten, nahiz eta arrainak eta krustazeoak ere lortu nahi izaten zituzten. Urdailaren edukien lehenengo analisisiek (Puissegur, 1935) intsektuen larbak (segur aski Plecoptera eta Trichoptera), intsektu txikiak (bereziki lurtarrak) eta krustazeoak identifikatu zituzten, baina ez arrainen hondarrak. Peyrek (1956) antzeko emaitzak lortu zituen eta Gammaridoak dietaren oinarria zirela ondorioztatu zuen, urte osoan zehar eskuragarri zeudelako. Gatibutasunean zeuden muturluzeez bareak eta irin-zomorroak (*Tenebrio molitor*), eta baita amuarrainak ere jaten zituztela esan zuen, zeinak guztiz jaten dituzten, hondarrik utzi gabe. Richard eta Viallardek (1969) gatibutasunean muturluzeez fauna urtar txikia —gastropodoak barne—

nahiago zutela esan zuten, baina harrapakin lurtarrak ere onartzen zituztela, zizareetatik artropodoetara. Ikertzaile haiek muturluzeek akuario guztiaren hondoa nola miatzen zuten deskribatu zuten, ahal izanez gero harrapakin txikiak baino handiak (5 vs. 15 cm) eta ez-mugikorrek baino mugikorrek hobetsiz, eta horiek uretatik kanpo janez. Muturluzeek jandako bazka-kantitatea oso handia zen, horrelako intsektiboro txiki batean esperoko litzatekeen bezala.

Santamarina eta Guitianek (1988) Galiziako zortzi muturluzeren hesteetako edukiak aztertu zituzten eta maizen jandako harrapakinak Ephemeroptera (aleen %35,8), Trichoptera (%32), Diptera (%24) eta Plecoptera (%4,3) zirela ikusi zuten. Kontsumitutako bolumenak neurtzerakoan, ordea, Trichoptera (%67,4) eta Ephemeroptera (%20,7) izan ziren talderik garrantzitsuenak. Baetidae, Leptophlebiidae, Simuliidae eta Limnephilidae ziren familiarik ohikoenak, azken familiarik izanik bolumenean ehuneko balio altuenak zituztenak. Jandakoa eskuragarri zutenarekin konparatuz, Santamarina eta Guitianek muturluzeek moluskuak eta kakalardo urtarrak (Dryopidae eta Elmidae) negatiboki hautatzen zituztela adierazi zuten. Bertrandek (1992) urtebetez Frantziar Pirinioetan bildutako 521 heste laginen emaitzak argitaratu zituen. Jandako taldeak Amphipoda, Ephemeroptera, Plecoptera, Trichoptera, Coleoptera eta Diptera izan ziren, eta euren maiztasunak urtaroen artean aldatu ziren. Muturluzeek harrapakin bentikoak, berezko energia balio altua zuten harrapakinak (estalkirik gabeko Triopteroak, vs. Limnephilidae) eta esklerifikazio maila baxuko harrapakinak hautatu zituzten positiboki. Santamarinak (1992) Galizian 2 heste aztertu zituen udan, 2 udazkenean eta 2 neguan. Trichoptera izan zen nagusi dietan, eta segidan Ephemeroptera, Amphipoda, Annelidae, Diptera eta Odonata. Santamarinak (1993) muturluzeek udaran nagusiki Sericostomatidae eta *Ephemerella* jaten zutela azpimarratu zuten, udazkenean Gammaridae, Lumbricidae, Philopotamidae eta Tipulidaera aldatuz, eta neguan Sericostomatidae, Limnephilidae, Lepidostomatidae eta Hydropsychidaera. Bestalde, ikertutako urtaro guztietan muturluzeek Hydrobiidae, Elmidae eta Chironomidaerekiko hautespen negatiboa erakutsi zutela, eta horiez gain udazkenean Ephemerellidae eta neguan Leptoceridae ere sailhesten zituztela behatu zuen. Castián eta Gosálbezek (1995) Nafarroako 46 heste lagin aztertu zituzten eta harrapakin garrantzitsuenak Ephemeroptera (Heptageniidae), Trichoptera (biomasan garrantzitsuenak), Diptera (Simuliidae, Chironomidae, Limoniidae eta Athericidae) eta Plecoptera zirela topatu zuten. Ephemeroptera eta Trichopteraren kontsumoa gutxi aldatu zen urtaroen artean, Plecoptera udazkenean eta udaberrian, eta Diptera neguan harrapakin ohikoagoak ziren bitartean. Espezie lurtarrak oso urriak izan ziren muturluzeen dietan (< %4). Castián eta Gosálbezek (1995) muturluzea nagusiki oso mugikorrek ez diren (Trichoptera) ornogabe handiez, eta txikiak izan arren ugariak diren beste taxon batzuek (Ephemeroptera larbak) bazkatzen den espezie estenofago gisa deskribatu zuten. Euren laginetan ez zuten arrairik identifikatu.

Pasadizo gisa, Quaglietta eta Bejak (2019), Portugalen, erdi-gatibutasunean, muturluzeek amuarrainak eta igelak harrapatzen zituztela azaldu zuten.

Metabarcoding bidezko dieta azterketak

Berriki, DNAREN Errendimendu Altuko Sekuentziazioa (EAS —*HTS, High Throughput Sequencing*—) bezalako metodo molekularrak iraultzaileak izan dira dieta azterketetan, gorotzetako DNAtik jandako aleak espezie mailan identifikatzea ahalbidetzen duelako, baita ikusmenaz bereizi ezin diren hondarretatik ere (Clare eta kideak, 2009; Valentini eta kideak, 2009; Bohmann eta kideak, 2014; Pompanon eta Samadi, 2015). Gillet eta kideak (2015) lehenak izan ziren DNA metabarcoding-a muturluzearen dieta aztertzeko erabiltzen. Frantziar Pirinioetako 16 gorotz laginen azterketak 11 familia eta 4 ordenetako (Diptera, Ephemeroptera, Plecoptera eta Trichoptera) 19 harrapakin espezie identifikatzea ahalbidetu zuen. DNA metabarcoding-aren abantaila nagusi bat Pirinioetako muturluzea gorotzen egile gisa identifikatzea ere ahalbidetzen zuela izan zen.

Metodo molekular horiek, ordea, zenbait muga dituzte. Alde batetik, DNA hesteetan zeharreko trantsizioaren bidez, zein gorotzak ingurumeneko egoeretara esposizioan egotearen ondorioz, degradatu egiten da (Deagle eta kideak, 2010; McInnes eta kideak, 2017; Oehm eta kideak, 2011; Symondson, 2002). Horrez gain, metodo molekularren sentikortasun altuak positibo faltsu ugari sortu ditzake bigarren mailako harraparitzaren, sarraski-janaren edo bestelako jatorrizko kutsaduraren ondorioz (Sheppard eta kideak, 2005). Hare gehiago, DNA metabarcoding-aren emaitzak erabilitako hasleen, PCR estrategien, sekuentziazio prozesuaren, erabaki bioinformatikoen edo datu-baseetan eskuragarri dagoen informazioa bezalako xehetasun metodologikoen arabera aldakorrak dira (Alberdi eta kideak, 2018; Brandon-Mong eta kideak, 2015; Divoll eta kideak, 2018; Piñol eta kideak, 2019). Dieta azterketetako positibo faltsuek eta negatibo faltsuek ondorio sendoak izan ditzakete harrapariaren ekologiaren interpretazioan zein ekosistemen kudeaketan (Hines eta Gessner, 2012). Hortaz, harrapakin taxon garrantzitsuen identifikazio arrakastatsua gako da harrapariaren paper ekologikoaren eta espezializazio trofikoaren inguruko zentzuzko ondorioak lortzeko (Schwarz eta kideak, 2018; Waraniak eta kideak, 2018).

Tesi hau hasi zenean metodo horiek ez ziren muturluzearekin erabiliak izan oraindik, eta beraz, ezagutza gutxi zegoen eskala fineko bere ekologia trofikoaren inguruan, zeina espeziearen kontserbaziorako gako izan zitekeen. Tesia egin bitartean, ordea, zenbait ikerketa argitaratu ziren: lehenengo, Biffi eta kideak (2017a) muturluzeen dieta deskribatu zuten Frantziar Pirinioetan, 383 gorotz

aztertzeke Gillet eta kideek (2015) probatutako hasleak erabiliz; ondoren, Biffi eta kideek (2017b) Frantziar Pirinioetako beste 184 gorotz lagin aztertu zituzten muturluzearen eta ur-satitsuaren (*Neomys fodiens*) dietak konparatzeko; eta berriki, Hawlitsch eta kideek (2018) muturluzearen Zamorako (Espainia) 41 gorotz aztertu dituzte, hasle desberdin batekin. Ikerketa hauek 4., 5. eta 6. Kapituluetan eztabaidatu dira.

Interakzioak beste espezie batzuekin

Muturluzeek habitata ur-satitsuarekin (*Neomys fodiens*), ur-zozoarekin (*Cinclus cinclus*) eta amuarrain arruntarekin (*Salmo trutta*) partekatu arren, euren dietak ez direla asko gainjarriko aurreikusi da (Santamarina, 1993). Ur-satitsuarentzako berriki egindako lanek egiaztatu egin dute aurrikuspina (Biffi eta kideak, 2017b). Bestalde, muturluzearen harrapariak dira igaraba arrunta (*Lutra lutra*, Adrian eta kideak, 1988; Callejo eta Delibes, 1987), lertxun hausgara (*Ardea cinerea*; González eta Román, 1988), amiamoko zuria (*Ciconia ciconia*; Hernández, 1988), zapelatz arrunta (*Buteo buteo*; Bustamante, 1985), urubi arrunta (*Strix aluco*; Nores, 2012) eta hontza zuria (*Tyto alba*; González eta Román, 1988; Purroy eta kideak, 1988). Amerikar bisoiak (*Neovison vison*) muturluzeen populazioetan sarraskia eragin duela uste da (Nores eta kideak, 2007), nahiz eta ez dagoen horren frogaririk. Azkenik, muturluzearen bizkarroien artean akaroak (*Eadidea desmanae*; Luckoschus, 1969), trematodoak (*Omphalometra flexuosa* var. *peyrei*, Vaucher, 1975; *Maritrema pyrenaica*, Jourdan, 1979; *Matovius galemydis*, Mas-Coma eta kideak, 1985) eta nematodoak (*Aonchotheca galemydis*, Álvarez-Mascato, 1991; *Paraucaria hispanica*, Álvarez eta kideak, 1994) daude.

Filogenia eta taxonomia

Geoffroy Saint-Hilairek (1811) Pirinioetako muturluzea *Mygale pyrenaica* gisa izendatu zuen. Horren ostean, Kaupek (1829) *Galemys* generoaren baitan ber-sailkatu zuen. Azkenik, Millerek (1912) finkatu zuen gaur egungo nomenklatura. Orain, muturluzeak Eulipothyphla ordenan, Talpidae familian eta Desmaninae subfamilian bilduta daude.

Datu molekularren arabera, muturluzearen eta sator arruntaren erroak duela 37 Mu bereizi ziren (Douady eta Douzery, 2003), Zenoziokoaren ekuatorean, izotz aro txiki batean, Eozenoaren amaierako garai hotz handienaren atarian. Banaketa horrek, muturluzeek espezializazio urtarrean aurrera eta bizimodu industrialean atzera egin zutela adierazten du. Fosilen erregistroak erakusten duenez, Miozenotik —duela 9,0 eta 5,3 Mu artean— Europa eta Asian muturluze

espezie pila egon ziren (Rümke, 1985).

Gaur egun, bi muturluze espeziek soilik diraute bizirik: Pirinioetako muturluzeak eta Errusiar muturluzeak (*Desmana mochata*, Linnaeus (1758)). Espezie horiek Desmaninae subfamilia osatzen dute (Cabria eta kideak, 2006) eta duela 10,05 Mu banatu zirela dirudi (Colangelo eta kideak, 2010). Beraien banaketak disjuntuak dira, eta erraza da espezieak euren artean bereiztea (Palmerim eta Hoffmann, 1983). Errusiar muturluzea handiagoa da, isatsa luzera guztian zehar du zanpatua, eta proportzionalki mutur laburragoa du.

Banaketa

Galemys fosilak Pliozenoaren amaieratik —duela 5,33 eta 2,58 Mu artean— Europa ia osoan dira ezagunak, Poloniatik Portugalera eta Hungariatik Ingalaterrara (Harrison eta kideak, 1988; Rümke, 1985). Genero honen banaketa, ordea, hego-mendebaldera murriztua izan zen Pleistozenoaren hasieran —duela 2,58 eta 0,78 Mu artean— (Arribas, 2004), eta gaur egun *Galemys pyrenaicus* eskualde Iberiar-Piriniarrera dago mugatuta. Kontrazio hori muturluze txikiek mendiko erreketan bizitzeko emandako espezializazioaren hazkuntzarekin azaldu liteke (Nores, 2012), segur aski Europako beste mendiek bezainbesteko glaziazio prozesua jasan ez zuen Iberiar Mendietan babeslekua topatu zutelako.

Azken hamarkadetan muturluzearen banaketa %50 baino gehiago murriztu da Iberiar Penintsulan eta Pirinioen iparraldean (MAGRAMA, 2013; Charbonnel eta kideak, 2015). Gaur egun Iberiar Penintsularen iparraldeko eremu menditarretara mugatzen da bere banaketa, Sistema Zentraletik eta Serra da Estrelatik Iberiar Sistemara eta Pirinioetara (Nores eta kideak, 2007). Euskal Herrian, Iberiar Penintsularen iparraldean, tesi hau egin den eremuan, Leitzarán, Urumea, Bidasoa, Larraun, Basaburua, Ultzama, Arga, Irati, Salazar eta Ezka erreketan ageri dira muturluzeen populazioak (Castién eta Gosálbez, 1992). Espeziearen egoera kaskarra da, kutsadura gutxitzeari esker kontserbazio egoerak hobera egin duen erreketan ere txikiagotu egin baitira bertako populazioak (Ihobe, 2012; Rubio, 2016; URA, 2017).

Mehatxu-faktoreak

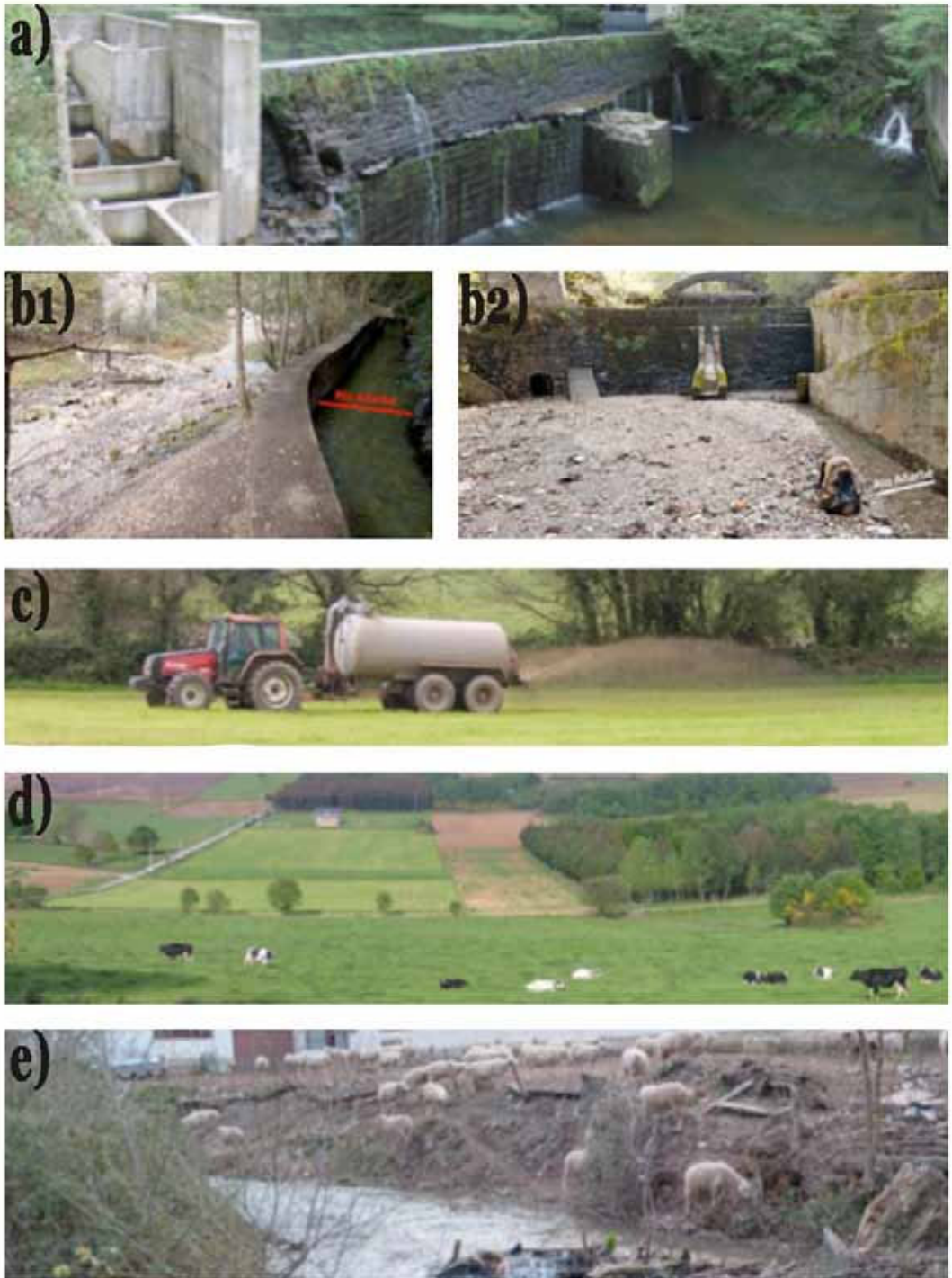
Muturluzearen kontserbaziorako mehatxu faktore nagusi gisa populazioen zatiketa, habitataren degradazioa eta bazka eskasia identifikatu dira (Nores, 2012), baina giza jarduera desberdinen ondorioak dira horiek (5. Irudia). Zatiketa eragile bat presak eta urtegiak dira. Muturluzeek ez dituzte erabiltzen

edo gutxi erabiltzen dituzte urtegiak, eta horiek emarien erregimenean dituzten efektuek errekan behera tarte luzeetan izan dezakete eragina. Muturluzeak bizi diren eremu menditar askotan ur-erazketa jarduera gehigarri bat da, bereziki instalazio hidroelektriko txikiekin lotua, eta desbiderapen-kanalen eraginpean dauden erreka tarte luzeetan izugarri emari baxuak egotea eragiten du (MAGRAMA, 2013; Queiroz eta kideak, 1996). Horrez gain, erazketa-kanalak heriotza-eragile garrantzitsuak izan daitezke muturluzeentzako (Chora eta Quaresma, 2001; Elozegi, 2010). Ibaien kanalizazioek eta rip-rap zein bestelako uholdeen defentsarako obra zibilek ere eraginak dituzte ibilgu eta ibai-ertzetan, habitataren narriadura eta erreken heterogeneotasunaren galera eraginez, eta muturreko egoeretan erreka tarteak guztiz desegokiak bihurtzea eragin dezakete. Ibaitezko landarediaren suntsiketa ere jarduera kaltegarri gisa aipatua izan da, habia egiteko lekuak murrizten dituelako, uraren tenperatura emendatzen duelako eta ornogabeen emankortasunean eraginak izan ditzakelako (Queiroz eta kideak, 1996). Uraren kutsadura mehatxu faktore bat izan daiteke, jakina, bereziki nekazaritza intentsiboaren eta produktu fitosanitarioen erabileraren ondorioz (kutsadura lausoa), zein hirigune, etxalde edo industriari loturiko kutsatzaileen jatorri puntualeko ekarpenaren ondorioz. Azken hamarkadetan, muturluzeen jatorrizko helmenaren gune askotan ur-kutsadura murriztu bada ere, konplexuagoa ere bihurtu da, ibai eta erreka askotan kutsatzaile berrien kopuru handiak topatu baitira (Petrovic eta kideak, 2016). Leku batzuetan uretako kirolak muturluzearentzako faktore kaltegarri gehigarri bat izan daitezke, janariaren ekoizpenean eragin dezaketelako edota nahi gabe animaliak molestatu ditzaketelako. Txintxarraren erazketa ere garrantzitsua izan daiteke, bereziki mendien behealdeko ibaien txirikordatutako guneetan, ibilguaren forman eta egonkortasunean eragin larriak izan ditzakelako (Brown eta kideak, 1998). Jakina da legez kontrako arrantzak ere muturluzeen ustekabeko heriotzak eragin ditzakela, trasmallo sareetan eror daitezkeelako (Queiroz eta kideak, 1996) edo ibaiak hipokloritoarekin pozoituta. Gainera, kanaberarekin arrantzatzen duten arrantzale batzuek, muturluzea amuarrainen stockentzako mehatxua delakoaren ustetan, harrapatu izan dute (Fernandes eta kideak, 2008). Bestalde, bildumazale amorratuen zuzeneko jazarpena mehatxua zen (Richard, 1976). Espezie inbaditzaileek, Amerikar bisoiak (*Neovison vison*) bereziki, eragin negatiboak izan ditzakete muturluzeen populazioetan (Fernandes eta kideak, 2008). Azkenik, klima aldaketa biodibertsitate guztiari, eta baita ur-gezatako espezieei ere, eragiten dion mehatxua da.

Kontserbazio-egoera

Atzerakadaren joera

Espezie honen populazio-tamainaren eta gainbehera-tasaren kalkulu zehatzak



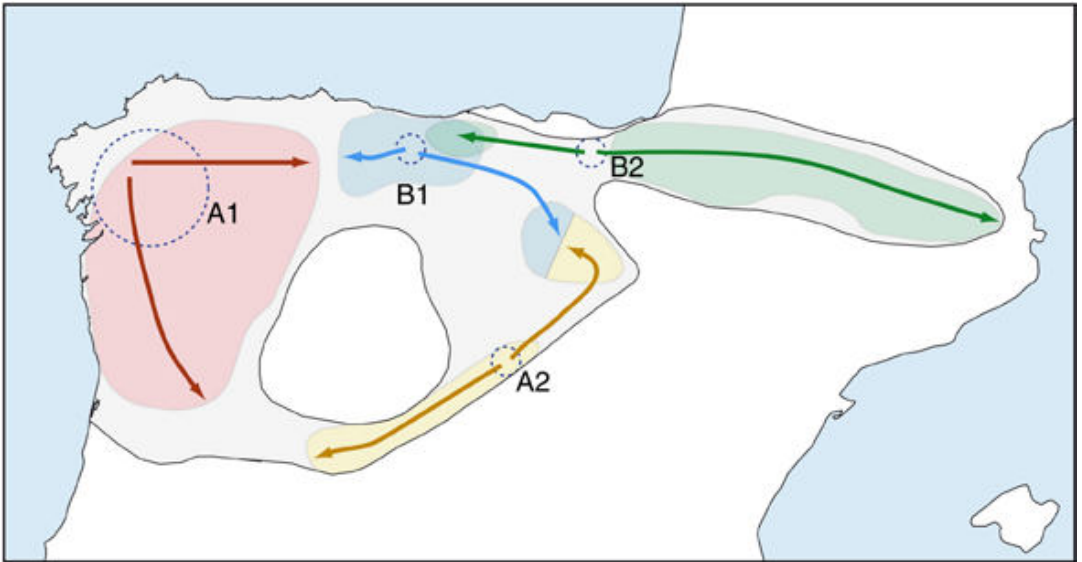
5. Irudia. Mehatxu faktore batzuen adibideak. (a) Presa bat Leitzaran ibaian; (b) Añarbe errekaen tarte bat ia guztiz lehortzen duen ur erauzketa bat; (c) Ullako nekazaritza intentsiboa; (d) Miñon giza erabilerarako lurren aldaketa; (e) ibai-ertzaren suntsiketa Tobian. Argazkiak: Jorge González-Esteban eta Amaïur Esnaola.

lortzea zaila bada ere (González-Esteban eta kideak, 2003a), kliman eta hidrogian oinarrituriko modeloek muturluzeen banaketa potentzialak txikiagotzen jarraituko duela iradokitzen dute (Araújo eta kideak, 2011). Gainera, Charbonnel eta kideek (2016) azken hiru hamarkadetako Pirinioetako muturluzearen banaketaren atzerakada klimaren, hidrogaren eta lurren erabileraren aldaketen bidez azaldu daitekeena baino askoz azkarragoa izan dela esan zuten. Horrek argi eta garbi erakusten du goian zerrendatutako horiek bezalako beste faktore batzuek eragin handia dutela espeziean.

Populazioen genetika

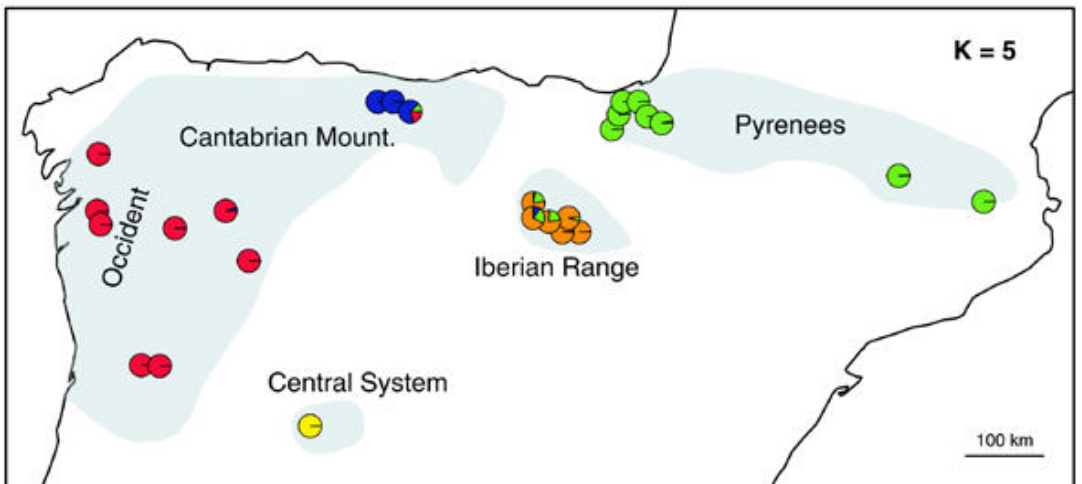
Fosilen hondarretatik deskribatutako lau *Galemys* espezieetatik (Rümke, 1985) *G. pyrenaicus* bakarrik gelditzen da bizirik. Nahiz eta zenbait autorek (Cabrera, 1914; Ellerman eta Morrison-Scott, 1951) bi subespezie bereizi izan dituzten (*G. p. pyrenaicus* Pirinioetan eta Kantauriar mendikatean eta *G. p. rufulus* Sistema Zentralean), azken ikerketek gezurtatu egin dute (González-Esteban eta kideak, 1999). Aitzitik, muturluzeak bi talde nagusitan multzokatzen diren muga geografiko argiko eta parapatria nabarmeneko lau klado genetikotan banatzen dira (6. Irudia; Igea eta kideak, 2013): A1 kladoa Iberiar Penintsulan ageri da, Portugaletik Galiziara eta Kantauriar mendikatearen erdialdera; A2 kladoa Sistema Zentralean eta Iberiar Sistemaren hegoaldean; B1 kladoa Kantauriar mendikatearen erdialdetik Iberiar Sistemaren iparraldera; eta B2 kladoa Kantauriar mendikatearen ekialdeko mendi-hegaletik Pirinioetara. Populazio hauetan topatutako dibertsitate genetikoaren balioak bat datoz glaziazio osteko Holozenoan babesleku glaziarretatik eremu desberdinetara emandako berkolonizaziotik esperotako patroiekin. Kontaktu-eremuetan ez ziren klado horien arteko trukeak behatu, nahiz eta ez dirudien muturluzeen sakabanatzeari eragingo zion ageriko oztoporik zegoenik (ikus ere Querejeta eta kideak, 2017).

Definitutako kladoen inguruan ordea desadostasuna dago. Querejeta eta kideek (2016) bost talde genomikotan oinarrituriko populazio-egitura posible bat aurkeztu zuten, mendikate nagusiekin bat egiten zuena: Pirinioak, Mendebaldea, Kantauriar Mendiak, Iberiar mendikatea eta Sistema Zentralea (7. Irudia). Datu nuklearrekin eraikitako taldeek nahiko ondo egiten zuten bat Igea eta kideen (2013) lerro mitokondrialen lehenengo banaketarekin (A eta B), baina desberdintasun aipagarri batzuk zituzten goi-mailako banaketetan. Hare gehiago, Frantziar Pirinioetan zentratuz eta datu nuklearrak erabiliz, Gillet eta kideek (2017) Igea eta kideek (2013) deskribatutako B2 kladoaren baitan genetikoki eta geografikoki desberdinak ziren hiru talde topatu zitezkeela erakutsi zuten (ekialdekoa, erdialdekoa, eta mendebaldekoa). Gillet eta kideek (2017) Frantziako taldeetan behatutako aniztasun genetikoaren maila baxuek —bertako endogamia koefiziente altuek eta populazioen tamaina-eraginkor txiki



6. Irudia. Pirinioetako muturluzearen *Galemys pyrenaicus* historia ebolutiboaren eta Igea eta kideek (2013) zehaztutako lau kladoen irudikapen eskematikoa. Eremu grisak espeziearen banaketa historikoa adierazten du, lau lerro mitondrialen gaur egungo eremuak kolore desberdinez erakutsi diren bitartean. Glaziazio garaiko babesleku hipotetikoak puntuzko zirkuluekin irudikatuta daude. Zirkuluen tamainak babeslekuaren garrantzi erlatiboa adierazten du eta geziek horietatik emandako kolonizazio-bideak seinalatzen dituzte. Igea eta kideen (2013) lanetik berreskuratutako irudia.

samarrek nabarmendu dutenez— eta heterozigotoen eskasiak, espeziearen iraungitze-arriskua hazten ari dela iradokitzen dute.



7. Irudia. Querejeta eta kideek (2016) proposatutako bost taldeak, neurri handi batean espeziea agertzen den mendikate nagusiekin bat egiten dutenak. Banakoak sektore-diagramen bidez adierazi ziren eta horietako bakoitzaren nahasketa-proportzioak kolore desberdinen bidez trazatu ziren. Ilundutako eremuak iturri desberdinen arabera espeziearen gaur egungo banaketa irudikatzen du. Occident = Mendebaldea, Cantabrian Mount. = Kantauriar mendikatea, Central System = Sistema Zentrala, Iberian Range = Iberiar mendikatea, Pyrenees = Pirinioak. Querejeta eta kideen (2016) lanetik berreskuratutako irudia.

Babes legala

Pirinioetako muturluzea Bernako Hitzarmenaren (II. Eranskina) (OJEC, 1982) eta Europako Habitaten Zuzentarauaren bidez (II. Eta IV. Eranskinak) (OJEC, 1992) babesturik dago. Pirinioetako muturluzearen populazioen gainbeheraren eraginez zerrenda gorri desberdinetan barneratzea ekarri zuen. Gaur egun, Zaurgarri gisa (A2ac+3c+4ac) zerrendatua dago Naturaren Kontserbaziorako Nazioarteko Batasunaren (IUCN) zerrenda gorrian (Fernandes eta kideak, 2008), Galzorian Euskal Autonomia Erkidegoan (EHAA, 2011) eta Interes Bereziko kategorian Nafarroan (Boletín Oficial de Navarra, 1995). Frantziar lurraldean 2007ko apirilaren 23ko ministro-ordenaren bidez (JORF, 2007) dago babestuta.

Tesi honen testuingurua

Pirinioetako muturluzearen egoera larria kontutan izanda, oraindik ezagumendu gabedia garrantzitsua zegoela uste izan zen, bereziki kontserbaziorako funtsezkoak ziren gaien inguruan, espeziearen ekologia espaziala eta trofikoak kasu. Ekologia espazialari zegokionean, muturluzeen habitataren inguruko azken lanek erreka tarteetan muturluzeen gorotzen presentziarekin lotutako tokiko habitat faktoreen inguruko informazioa eskaini zuten, baina ez zituzten mikrohabitat mailako okupazioa kontrolatzen zuten faktoreak identifikatu, zeina gakoa den espeziearen beharrak ulertzeko. Alde batetik, gorotz laginek espeziearen presentzia datuak soilik eskaintzen dituzte —negatibo faltsuak barne—, baina ez dute habitat mota desberdinen erabileraren intentsitatea neurtzea ahalbidetzen. Bestalde, ordura arteko ikerketek muturluzearen banaketak ingurumeneko faktore desberdinekin zuen lotura aztertu zuten — baliteke horietako batzuek elkarren arteko korrelazioak izatea —, baina ez zituzten espeziearen mikrohabitat mailako banaketaren funtsezko faktoreak zehazten. Bestalde, hautespen-patroiak espazioan eskuragarritasuneko aldean arabera aldatzen zirenez (Ayllón eta kideak, 2010; Boyce eta kideak, 2016), euren habitat-zaletasunak eragiten dituzten faktore gakoak ulertzeko beharrezkoa da muturluzeez ingurune desberdinetan ematen dituzten jokabidezko erantzunak aztertzea. Ekologia trofikoari zegokionean, tesi hau baino lehen egindako lanek muturluzeez zein harrapakin talde nagusi jaten zituzten erakutsi zuten. Lan horiek ordea ez zuten bereizmen taxonomiko fineko informazioa eskaini, eta ez zituzten muturluzeen harrapakinak euren ezaugarri morfofuntzionalen eta morfoekologikoen arabera ikertu. Bestalde, muturluzearen moldapen/muga sakonagoekin lotuta egon zitekeen tokiko habitat-erabilera bazka eskuragarritasunaren edo beste faktoreren baten arabera zen argitzea erabakigarria zela iruditu zitzaigun.

Tesiaren helburuak

Goian aipatutako arrazoiketan oinarrituz, Doktorego Tesi honen helburua Pirinioetako muturluzearen kontserbaziorako ezagumendu zehatzagoa lortzeko bere ekologia espazial eta trofikoaren inguruko ideia bat ematea da. Tesiaren helburu zehatzak honakoak dira:

- 1) Muturluzearen bizi-eremuaren baitako bazka-habitat zaletasunak zehaztu, bere habitaten egokitasunean eragiten duten faktoreak identifikatu ahal izateko.
 - Habitat hautespena ematen ote den eta positiboki eta negatiboki zein habitat hautatzen dituen (urlasterrak, ur-bareak eta putzuak) frogatu.
 - Habitat hautespena bi ibai desberdinen artean desberdina ote den egiaztatu.
- 2) Dieta eta muturluzearen beraren identitatea modu ez inbasorean metabarcoding bidez zehazteko hasle desberdinak frogatu.
 - Gorotz laginetatik filogenetikoki anitza den dietaren eta harrapariaren identitatearen karakterizazioan hasle desberdinek nola eragiten duten ebaluatu.
 - Muturluzearen dieta zehazteko hasleen konbinazio egokiena zehaztu.
- 3) Muturluzearen edozein habitat zehatzekiko zaletasunak bazka eskuragarritasunari edo bestelako arrazoi batzuei erantzuten dien frogatu, dieta eta hiru habitatetako (urlasterrak, ur-bareak eta putzuak) harrapakin eskuragarritasuna konparatuz.
 - Muturluzearen dieta deskribatu kontsumitutako espezieen mailan.
 - Muturluzearen harrapakin-hautespena deskribatu.
 - Kontsumitutako eta hobetsitako harrapakinen eskuragarritasunetan habitaten artean alderik ote dagoen aztertu eta alde horiek habitat-hautespena azaltzen ote duten ikusi.
 - Harrapakin-hautespena bi erreka desberdinen artean desberdina ote den egiaztatu.
- 4) Muturluzearen bazka-zaletasunak harrapakinen zein ezaugarrik (trait-ek) gidatzen dituen aztertu eta analisi horrek muturluzeen urlasterrekiko zaletasuna argitzen lagundu ote dezakeen frogatu. Horretarako, dieta eta hiru habitatetako (urlasterrak, ur-bareak eta putzuak) harrapakin

eskuragarritasuna konparatu, harrapakinak ezaugarri biologikoak erabiliz sailkatuta.

- Muturluzearen dieta deskribatu harrapakinen ezaugarriak erabiliz.
- Muturluzearen harrapakin-hautespena deskribatu harrapakinen ezaugarriak erabiliz.
- Harrapakinen ezaugarriak erabiliz kontsumitutako edota hobetsitako harrapakinetan habitaten artean alderik ote dagoen aztertu eta alde horiek habitat-hautespena azaltzea ahalbidetzen ote duten ikusi.
- Harrapakinen ezaugarriak erabiliz harrapakin-hautespena bi erreka desberdinen artean desberdina ote den egiaztatu.

2. Kapitulua



Ziztu-premia:
galzorian dagoen
Pirinioetako muturluze erdi-urtarraren
(*Galemys pyrenaicus*)
urlasterrekiko hautua
bi erreka desberdinetan

Argitalpena

Kapitulu honetan oinarrituriko artikulu bat *Aquatic Conservation: Marine and Freshwater Ecosystems* aldizkarian izan da argitaratua:

Esnaola, A., González-Esteban, J., Elosegi, A., Arrizabalaga-Escudero, A., eta Aihartza, J. (2018). Need for speed: Preference for fast-flowing water by the endangered semi-aquatic Pyrenean desman (*Galemys pyrenaicus*) in two contrasting streams. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28(3), 600-609.

Laburpena

1. Pirinioetako muturluzea edo ur-satorra (*Galemys pyrenaicus*) galzorian dagoen ugaztun intsektiboro erdi-urtarra da, Iberiar Penintsulako iparraldean eta Pirinioetan endemikoa dena. Populazioen dentsitate baxuak, jokabide iheskorak eta jarduera gautarrak bere beharrian ekologikoen ezagutza oraino mugatua izatea dakar. Banaketa-eremu ia osoan jasaten ari den populazioaren murrizketa etengabeak —baita uraren kalitateak nabarmenki hobera egin duen erreketan ere— kontserbaziorako mehatxu nagusia beste faktore batzuk direla iradokitzen du. Hala ere, okupatzen duten eremuaren baitako habitat zaletasunaren inguruko informazio gabezia dago gaur egun (adb. ibaien mikrohabitat mailako ezaugarriekiko), eta hau gakoa da haren habitata ebaluatu edo hobetzeko.

2. Ikerketa honek irrati-telemetria datuak erabili ditu muturluzeen mikrohabitat mailako espazioaren erabilera zehazteko, eta ingurumenaren baldintzen arabera erabilera nola aldatzen zen ikusteko.

3. Euskal Herriko (Iberiar Penintsulako iparraldea) bi erreka desberdinetan ikertu ziren muturluzeak: Elama, erreka ia ukitu gabea, eta Leitzaran, jarduera hidroelektrikoaren eraginpean dagoen ur garbiko ibaia. Hamabost eta hamasei muturluze harrapatu eta jarraitu ziren Elaman eta Leitzaranen, hurrenez hurren, irrati-telemetria bidez, eta gauean zeharreko jarduera puntuak hiru habitatetako bati esleitu zitzaizkion: urlasterrei, ur-bareei edo putzuei. Habitat-erabilera eskuragarritasunarekin konparatu zen erreka bakoitzeko eta erreken araberrako habitat-hautespena neurtzeko.

4. Muturluzeek positiboki hautatu zituzten urlasterrak eta negatiboki putzuak, Leitzaran ibaian hautespena sendoagoa izanik Elaman baino.

5. Urlasterrek muturluzeen bazkatzeko habitat gisa duten garrantzi ekologikoa nabarmentzen dute emaitzek, kontserbaziorako ezinbesteko baliabidea direla erakustez gainera. Urlasterren azalera murrizten duten ibilguaren moldaketek espezie honentzako habitat-kalitatean eragiten duela iradokitzen du horrek. Gainera, jarduera hidroelektrikorako ematen diren ur-erauzketak segur aski muturluzeentzako kaltegarriak izango dira, urez gabetutako erreka zatietan emaria eta ur-fluxuaren abiadura murrizten baita.

Hitz gakoak

erreka, galzorian dagoen espeziea, habitat-kudeaketa, hidroelektrika, jokabidea, ugaztunak, ur-satorra

Sarrera

Pirinioetako muturluzea edo ur-satorra *Galemys pyrenaicus* (Geoffroy, 1811; 1. Irudia) galzorian dagoen ugaztun intsektiboro erdi-urtarra da, Iberiar Penintsulako iparraldean eta Pirinioetan endemikoa dena (Palmerin eta Hoffman, 1983). Erreka hotzetan bizitzera espezializaturik dago, arraun-formako atze-hanka eta lateralki konprimatutako isats luzearekin, igeriketa eta murgilaldietarako moldatuta (Richard, 1986). Pirinioetako muturluzea Bernako Hitzarmenaren (II. Eranskina) eta Europako Habitaten Zuzentarauaren bidez (II. Eta IV. Eranskinak) babesturik dago (Council of the European Communities, 1992). Bere banaketa-eremua arras murriztua izan da azken hamarkadetan, eta horrek Naturaren Kontserbaziorako Nazioarteko Batasunaren (IUCN) zerrenda gorrian Zaurgarri gisa sartzeari ekarri du (Fernandes eta kideak, 2008). Beherakada honen atzean dauden arrazoiak ez daude argi, baina badirudi batez ere uren kutsadura, habitaten andeketa (kanalizazioak, ibaiertzen narriadura, emari aldaketak), eta ibaien fragmentazioaren gisako faktore antropogenikoekin lukeela lotura (Charbonnel eta kideak, 2016; Queiroz eta kideak, 1996; Williams-Tripp eta kideak, 2012). Azpimarratu behar da beherakada hori Euskal Herria bezalako herrialdeetan ere ematen ari dela (Ihobe, 2011; Rubio, 2016; Ura, 2017), bertan saneamendu eta ur-arazketa zerbitzuei esker kutsadura gutxitzea lortu den arren. Beherakada eragiten ari diren faktore potentzialak ur-kalitatea ez beste batzuk direla iradokitzen du horrek, zeinen artean habitat fisikoaren andeketa nagusitzen den.

Ehundaka metrotako luzera izaten dute muturluzeen bizi-eremuek eta ohikoena jarduera-erritmoa bimodala izatea da, egunean zeharreko jarduera-aldi



1. Irudia. Pirinioetako muturluzea (*Galemys pyrenaicus*) Elama errekan. Argazkia: Jorge González-Esteban.

labur batekin eta gauean zeharreko beste aldi nagusi batekin (Stone, 1985, 1987a). Gauean zeharreko bazka-ereduak, osterara, jarduera goreneko bi edo hiru aldi erakusten ditu (Melero eta kideak, 2014). Oraindik ez da ziurra ur-satorrak jokabide lurraldekoia aurkezten duen, ez eta banakoek gordelekuak euren artean sexuarekiko, adinarekiko edo bizi-eremu berean dauden banako kopuruarekiko modu independentean partekatzen dituzten ere (Melero eta kideak, 2012). Gordeleku gisa, muturluzeek batez ere arroken arteko, harrizko hormetako edo ibai-ertzeko zuhaitzen sustraipeko zulo naturalak erabiltzen dituzte; hauek ez dute kanpotik identifikatu ahal izateko ezaugarri nabarmenik izaten (Stone, 1987a). Ornogabe urtarrez bazkatzen dira batez ere (Biffi eta kideak, 2016; Santamarina eta Guitian, 1988). Faktore asko daude ur gezatako ornogabeen ugaritasun eta konposizioa baldintzatu dezaketenak, hala nola kutsadura, lehortea, edo habitat fisikoaren aldaketak (Cowx eta kideak, 1984; McIntosh eta kideak, 2002; Wood eta kideak, 2000). Ornogabeek multzokako banaketa izan ohi dute ibaietan, urlaster, ur-bare eta putzuen gisako habitat moten (Dewson eta kideak, 2007) zein sedimentuko bikorren tamainaren baitan (Elosegi eta kideak, 2011) aldatzen baita hauen konposizioa eta ugaritasuna. Hortaz, erreketako ornogabeen ugaritasun eta dibertsitateak okerrera egin dezakete ur-erazketen gisako estres-eragileen ondorioz (McIntosh eta kideak, 2002).

Charbonnel eta kideek (2015) erreken emariak eta substratuen heterogeneotasunak muturluzeen okupazioan eragin positiboa zutela iradoki zuten, Frantziako Pirinioetako arro batean aplikatutako okupazio eredueta oinarrituta. Muturluzeak erreofiloak dira, antza, ur-fluxu bizkorak hobesten baitituzte, eta horregatik diogu “ziztu-premia” dutela. Charbonnel eta kideek (2015) proposatu zuten jokabide hori urlasterretako ornogabeen ugaritasun eta aberastasun handiagoak eragindakoa zela, Richard-ek (1986) azalpen mekanikoagoa proposatzen zuen bitartean, uraren abiadura handiak animalari bere flotagarritasun naturalari aurre egiten lagunduko ziolakoan. Morueta-Holme eta kideek (2010) emariaren garrantzia azpimarratu zuten, ur gehiago izateak habitat baldintza egokiagoak sortu eta bazka-eskuragarritasuna emendatzen dituelako. Biffi eta kideek (2016) eskala lokalean muturluzeek okupatutako ibai-tarteetan neurtutako habitat faktoreen inguruko informazioa eskaini zuten, baina ez zuten mikrohabitat mailan okupazioa kontrolatzen zuten faktoreen inguruko informaziorik eman: azken hori ezinbestekoa da muturluzeen beharrianak ulertzeko. Bestalde, espazialki habitat-eskuragarritasunaren arabera habitat-hautespen patrioiak aldatzen direla ikusirik (Ayllón eta kideak, 2010; Boyce eta kideak, 2016), muturluzeen habitat-hautespenean eragiten duten faktore gakoak hobeto ezagutzeko beharrezkoa da muturluzeen jokabidezko erantzunak ingurune desberdinetan ikertzea. Orain arteko ikerketa gehienek bereizmen espazial eskasa erdietsi dute, ingurumeneko datuak ez baitira landan bertan bildu. Horrez gainera, Pirinioetako muturluzearen habitataren ikerketa ia denek

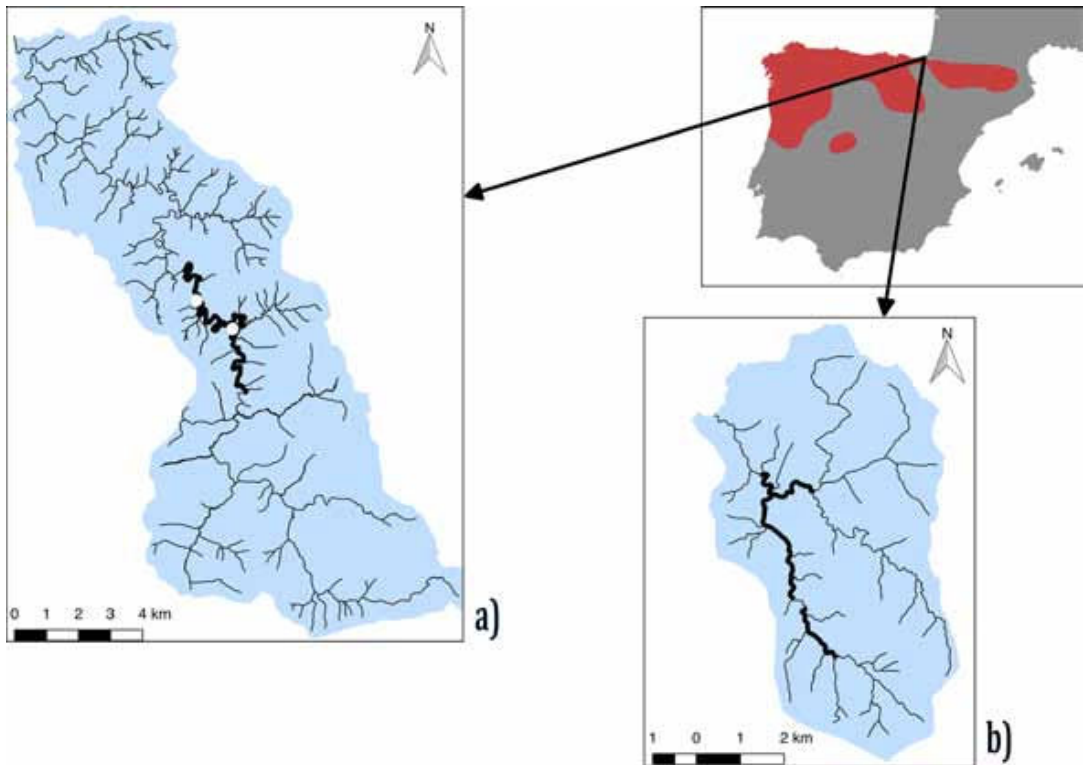
gorotzen detekzioa erabili dute presentziaren arrasto gisa (Barbosa eta kideak, 2009; Charbonnel eta kideak, 2015, 2016; Morueta-Holme eta kideak, 2010; Williams-Tripp eta kideak, 2012). Horietako ikerketa bakarra ere ez da irrati-telemetria datuak erabiliz egin, eta honek beharrezko informazio berritzailea eskaini lezake banakoak identifikatu eta mugimenduen jarraipenak egiteko.

Ikerketa honen helburua Pirinioetako muturluzearen bizi-eremuaren baitako bazka-habitataren lehentasunak ikertu eta habitataren egokitasunean eragiten duten faktoreak identifikatzea zen. Honakoa proposatu zen: (i) muturluzeek urlasterrak dituzte nahiago, bazka-eskuragarritasunari dagokionean emankorragoak direlako (Dewson eta kideak, 2007) eta harrapari flotatzaile batentzako bazkatzea errazagoa delako bertan; eta (ii) habitat lehentasun patriiak, edo habitat-hautespenaren intentsitatea, errekaen ezaugarriekin aldatuko dira. Ondorioz, ikerketa hau bi erreka desberdinetan egin zen: Elaman, ia ukitu gabeko erreka, eta Leitzararen, jarduera hidroelektrikorako ur-erazketen eraginpean dagoen ibaia.

Metodoak

Ikerketa-eremua

Ikerketa hau Iberiar Penintsularen iparraldeko mendiko bi erreketan eraman zen aurrera (Euskal Herrian; 2. irudia). Europar Batasunaren Natura 2000 sarearen baitan babestuta daude biak (Council of the European Communities, 1992), eta biek dute egoera ekologiko ona Uraren Europako Zuzentarauaren arabera (Council of the European Communities, 2000), baina ingurumen-presio desberdinak pairatzen dituzte. Elama (3. irudia) bigarren ordenako mendiko erreka bat da, biztanlerik gabeko granito eta eskistozko 1415 ha-ko arroa estaltzen duena, 1919-tik natura-erreserba gisan zorrozkari kudeatua izan dena, eta ondorioz pago eta haritzez osaturiko baso-estaldura zabala duena (Castro, 2009). Gaur egun Elamaren arroan ez da baso-jarduerarik ematen. Bestalde, Leitzarari (4. irudia) laugarren ordenako ibaia da, kareharri, arbel eta hareharrizko 12 402 ha-ko arroa estaltzen duena. Elaman ez bezala, guztira 3150 biztanle dituzten bi herri daude Leitzararen arroaren goiko aldean, nahiz eta horren ostean errekek biztanlerik gabeko 25 km inguruko haran luzea zeharkatzen duen, non basogintza eta jarduera hidroelektrikoetarako ur-erazketak diren giza-jarduera nagusiak (Izagirre eta kideak, 2013). Jarduera hidroelektrikorako presa baxuek (2–4 m-ko altuera) ura ibilgutik desbideratu eta hainbat kilometro luze diren desbiderapen kanaletara bideratzen dute, azkenik turbina hidrauliko batzuetatik igaro eta ura ibilgura itzuliz. Presa hauek ura metatzeko ahalmen



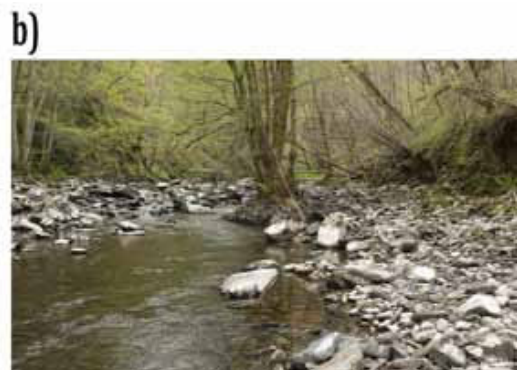
2. Irudia. Ikerketa-eremua: (a) Leitzaran eta (b) Elama erreken arroak. Erreka bakoitzean ikertutako tartea beltz ilunagoz irudikatu da. Puntu txuriek ikertutako tartean dauden presak adierazten dituzte. Pirinioetako muturluzearen Iberiar Penintsulako banaketa (Fernandes eta kideak, 2008) gorritz adierazi da.

txikia dute, baina ur gehiena desbideratzen dute. Hidroelektrika emakida gehienek emari ekologikoa utzi behar dute, zeina hilabete bakoitzerako kalkulaturako emariaren batzbestekoaren %10-ean zehazten den normalean (BOE, 2016). Hala eta guztiz ere, kontzesio zahar batzuek ez dute oraindik emari ekologikoaren inguruko betekizunik, eta hori dela eta, erreka tartek ia osorik agortzen dituzte. Eremuko instalazio hidroelektrikoek ia urte osoan zehar jarduten dute, udan edo udazkenaren hasieran soilik geratuz, mantentze-lanak egiteko edo ur-kudeatzaileek emari ekologikoak bermatzera behartzen dituztelako (Iñaki Bañares, Gipuzkoako Foru Aldundia, komunikazio pertsonala, 2017ko iraila). Instalazio hidroelektrikoak elkarren segidan egoten dira, turbina hidroelektrikoaren ihesbidetik oso hurbil egonik hurrengo presa. Hortaz, jarduera hidroelektrikoaren eraginpean dauden erreketan desbiderapen kanalen eraginpean dauden tartek ugariagoak izaten dira emari naturaleko tartek baino. Leitzaran ibaiaren erdiko eta beheko tartetan ibilgu nagusiaren %70 inguru desbiderapen kanalen eraginpean dago, eta %4 gehigarri bat presen goialdeko gune geldoetan dago metaturik (Izagirre eta kideak, 2013).



3. Irudia. Elama erreka, Euskal Herria. Argazkia: Amaiur Esnaola.

Ikerketa Elama errekaiko 4 km-ko tartean ($43^{\circ}12'40''\text{N}$, $1^{\circ}48'36''\text{W}$ eta $43^{\circ}11'14''\text{N}$, $1^{\circ}48'4''\text{W}$ artean; batazbesteko altitua, 330 m; batazbesteko zabalera, 7,08 m), eta Leitzarar ibaiko 10 km-ko tartean egin zen ($43^{\circ}8'57''\text{N}$, $1^{\circ}57'26''\text{W}$ eta $43^{\circ}6'55''\text{N}$, $1^{\circ}56'9''\text{W}$ artean; batazbesteko altitua, 290 m; batazbesteko zabalera, 12,42 m) (2. irudia). Tartearen luzeraren arteko aldeak Leitzarar ibaian muturluzeak harrapatzeko zailtasun handiagoari dagozkio.



4. Irudia. Leitzarar ibaia, Euskal Herria: (a) muturluzeek okupaturako tartea bere emari naturalarekin (2016ko apirilaren 21a) eta (b) tarte berdina bost egun beranduago (2016ko apirilaren 25a), zentral hidroelektriko batek emari gehiena desbideratu ostean. Bi argazkien arteko aldeak ur-erazketek habitat hidromorfologikoen banaketan, bereziki ibilgu bustiaren azalera osoaren eta urlasterren azalaren murrizketan, duten eragina erakusten dute. Argazkia: Jorge González-Esteban.

Habitat-eskuragarritasuna eta ezaugarriak

2016ko iraila eta urrian, GPS baten eta mapa topografikoen laguntzaz, oinez ikuskatu ziren bi erreketako tarte guztiak. Overton eta kideen (1997) lana jarraituz, hiru habitat mota identifikatu ziren: urlasterrak, ura bizkor, zurrunbilotsu, azalera irregularrez eta zurixka duten lekuak; ur-bareak, ura ia laminarki eta sakonera erregularra dutenak; eta putzuak, ibilguaren sakonuneetan, emari motelekoak.

Erreka tarte bakoitza azpi-tarteetan banatu zen habitat dominatzailearen arabera: urlasterra, ur-barea edo putzua. Habitat moten arteko mugak ibilguaren hondoko maldaren hausturak identifikatuz antzeman ziren. Erreka tarte bakoitzeko habitat mota bakoitza 10 zehar-trantsektu eginez ezaugarritu zen. Zehatzago, trantsektu bakoitzean ibilgu bustiaren zabalera neurtu zen, eta tarte erregularretan (0,5 m-ro Elaman; 1 m-ro Leitzaranean) uraren sakonera eta sakonera maximoaren 0,6-ko abiadura (Martin Marten Z30 korrontimetroa, Bartzelona, Espainia) neurtu ziren. Substratua ere karakterizatu zen, hondoa ikuskatuz substratu tamainaren araberrako kategoriak esleituz: harea, txintxarra, harkoskoa, uharria, harrizarra, eta besteak (arroka, arroka-ama, edo organikoa) (Díez eta kideak, 2000). Neurketa horietan, ikerketa-tarteak plastikozko zintaz markatu ziren 25 m-ro, GPS bitartezko kokapenak hobetze aldera.

Muturluzeen harrapaketa eta jarraipena

Ernari edo kumeekin zeuden emeak molestatzea ekiditeko, muturluzeen estaltze eta ugalketa garaitik (urtarrietik abuztura; Palmerin eta Hoffman, 1983) kanpo egin zen ikerketa hau. Muturluzeak 2016ko iraila eta urria artean gauzez harrapatu ziren, ibilguan erdi-urperatutako alanbre-sarezko nasak erabiliz, zeinak 3 orduro begiratzen ziren estresa gutxitze aldera (González-Esteban eta kideak, 2003a). Erreka bakoitzean bi-hiruna gauko bi harrapaketa-saio egin ziren, gau bakoitzean 12 tranpa erabiliz. Harrapaketen maiztasuna baxuagoa zen Leitzaranean, eta beraz, animalia kopuru berdintsua harrapatzeko esfortzu handiagoa egin behar izan zen bertan (lau gau Elaman, sei Leitzaranean).

Harrapatutako ur-satorren adina eta sexua begiz zehaztu ziren (González-Esteban eta kideak, 2003b; González-Esteban eta kideak, 2002). Bizkar-aldeko ilea guraizeekin moztu (gutxi gorabehera 1 cm²) ondoren, irrati-igorgailuak (0,75 g-ko pisua, A2435, Advanced Telemetry Systems, <https://atstrack.com>) Dermabond™® erabiliz itsatsi zitzaizkien eta animaliak harrapatutako leku berean askatu ziren. Prozesuak 15-20 minutu iraun ohi zuen animalia bakoitzeko.

Markatutako muturluzeak bost gauz jarraitu ziren, ahalik eta denbora tarte

laburrenean banako bakoitzaren jardueraren inguruko informazio nahikoa eskuratu ahal izateko. Gainera, emaitzak nahas zitzaketen faktoreak saihesten ziren horrela; adibidez, irrati-igorgailuak erortzea eta ingurumenean aldaketa handiak gertatzea (eguraldiaren gora-behera gogorak kasu). Irrati-telemetry bidezko jarraipenak oinez egin ziren, bi irrati-hartzaile eta antena mota erabiliz (TRX1000S, Wildlife Materials Inc., Carbondale, IL, USA, hiru elementuzko eskuzko Yagi antenarekin; AOR AR8200 Mark III B, Universal Radio Inc., OH, USA, H motako antenarekin). Gauean zeharreko jarduera ezagutzeko, muturluzeak ahalik eta distantzia laburrenera jarraitu ziren. Jarduera- eta atsedendaldia irrati-seinaleen aldaketei esker bereizi ahal izan ziren. Autokorrelazio espaziala saiheste aldera, gutxienez 5 minutuko tarteaz jaso ziren elkarren segidako kokapen-puntuak, denbora tarte horretan banako bakoitzak edozein habitat motatara iristeko gaitasuna zuela ikusirik (Stone, 1987). Atsedendaldiko kokapenak analisisietatik baztertu ziren.

Muturluzeen harrapaketa eta maneirako protokoloek ikerketako eta irakaskuntzako animalien traturako arauak bete zituzten (Animal Behaviour Society, 2012). Ikerketak lekuko betebeharrak juridikoak bete zituen eta Euskal Herriko Unibertsitateko Animalien Ongizaterako Etika Batzordeak onartua izan zen (erref. CEBA/M20/2016/022).

Datuen analisisia

Habitaten informazioa eta irrati-telemetry puntuak GPS sistema batetan jaso ziren (ArcView 3.2; ESRI, Redlands, CA, USA). Banako bakoitzaren habitat-eskuragarritasuna eta bizi-eremua (luzera m-tan, azalera m²-tan) ArcView 3.2-rako Animal Movement 2.0.β luzapena erabiliz (Hooge eta Eichenlaub, 1999) lehentasunezko bazka-eremuak kalkulatu zehaztu ziren, jarduera puntuak soilik hartuz kontutan. Kokapenen kopururik eta antolaketa espazialek eratorritako erabilera-probabilitatean eta animalia batek eremu zehatz batean igarotako denbora-tarte erlatiboan oinarrituriko (Worton, 1989) kernel bizi-eremuen bidez (Kernel Density Estimation (KDE) %95) zehaztu ziren lehentasunezko bazka-eremuak. Kernelak kernel finkoen metodoa erabiliz kalkulatu ziren eta leuntze faktorea karratu minimoen balidazio gurutzatua erabiliz estimatu zen. Bizi-eremuen (%95KDE; luzeran eta azaleran) eta urlaster, ur-bare eta putzuen azalaren (%95KDE-tik) ikerketa-guneen arteko aldeak estatistikoki aztertu ziren Mann-Whitney-ren U test ez-parametrikoa erabiliz.

Habitat-eskuragarritasunaren eta habitat-erabileraren arteko independentzia χ^2 doikuntzaren egokitasunaren bidez (egiantzekotasunaren algoritmoaren G ratioa) aztertu zen. Batezbesteko hautespen-ratioaren w_i (Manly eta kideak, 2002) arabera lehentasun erlatiboaren arabera sailkatu ziren habitat

motak: behatutako animalia bakoitzak erabilitako habitat mota bakoitzaren proportzioen, eta aurrez ezagututako populazio bakoitzeko habitat bakoitzaren eskuragarritasunaren arteko ratioen batezbestekoa, erreka bakoitzeko animalia-populazio osoarentzako. Habitat zehatz baten hautespena positiboa edo negatiboa zela zehazteko, Bonferroniren konfiantza tarteak eraiki ziren, Manly eta kideek (2002) egin bezala, banako bakoitzeko habitat-eskuragarritasuna eta erabilera estimatzeko. Erreken arteko konparaketak batezbesteko hautespen-ratioak erabiliz egin ziren, Bonferroniren konfiantza tarteak kontutan hartuz. Aldibereko konparaketa kopuruarekin zuzendutako 0,05 α balioa zehaztu zen proba guztientzako. Konfiantza tarteak ere aldibereko konparaketa kopuruarekin zuzendu eta %95-eko mailan kalkulatu ziren. Banako bakoitzaren hautespena erreka bakoitzerako eraikitako hautespen-diagrama banaren bidez irudikatu zen (R-ko SOILTEXTURE paketea).

Emaitzak

Habitat-eskuragarritasuna eta ezaugarriak

Uraster eta ur-bareek antzeko eskuragarritasuna zuten Elaman (0,48 eta 0,49-ko proportzioak, hurrenez hurren; 1. taula), eta Leitzarane berriz ur-bareak ziren nagusi (0,62; 1. taula). Putzuak ziren ugaritasun txikiena zuten habitatak bi erreketan (0,03 Elaman, eta 0,11 Leitzarane; 1. taula) Oro har, urlasterrak, ur-bare eta putzuak baino pixka bat estuagoak, sakonera dezente txikiagoak, eta azkarragoak ziren. Uraren sakonera Leitzarane handiagoa zen oro har, nahiz eta uraren abiaduraren erreken arteko aldeak txikiak izan. Elaman harrizarrak ziren nagusi urlasterretan, uharriak ur-bareetan eta bestelako substratuak putzuetan. Leitzarane, aldiz, harrizarrak urlaster eta ur-bareetan ziren nagusi eta bestelako substratuak putzuetan.

Harrapaketak

Hogeita hamahiru muturluze harrapatu eta jarraitu ziren arrakastaz: zazpi eme eta zortzi ar Elaman, eta hamar eme eta sei ar Leitzarane (2. taula).

Bizi-eremuen tamaina eta habitat-hautespena

Oro har, 200 jarduera puntu baina gehiago erregistratu ziren banako bakoitzeko, eta launa gau baina gehiago jarraituak ziren animaliak (5. irudia; 2. taula).

1. Taula. Elama eta Leitizaran erreketako habitataren ezaugarriak..

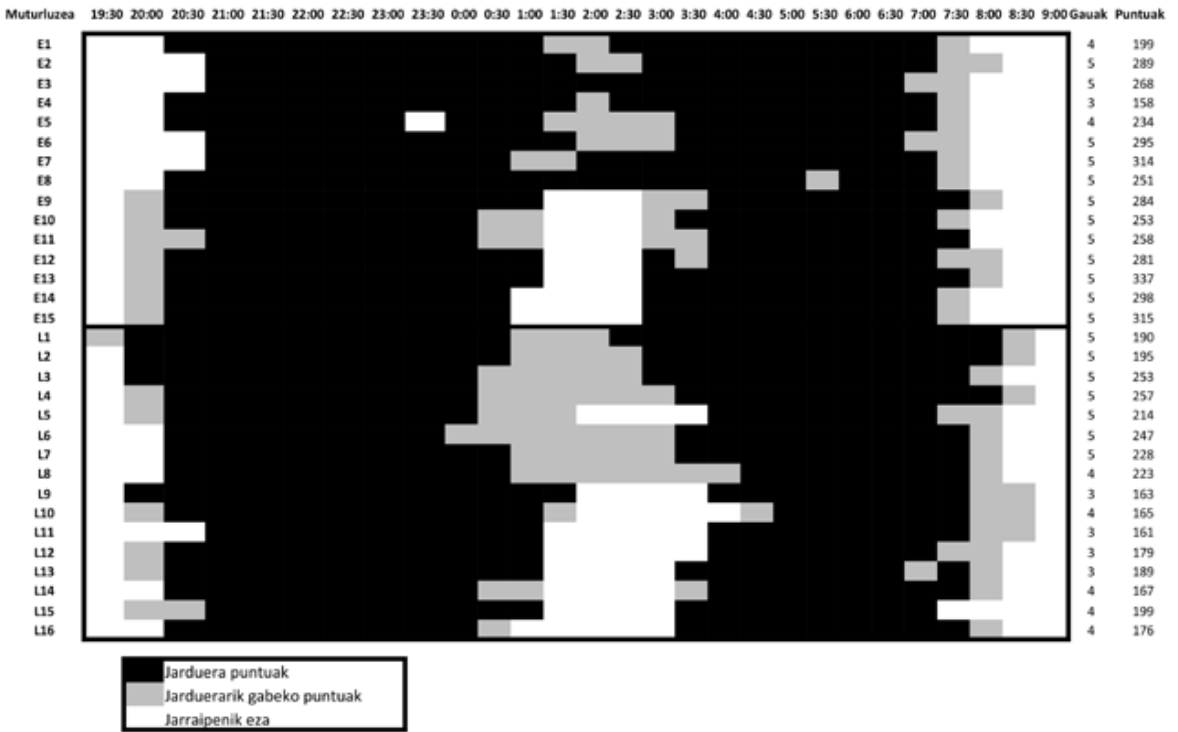
Erreka	Habitata	Eskuragarrit. zabalera (m)	Ibilguaren batezbestea ± DE	Sakonera (cm) batezbestea ± DE	Uraren abiadura (ms ⁻¹) batezbestea ± DE	Harea (%) ± DE	Txintxarra (%) ± DE	Harkoskoa (%) ± DE	Uhartia (%) ± DE	Harrizarra (%) ± DE	Besteak (%) ± DE
Elama	Rifle	0,48	6,7 ± 2,4	9,13 ± 2,78	0,31 ± 0,13	0,8 ± 2,6	5,7 ± 8,1	10,1 ± 10,1	28,5 ± 20,3	32,6 ± 20,6	22,2 ± 29,8
	Run	0,49	7,4 ± 1,3	16,79 ± 4,10	0,09 ± 0,03	6,7 ± 9,7	18,6 ± 13,2	19,0 ± 15,8	29,6 ± 13,2	21,8 ± 16,7	4,3 ± 6,6
	Pool	0,03	7,1 ± 2,8	57,74 ± 16,59	0,03 ± 0,02	18,6 ± 15,8	9,4 ± 8,6	12,4 ± 10,9	14,3 ± 16,1	14,3 ± 11,7	31,0 ± 19,4
Leitizaran	Rifle	0,27	11,5 ± 2,8	11,28 ± 2,81	0,36 ± 0,11	0	2,5 ± 7,9	0	25,1 ± 9,1	65,3 ± 14,5	7,1 ± 12,8
	Run	0,62	12,1 ± 2,0	28,86 ± 9,95	0,07 ± 0,04	5,6 ± 9,1	12,0 ± 10,2	5,7 ± 8,9	20,3 ± 21,6	34,7 ± 28,6	21,6 ± 23,7
	Pool	0,11	13,6 ± 4,7	73,56 ± 27,01	0,02 ± 0,02	2,5 ± 4,3	12,9 ± 10,7	7,7 ± 9,6	19,1 ± 21,1	14,1 ± 13,9	43,6 ± 27,5

Eskuragarritasunak ('Eskuragarrit. ') habitat mota bakoitzak erreka bakoitzeko ikerketa area osoan duen proportzioa erakusten du

2. Taula. Elama eta Leitizaran erreketan jarraitutako animalien xehetasunak.

Erreka	Muturluzeen harrapaketa gau kopurua	Jarraitutako muturluzeak	Emeak	Arrak	Jarduera puntuak: batezbestea ± DE	Jarraipen gau batezbestea ± DE	BBEL (m) (%95KDE)	BBEA (m ²) (KDE95%):	Urlasterren azalera (m ²) (%95KDE):	Urbareen azalera (m ²) (%95KDE):	Putzuen azalera (m ²) (%95KDE):
Elama	4	15	7	8	268,9 ± 44,9 ± DE	4,73 ± 0,57 ± DE	328 ± 72	2215,6 ± 345,3	1103,3 ± 394,7	1038,7 ± 494,0	73,6 ± 78,6
Leitizaran	6	16	10	6	200,4 ± 32,9 ± DE	4,19 ± 0,83 ± DE	368 ± 231	6176,9 ± 5201,9	2379,3 ± 1298,1	3295,2 ± 3497,8	502,5 ± 761,9

BBEL (%95KDE): banakoen bazka-eremuaren luzera (m) (%95seko kernela); BBEA (%95KDE): banakoen bazka-eremuaren azalera (m²) (%95seko kernela). Eskuragarri zeuden urlaster, ur-bare eta putzuen azalerak (%95seko kernela) ere gehitu dira.

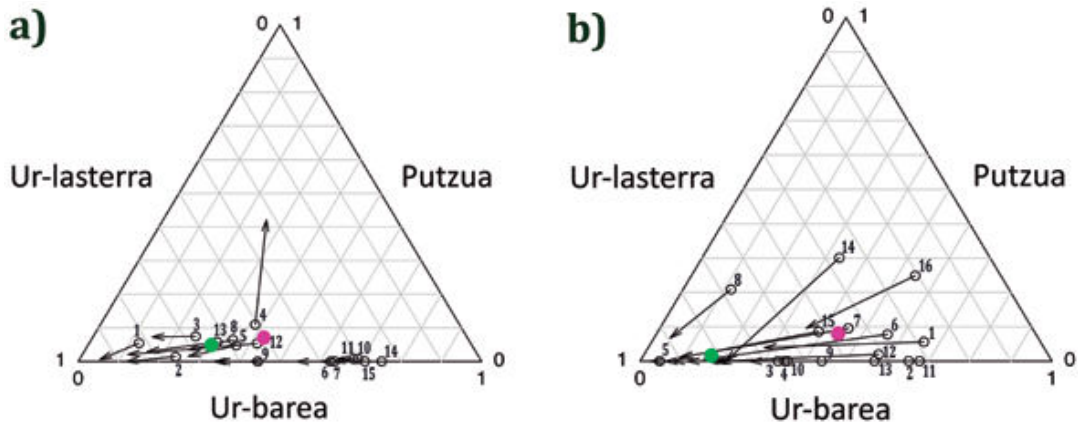


5. Irudia. Animalia bakoitza gauz irrati-telemetry bidez jarraitua izan deneko denbora tartek: gune beltzek animaliak aktibo zeudeneko tartek adierazten dituzte, gune grisek jarduerarik gabeko tartek, eta gune zuriek daturik jaso ez zeneko tartek. ‘Muturluzea’ zutabea agertzen diren kodeek bi erreketako banakoak adierazten dituzte (E, Elama; L, Leitzarar). Animalia bakoitza zenbat gauz jarraitu den (‘Gauak’) eta zenbat jarduera puntu erregistratu diren ere (‘Puntuak’) adierazi da eskuineko zutabeetan.

3. Taula. Habitat-erabilera, habitat-erabileraren Bonferroniren konfiantza tartek, habitat-eskuragarritasuna eta batezbesteko hautespen-ratioaren (Manly eta kideak, 2002) Bonferroniren konfiantza tartek Elama eta Leitzarar erreketan. .

Erreka	Habitata	Habitat-erabilera u_i	Habitat-erabileraren proportzioen (α_i) Bonferroniren konfiantza tartek		Habitat- eskuragarritasuna (%95KDE) Π_i	Hautespena	Batezbesteko hautespen-ratioaren (\hat{w}_i) Bonferroniren konfiantza tartek	
			Max.	Min.			Lower	Upper
			Elama	Urlasterra			2176	0,615
Ur-barea	1130	0,310		0,352	0,466	Negatiboa	0,642	0,729
Putzua	110	0,024		0,040	0,033	Nulua	0,864	1,308
Leitzarar	Urlasterra	2289	0,818	0,856	0,490	Positiboa	1,626	1,719
	Ur-barea	406	0,130	0,166	0,441	Negatiboa	0,293	0,396
	Putzua	40	0,009	0,021	0,068	Negatiboa	0,046	0,384

‘Habitat-erabilerak’ 5 minutuan behin edo maiztasun baxuagoz jasotako jarduera puntuak —kokapenak— adierazten ditu. ‘Habitat-eskuragarritasunak’ erreka bakoitzean habitat mota bakoitzak duen proportzioa adierazten du (%95eko kernela).



6. Irdia. Elama (a) eta Leitzarán (b) erreketako habitat-hautespenaren diagramak. Zirkuluek animalia bakoitzak eskuragarri dituen habitaten proportzioak adierazten dituzte (habitat-eskuragarritasunaren proportzioak kontutan hartuz, 0 eta 1 artean mikrohabitat bakoitzeko), geziek erabiliko habitaten proportzioa adierazten duten bitartean (habitat-erabileraren proportzioak kontutan hartuz, 0 eta 1 artean mikrohabitat bakoitzeko). Puntu arrosek erreka bakoitzeko batezbesteko habitat-eskuragarritasuna adierazten dute eta puntu berdeek erreka bakoitzeko batezbesteko habitat-erabilera.

Erreken arteko bizi-eremuen luzerak (%95KDE) ez ziren modu esanguratsuan desberdinak (banakoen bazka-eremuaren luzera, BBEL; $w = 135$, $P = 0,5665$), baina azalerak nabarmenki handiagoak ziren Leitzaránen Elaman baino (banakoen bazka-eremuaren azalera, BBEA; $w = 26$, $P < 0,0001$; 2. taula). Leitzaránen urlasterrek ibilguaren proportzio txikiagoa estaltzen zuten arren, bizi-eremuen baitako urlasterren azalera osoa handiagoa zen bertan (%95KDE; $w = 29$, $P = 0,0003$; 2. taula), ur-bareen azalera osoa bezalaxe (%95KDE; $w = 63$, $P = 0,0255$; 2. taula), nabarmenki handiagoa baitzen ibai hau. Putzuen azalera osoen artean aldiz ez zegoen alde esanguratsurik bi erreken artean (%95KDE; $w = 109$, $P = 0,6663$; 2. taula).

Elaman aktibitateko kokapenen %63,7 urlasterretan erregistratu ziren, %33,1 ur-bareetan, eta %3,2 soilik putzuetan (3. taula). Urlasterrek eskuragarritasunari dagokiona baino gehiago erabili ziren (hots, habitat-hautespen positiboa zegoen; $\chi^2 = 361,2$, d.f. = 30, $P < 0,0001$) eta ur-bareak aldiz kontrara, putzuekiko hautespen datuak estatistikoki esanguratsuak ez ziren artean (3. taula). Muturluze batek ez zuen eredu orokorra jarraitu Elaman eta putzuak positiboki hautatu zituen (6. irudia).

Leitzaránen aktibitateko kokapenen %83,7 urlasterretan jaso ziren, %14,8 ur-bareetan eta %1,5 putzuetan. Urlasterrek positiboki hautatu ziren ($\chi^2 = 819,9$, d.f. = 32, $P < 0,0001$), eta ur-bare eta putzuak aldiz negatiboki (6. irudia; 3. taula). Orohar, Elaman baino zorrotzagoa zen habitat-hautespena Leitzaránen,

batezbesteko hautespen-ratio altuagoak (1,70 *versus* 1,26) eta 6. irudiko puntu arrosen eta berdeen arteko distantzia handiagoak adierazi bezala.

Gordelekuetatik kanpo zeuden artean, muturluzeen mugimenduek eredu irregularrak aurkezten zituzten. Orokorrean, ehundaka metro gutxitako erreka tarteetan ibiltzen ziren banakoak gauero, hurrengo bazkalekura mugitu baina lehen, euren bazka-jarduera hainbat minutuz gune zehatzetan (nagusiki urlasterretan) kontzentratuz. Hala ere, animalia batzuek agudo egiten zituzten ehundaka metro batzuk habitatik urlaster batetara, minutu gutxian, gero bertan 1-3 orduz egoteko. ‘Leku-bero’ horietan hautemandako irrati-seinaleen intentsitate-aldaketek metro gutxi batzuetara mugatutako etengabeko mugimenduak adierazten zituzten. Egonaldi luzeak urlasterretan ematen ziren bereziki, ibilgua adarkatu eta adarrak eta hostoak metatzen zituzten harrizarrak edo eroritako enborrak agertzen ziren guneetan.

Eztabaida

Ikerketa honek Pirinioetako muturluzeak urlasterrak positiboki eta putzuak negatiboki hautatzen dituela erakutsi du. Horrez gain, habitat-hautespena erreken arabera aldatzen dela ere erakutsi du, jarduera hidroelektrikoaren eraginpean zegoen errekan hautespen handiagoa azalduz. Segur aski muturluzeen habitataren kalitatean eragiten duten faktoreen eta galzorian dagoen animalia honen kontserbazio egoera hobetuko duten kudeaketa ekintza potentzialen inguruko balio handiko informazioa gehitzen dute emaitza hauek.

Habitaten hondamena eta uren kutsadura bezalako giza presioei egotzi zaie Pirinioetako muturluzearen populazioaren atzerakadaren errua (Queiroz eta kideak, 1996; International Union for Conservation of Nature (IUCN), 1995). Bereziki, banaketa oztopatzen duten presa edo oso eraldatuta egoteagatik muga biogeografiko ugari dituzten sare dendritikoetako ibai-tarteetako hesi artifizialei egotzi izan zaizkie galera lokalak (Morueta-Holme eta kideak, 2010). Espezie honen Frantziako banaketa historikoa eta egungo banaketa konparatuz, Charbonnel eta kideek (2016) klimaren eta erabilera hidrologikoaren zein lur-erabileren aldaketen ondoriozko habitataren egokitasunaren beherakadaren eraginezko hedaduraren murrizketa uste baina handiagoa zela ondorioztatu zuten. Beherakada hori habitataren zatiketaren, kutsaduraren, harrapakin-eskuragarritasunaren aldaketen, edo muturluzeen harrapari ziren espezie inbaditzaileen (adb. Amerikar bisoia *Neovison neovison*) gisako faktore gehigarrien eraginez eman zela espekulatu zuten, nahiz eta ez zuten faktore potentzialen zerrenda horren garrantzia erlatiboaren inguruko iradokizunik gehitu. Hori dela eta, muturluzearen kontserbaziorako neurrien eskasian islatua

ikusten den bezala, oraindik ere generikoegia da zerrenda hori kontserbaziorako fundazio gisa. Adibide gisa, bai Frantziako Pirinioetako Muturluzearen Kontserbaziorako Ekintza Planean (Némoz eta Bertrand, 2008) eta baita Espainiako Muturluzearen Kontserbaziorako Estrategian ere (Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA), 2013), ikerketa eta zientziarako ekintza asko aurreikusi arren, ez dute ia habitataren hobekuntzara bideraturiko jarduerarik proposatzen.

Emariaren abiadura Pirinioetako muturluzearentzako ingurumeneko faktore garrantzitsu gisa plazaratu izan da (Charbonnel eta kideak, 2015). Biffi eta kideek (2016) Nitxo Ekologikoaren Faktoreen Analisia (*Ecological Niche Factor Analysis, ENFA*) egin zuten gorotzen detektagarritasunean oinarrituz. Pirinioetako muturluzearen habitata ibilguaren baitako aldagaiek (substratuaren heterogeneotasunak, urlasterrek, sedimentu finen ausentziak) eta ibaiertzaren ezaugarriek (ibaiertz harritsuek) ezaugarritzen zutela ondorioztatu zuten, muturluzeek egitura irten askoko eta babesleku heterogeneotasun handiko gunek nahiago izanik. Hala ere, euren emaitzak sobera alboratuta daude; izan ere ibai-tarte horiek dira zehazki muturluzeen gorotzak detektagarrien diren gunek. Egitura irtenik gabeko ibai-tarteetan ibaiertzeko babeslekuetan egiten dute kaka muturluzeek eta leku horietan oso zaila izaten da gorotzak detektatzea; beraz, ausentzia faltsuak ondorioztatzen dira (Charbonnel eta kideak, 2015; González-Esteban eta kideak, 2003a; Nores eta kideak, 1999). Era horretako ikerketek adierazterik duten bakarra muturluzearen gorotzak errazago non detektatu daitezkeen da, ezin baitute bizi diren ibaietako espazioa nola erabiltzen duten erakutsi. Hemen aurkezten den lanak zulo hori estaltzen du hein batean, abiadurarekiko behar iraunkor bat erakutsiz, hots, urlasterrekiko hautespen sendoa eta putzuekiko saihaspen garbia, gure lehenengo hipotesia berretsiz. Banako bakarrak erakutsi zuen putzuekiko zaletasuna. Banako horren bizi-eremuaren ikuskapen hurbila egin ostean ezin izan zen jokabide horren azalpen zehatzik topatu. Harrapakin-eskuragarritasun handiko guneen ondorioz izan liteke, baina kokapenen zehaztasuna tarteko, hipotesi hori ezin da aztertua izan, ez bada bentosaren laginketa oso suntsikor bat egiten dela.

Banako horretaz gain, gainontzeko ale guztiek mikrohabitat azkarrekiko zaletasuna erakutsi zuten, seguraski bazkari, babeslekuei edo hidraulikari lotuta. Bazkari dagokionean, urlasterrek ur-abiadura motelagoko beste habitatek baino ornogabeen dibertsitate eta biomasa handiagoak eduki ditzaketela plazaratu izan da (Dewson eta kideak, 2007; Hussain eta Pandit, 2012). Badirudi, gainera, bigarren mailako ekoizpen altuagoa ere izan dezaketela (Buffagni eta Comin, 2000; Polis eta kideak, 1997), nahiz eta joera unibertsal izatetik urrun dagoen (Wohl eta kideak, 1995). Bestenaz, urlasterrek ur-bare eta putzuek baina aterpe egokiagoa eskain lezakete, muturluzeak urpean harrapatutako harrapakina jan bitartean ezkutaleku gisa erabili ditzakeen (Niethammer, 1970) edo emariaren

bat-bateko igoeren (igoera natural edo artifizialen) aurrean babeslekua eskaini diezaioketen harriztar irtenak ohikoak izaten direlako bertan. Horrez gainera, urlasterretako uraren abiadura azkarrak muturluzeei euren flotagarritasun naturalari aurre egitea erraztuko lieke, euren hatzapar indartsuak erabiliz hondoan ibiltzea baimenduz (Richard, 1986).

Lan honen bigarren hipotesia ere berretsia izan zen, muturluzeek habitata desberdin erabili zutelako errekaen ingurumeneko baldintzen arabera. Animaliek, bizirik irauteko habitat desegokiak erabiltzera behartuak suertatzen diren kalitate gutxiagoko inguruneetan hautakortasun baxuagoa izango zutela espero zitekeen arren (Goiti eta kideak, 2003; baina ikusi Garshelis, 2000, edo Railsback eta kideak, 2003), kontrakoa izan zen behatutakoa. Muturluzean behatutako ereduaren arrazoia Leitzaranean, jarduera hidroelektrikorako ur-erazketen eraginpean dagoen ibaian, gutxien hobetsitako habitatak oraindik ere okerragoak zirelako, edo Elamako populazioaren dentsitate handiagoaren eraginez bertan hautespena txikiagoa zelako izan liteke. Izan ere, Elaman adinako animalia kopurua Leitzaranean harrapatzeko izandako zailtasunak bertako populazioaren dentsitatea baxuagoa dela iradokitzen du. Esan beharra dago bazka-eremuak Leitzaranean Elaman baino handiagoak zirela, eta horrek habitataren egokitasun kaskarragoa, edo populazioaren presio altuagoa nabarmentzen du. Bestalde, Elaman behatutako bazka-eremu txikiagoek erreka honetako putzuen erabilera populazioaren presio altuagoaren edo espezie barneko lehiaren eragina ez zela iradoki zuten. Honela, emaitza orokorrekin, muturluzeak gutxien hobetsitako habitatak, harrapakinen eskuragarritasunari edo ezaugarri fisikoei (adb. sakonera txikiagoa, emari motelagoa) zegokienean, Elaman egokiagoak zirela adierazi zuten Leitzaranean baino.

Arrazoia edozein delarik ere, Pirinioetako muturluzeak urlasterrak positiboki hautatzen dituela erakutsi dute emaitzek, ur-bare eta putzuen aldean, eta are gehiago ur-erazketen eraginpean dagoen errekan. Ur-erazketek ibilgu bustia murrizten dute (Stanley eta kideak, 1997), aldaketa hidraulikoak eragiten dituzte (McIntosh eta kideak, 2002), ornogabe bentonikoen populazioak txikiagotzen dituzte (James eta kideak, 2008), eta ibai-ekosistemaren funtzionamenduan inpaktuak eragiten dituzte (Arroita eta kideak, 2017). Ur-erazketek, gogokoen duten mikrohabitataren eskuragarritasuna murriztuz, galzorian dagoen Pirinioetako muturluzearengan ere efektu kaltegarriak izan ditzaketela iradokitzen dute emaitzek. Aymerich-ek (2004) emari-erregimenaren murrizpen artifizialak (adb. ekoizpen hidroelektrikoaren edo ureztaketaren eraginez) Pirinioetako muturluzean kontrako inpaktua eragin zezakeela proposatu zuen, bereziki emariaren murrizpenaren aurrean sentikorrako diren mendiko erreketan. Horrez gain, jarduera hidroelektrikorako desbiderapen-kanaletan behatu izan dira muturluzeak (MAGRAMA, 2013), segur aski ibilguan behera lurralde berrien bila bidaiatzerakoan ur gutxi zeraman ibilgua hartu beharrean

kanalean jarraitu izanagatik. Oraindik ez dago argi animalia hauei zer gertatzen zaien, baina turbinetan hiltzea ez litzateke ustekabeko emaitza izango (Elosegi, 2010). Jakina da murriztutako emariak oro har, eta ur desbiderapenek bereziki, ibaietako beste espezie batzuegan ere efektu kaltegarriak dituztela; salmonidoak (Bradford eta Heinonen, 2008; Heggenes eta kideak, 1996) eta ur-zozoa (Chen eta Wang, 2010), urlasterrak faboratzen dituen (Logie eta kideak, 1996) eta nonbait bazka-nitxoa muturluzearen antzera ustiatzen duen (Santamarina, 1993) hegazti generoa, barne.

Aurkikuntza hauen kudeaketa-ondorioei dagokienean, muturluzeak dauden ibaietako emari ekologikoen esleipenak berrikusi egin beharko liriatekeela iradokitzen dugu, bereziki Leitzaran ibaia bezalakoetan, Natura 2000 sarearen baitan Kontserbazio Berezirako Eremu (KBE) gisa izendatuta daudelako. Gaur egun erabiltzen diren formula hidrologiko sinpleekin fidatu beharrean, ikerketa hidrauliko zehatzei ekin beharko litzaieke, desbiderapen kanalen eraginpean dagoen tarte bakoitzean emariak urlasterren ugaritasun eta banaketan nola eragiten duen zehaztu eta alde batetara utzi ezin den inflexio-punturik ba ote dagoen hautemateko, hots, desbideratutako zein emari-atalasetik aurrera murrizten den bizkor habitat-eskuragarritasuna. Arroita eta kideek (2017), Elamaren antzeko erreka batean emariaren manipulazio esperimentalak erabiliz, emari ekologikoen, egun Euskal Herrian izendatzen diren bezala, emariaren abiadura eta urlasterren ugaritasuna gogorki murrizten dituztela —eta hortaz ekosistemen funtzionamenduan ikaragarri eragiten dutela— frogatu zuten. Beraz, litekeena da Euskal Herriko (Ente Vasco de la Energía (EVE), 1995) zein beste zenbait lekutako iturburuko erreketako desbiderapen sistema ugariak muturluzearen habitataren kalitatean gogorki eragiten aritzea.

Emari ekologikoen gain, urlasterren eskuragarritasuna murrizten duten kudeaketa jarduerak ekidin beharko liriateke. Gipuzkoan, Leitzaran ibaia kokatzen den 2000 km² baina txikiagoa den lurraldean, 900 presa txiki baina gehiago daude (BOE, 2016; EVE, 1995). Horietako egitura gehienak errota edo burdinola bezalako iraganeko jardueren ondareak dira, eta oro har geldirik dagoen ur-azalera handiak sortzen dituzte, segur aski putzuen gisan muturluzearentzako oso erabilgarriak izango ez direnak. Aukera dagoen lekuan bota egin beharko liriateke presa horiek, erabileran daudenak edo ondare-balioa dutenak soilik mantenduz. Kanalizazioak ere muturluzearen habitatarentzako kaltegarriak direla deskribatu izan da (MAGRAMA, 2013), nahiz eta frogak enpiriko gutxi izan. Lan honen emaitzek iradokitzen dute kanalizazioak bereziki izan daitezkeela kaltegarriak emariaren abiadura eta heterogeneotasuna murrizten dituztenean, eta hori arrunta izan ohi da emariaren garraio-ahalmena handitzeko diseinatzen diren zehartebaki trapezoidaleko kanalizazio artifizialetan. Deflektoreak eta urlasterren gisako guneak sortzeko egur handien sarrera salmonidoen habitata

hobetzeko sarri erabilia izan den jarduera da (Nagayama eta Nakamura, 2010), eta muturluzearen habitata hobetzeko errestiturazio jardueren artean legoke.

Ikerketa honek abiadura handiko urlasterren azalera Pirinioetako muturluzearen habitataren kalitatean faktore gakoa dela adierazten du, abiadura beharra duen espezie honen kontserbazio egoera hobetzeko jarduera zehatzak zein liratekeen azpimarratuz. Jarduera horiek, muturluzearen populazioaren egoera zatikatua kontutan hartuta, ikerketa genetikoak eta banakoen translokazioak konbinatu beharko lituzkete, beti ere espezie honen galzoriko egoera hobetu nahi bada.

Eskerrak

Iñigo Mendiola eta Aitor Lekuona (Gipuzkoako Foru Aldundia), eta Asunción Yarzabal eta Iñaki Uranga (Donostiako Udaletxea) eskertu nahi ditugu, lan hau egin ahal izateko eman diguten laguntzarengatik; Egoitz Salsamendi eta Inazio Garin (Euskal Herriko Unibertsitatea) landa-lanean laguntzeagatik, eta Joan Lluís Riera (Bartzelonako Unibertsitatea) grafikoekin laguntzeagatik. Eskerrak John S. Richardson irakasleari (Basogintza Fakultatea, British Columbia Unibertsitatea) ingelesa gainbegiratzeagatik. Autoreek interes-gatazkarik ez dagoela aitortzen dute.

3. Kapitulu



Gorotzetatik abiatutako dieta azterketa:
Galemys pyrenaicus
Pirinioetako muturluzearentzako
metabarcoding-erako hasleen hautaketa

Argitalpena

Kapitulu honetan oinarrituriko artikulu bat *PLoS ONE* aldizkarian izan da argitaratua:

Esnaola, A., Arrizabalaga-Escudero, A., González-Esteban, J., Elozegi, A., eta Aihartza, J. (2018). Determining diet from faeces: Selection of metabarcoding primers for the insectivore Pyrenean desman (*Galemys pyrenaicus*). *PLoS ONE*, 13(12), e0208986.

Laburpena

Teknika molekularrek gorotzetatik dieta-azterketa ez inbasoreak egitea ahalbidetzen dute, galzorian dauden edo iheskorak diren espezieen beharrian ekologikoak ezagutarazteko tresna baliotsua eskainiz. Genomika, populazioen genetika, dieta-azterketa, edo ugalketa-analisen gisako gai garrantzitsuetan aurrera egiten laguntzen dute, ezinbesteko ezagutza eskainiz kontserbazio-biologian. Hala ere, teknika molekularrek metodo orokorrak ikerketen helburu zehatzen arabera egokitzea eskatzen dute, substratu eta espezieen arabera egokitzapenekin batera. Ikerketa pilotu honetan eskuragarri dauden hasle desberdinak probatu ditugu *Galemys pyrenaicus* Pirinioetako muturluzea (É. Geoffroy Saint-Hilaire, 1811, Talpidae) intsektiboro generalista urtarraren gorotzen metabarcoding bidezko dieta-analisia optimizatzeko, espezie horren kontserbazio-biologiaren inguruko ezagutza hobetzeko pauso gisa. Hogeita lau gorotz-lagin landan bildu, euren DNA erauzi, eta COI barkode eskualde estandarreko zatiak bost hasle erabiliz (Brandon-Mong, Gillet, Leray, Meusnier eta Zeale) PCR bidez anplifikatu ziren. PCRz lortutako anplikoak Illumina MiSeq platafomran sekuentziatu, sekuentziak prozesatu, UPARSE algoritmoa erabiliz OTU-etan (*Operational Taxonomic Units*) taldekatu eta NCBI datu-basearen kontra BLAST-eatu ziren. Nahiz eta hasle guztiek arrakastaz anplifikatu zituzten euren itu ziren zatiak, sekuentzia-irakurketa, OTU gordin eta taxonomia esleitutako OTU kopuru nahiko desberdinak eman zituzten. Hasleek modu erregularrean identifikatu zituzten ugariak ziren harrapakin taxon gutxi batzuk, segur aski Pirinioetako muturluzearen oinarrizko bazka adierazten zutenak. Dena den, ez zetozen bat hain arruntak ez ziren harrapakin taldeei zegokienean. Oro har, Gillet eta Zeale-ren hasleen konbinazioa izan zen taxonomikoki harrapakin aukera zabalena eta muturluzea bera ere identifikatu ahal izateko errentagarriena; etekin hori, hurrenez hurren, Leray, Brandon-Mong eta Meusnier-en hasleen emaitzak gehituz hobetzen zen. Azterketa honen emaitzak, galzorian dagoen espezie baten bazka eta habitat beharrianen karakterizazio egokiagoa ahalbidetzen dutenez, esanguratsuak dira bere kontserbazio-biologiarako.

Hitz gakoak

COI genea, dieta, ekologia molekularra, galzorian dauden espezieak, harrapari-harrapakin elkarrekintzak, HBS—Hurrengo belaunaldiko sekuentziazioa (*NGS—next generation sequencing*)

Sarrera

Zaila da kontsumitzaileen askoren dieta zehaztea: zuzeneko behaketak erronka metodologikoak izan daitezke eta ikusmen bidez gorotzetako harrapakinen hondarrak identifikatzea zaila (Clare eta kideak, 2009), bereziki jale generalisten kasuan, askotariko espezieez bazkatzen direlako. Kontsumitzaileak normalean ez dira zoriz bazkatzen eta harrapakinen hautua euren babes- edo ihes-estrategiek, nutrizio-kalitateak, edo harrapari eta harrapakinen banaketa espazialak eta denboran zeharreko banaketak baldintzatua egon daiteke, beste zenbait faktoreen artean (Symondson, 2002). DNA metabarcoding-a bezalako tresna molekular berriek dieta-azterketa ez inbasoreak ahalbidetzen dituzte, eta horrez gainera gorotza egin duen kontsumitzailea identifikatzea ere ahalbidetzen dute, honela, identifikazio erroreak saihestuz. Teknika berri hauek gorotzetatik erauzitako ingurumen-DNA (eDNA) aztertzen (Bohmann eta kideak, 2014; Sheppard eta Harwood, 2005; Taberlet eta kideak, 1999) eta gorputz bigun edo gogorreko harrapakin espezieak espezie mailaraino identifikatzen uzten dute, teknika morfologiko tradizionalekin ezinezkoa zena (Razgour eta kideak, 2011). Bestalde, DNA metabarcoding-ak dieta-azterketen bereizmena handia izatea errazten du, kontsumitzaileen bazka- eta habitat-beharrizanak are gehiago ezagutarazi eta sare trofikoaren egiturak ezagutarazteko tresna baliotsua eskainiz (Pompanon eta kideak, 2012), zeina bereziki erabilgarria den espezie iheskorrentzako eta galzorian dauden espezieentzako (Casper eta kideak, 2007; Greenstone eta kideak, 2005; Piggot eta Taylor, 2003; Valentini eta kideak, 2009). Espresuki, teknika berritzaile hauek dieta ikerketak espezieen kontserbaziorako erabilgarriak izatea dakarte. Adibidez, gorotzetako DNAREN metabarkodeen iragazpen errentagarriak landare dibertsitate zabala (99 taxa) detektatu ahal izan zuen *Lepus corsicanus* Italiako untxi zaurgarriaren dietan, hondakin solidorik uzten ez duten edo ezaugarri taxonomiko diagnostikorik ez duten aleak tartean egonik (Buglione eta kideak, 2018). Era berean, Schwarz eta kideek (2018) *Phoca vitulina* itsas txakur arruntaren arren eta emeen dietan lekuaren eta urtearen araberako desberdintasun sendoak dokumentatu zituzten, segur aski izokina bezalako harrapakin komertzialetan eragina izango dutenak. Bestalde, *Plecotus macrobullaris* saguzar alpetar belarrihandiaren ekologia trofikoa bere gorotzetan azterketa molekularrez identifikatutako harrapakinen beharrizan ekologikoetatik ondorioztatu zen, zeina ezinezkoa izango litzatekeen irrati-telemetry metodo tradizionalen bide (Alberdi eta kideak, 2012). Are gehiago, kontsumitutako harrapakinen azterketak *Rhinolophus euryale* ferra-saguzar mediterranearrak zuzenean ehizatzen duen habitaten beharra izateaz gain, ehizatzen ez duten baina euren harrapakinen larbentzako nahitaezkoak diren habitaten beharra ere baduela erakutsi zuen (Arrizabalaga-Escudero eta kideak, 2015). Horrelako emaitzek espezieen kudeaketarako jarraibideak ezartzen laguntzen dute.

Errendimendu Altuko Sekuentziazio (EAS) metodoak eraginkorrak dira harraparien dieta-azterketetan (Clare eta kideak, 2013), harrapariaren alde aurretiko identitatearen inguruko ezagutzarik izan gabe (Clare eta kideak, 2009; Valentini eta kideak, 2009; Pompanon eta Samadi, 2015) oso degradatuak, zatikatuak eta desberdinak diren DNA zatien azterketa ahalbidetzen dutelako. Hala ere, zenbait muga metodologiko izan behar dira kontutan. Alde batetik, jakien hesteetan zeharreko trantsizio-denborak eta gorotzek ingurumeneko baldintzetara duten esposizioak (adb., tenperaturaren oszilazioek, eguzkiak, euriak, hezetasunak, eta onddoen erasoek) eragina dute gorotzetako DNA-ren kalitatean (Deagle eta kideak, 2010; Oehm eta kideak, 2011; Symondson, 2002). Beste aldetik, metodo molekularren sentikortasun altuak positibo faltsu ugari sortu ditzake, bigarren mailako harrapakaritzaren, sarraski-janaren edo iturri ezberdinetako kutsaduraren eraginez (Sheppard eta kideak, 2005). Azkenik, dietaren karakterizazioaren osotasuna hasleen estaldura taxonomikoa, kontsumitutako harrapakin espezieen espektroa, PCR estrategia, sekuentziazio-prozesua, erabaki bioinformatikoak edo datu-baseetan eskuragarri dagoen informazioa bezalako xehetasunen araberakoa da (adb. Alberdi eta kideak, 2018; Brandon-Mong eta kideak, 2015; Divoll eta kideak, 2018).

Animalia lehortarrak itu dituzten ikerketek mitokondrioko c zitokromo I oxidasaren (COI) eskualdeko markatzailetan oinarritzen dira, honek kopia kopuru handia eta espezie mailako identifikazioa ahalbidetzeko aldakortasuna dituelako (Elbrecht eta Leese, 2017; Hebert eta kideak, 2003). Noski, COI-ak gaur egungo erreferentziazko datu-base osatuenetariko bat du, eta bertan ornogabe taxa asko ongi ordezkatuak daude, bereziki GenBank (<http://www.ncbi.nlm.nih.gov>) eta BOLD System (<http://www.boldsystems.org/>) datu-baseetan. Hala ere, nahiz eta oso zatikatuta dauden DNA sekuentziak — gorotzetan topatzen direnak bezalakoak — identifikatzeko COI-a nahiko laburra izan, markatzaile laburragoak arrakastaz erabili izan dira (adb. Deagle eta kideak, 2006). Markatzaileen hautaketa DNA-ren erreferentziazko datu-baseek bideratua egoten da normalean, baina ikerketaren galderaren araberakoa ere bada (Creer eta kideak, 2016). Zentzu horretan, Alberdi eta kideak (2018) dieta-azterketen emaitzak hautatutako eskualde markatzaileen, anplikoiairen tamainaren, hasleen, edota lortu nahi den maila taxonomikoaren araberakoak izaten direla erakutsi zuten. Harrapakin espezie batzuk hasle askok detektatzen dituzten bitartean, beste batzuen detekzioa hasleen araberakoa izaten da. Eskualde luzeegiak anplifikatzekotan, espezie batzuk ez dira identifikatuko, negatibo faltsuak eraginez. Eragin horrek laginen edo banakoen arteko desberdintasunak puztuko ditu, aldakortasun intraespezifikoren inguruko informazio partziala emanaz. Hortaz, eskualde markatzaileen eta hasleen hautaketa erabaki kritikoa da edozein DNA metabarcoding-ikerketetan, hasleen luzera edo espezifikotasuna bezalako faktoreek emaitzetan eragin handia dutelako (Elbrecht eta Leese, 2017; Gibson eta kideak, 2014).

Dieta-azterketetan positibo faltsuek eta negatibo faltsuek ondorio gogorak izan ditzakete harrapariaren ekologiaren interpretazioan eta ekosistemen kudeaketan (Hines eta Gessner, 2012). Harrapakin garrantzitsuen identifikazio arrakastatsua gakoa da edozein harrapariaren —edo eurek kontsumitutako harrapakin (Schwarz eta kideak, 2018; Waraniak eta kideak, 2018)— eginkizun ekologikoaren, espezializazio trofikoaren edo kontserbazioaren inguruko ondorio sakonak lortzeko. Horrez gain, gorotzetatik dieta eta harrapariaren identitatea aldi berean identifikatzearen beharrak erabaki metodologikoak oraindik ere zorrotzagoak izatea dakar, jomugan espektrro filogenetiko zabalagoa izateagatik.

Hemen DNA metabarcoding-ikerketa bat egin da, harrapari urtar baten dieta eta identitatea modu ez inbasorean zehazten duten hasle ezberdinak konparatzeko. Bereziki, hasle desberdinek —edo euren arteko konbinazioak— dietaren eta harrapariaren identitatearen karakterizazio filogenetikoan nola eragiten duten ebaluatzea izan da helburua.

Pirinioetako muturluzea (*Galemys pyrenaicus*, É. Geoffroy Saint-Hilaire, 1811, Insectivora, Talpidae) hautatu da ereduzko espezie gisa, mendiko erreka hotz eta garbietan bizi den, erdi-urtarra eta generalista den ugaztun intsektiboroa (1. irudia). Endemikoa da Iberiar Penintsularen iparraldean eta Pirinioetan, baina bere banaketa-eremua gogorki murriztua izan da azken hamarkadetan, eta



1. Irudia. Pirinioetako muturluzea Elama errekan. Argazkia: Joxerra Aihartza.

horrek Naturaren Kontserbaziorako Nazioarteko Batasunaren (IUCN) zerrenda gorriaren kategorietan Zaurgarri gisa barneratzea ekarri du (Fernandes eta kideak, 2008). Azken ikerketek muturluzea nagusiki ornogabe urtarrez bazkatzen dela (Biffi eta kideak, 2017a; Hawlitschek eta kideak, 2018) eta urbare eta putzuen aldean urlasterrak nahiago dituela (2. Kapitulu; Esnaola eta kideak, 2018b) erakutsi dute. Hala ere, galzorian dagoen espezie hau egokiro kudeatzeko informazio gabezia dago oraindik (MAGRAMA, 2013; Némoz eta Bertrand, 2008). Hain zuzen ere, ezezaguna da oraindik zein puntutaraino dagoen muturluzearen dieta harrapakin-eskuragarritasunaz baldintzatua, eta zein harrapakin mota hautatzen dituen; hare gehiago, oraindik ez dago argi muturluzeen erreken baitako habitat-hautespenak (2. Kapitulu; Esnaola eta kideak, 2018b) harrapakinaren eskuragarritasunaren diferentzia edo beste faktoreren bat islatzen duten. Galdera horiek, eta beste batzuek, dieta-azterketa zehatzen beharra azaleratzen dute.

1. Taula. Aztertutako gorotz-laginen kokapena.

Arroa	Erreka	Gorotz kopurua	Kaka-leku artifizialetan bilduak ^a	Koordenatuak ^b
Urumea	Asura	3	Ez	43°08'03"N 1°48'10"W
				43°07'46"N 1°47'07"W
				43°08'21"N 1°49'39"W
Ollin		3	Ez	43°07'44"N 1°51'01"W
				43°07'44"N 1°51'01"W
				43°07'37"N 1°51'00"W
Añarbe		3	Ez	43°13'09"N 1°51'17"W
				43°13'18"N 1°51'05"W
				43°13'18"N 1°51'05"W
Elama		9	Bai	43°12'37"N 1°48'38"W
				43°12'37"N 1°48'38"W
				43°12'37"N 1°48'38"W
				43°10'59"N 1°47'59"W
				43°10'59"N 1°47'59"W
				43°10'58"N 1°47'56"W
				43°10'58"N 1°47'56"W
				43°10'57"N 1°47'56"W
				43°10'57"N 1°47'56"W
Oria	Leitzaran	6	Bai	43°08'59"N 1°57'19"W
				43°08'59"N 1°57'19"W
				43°08'59"N 1°57'19"W
				43°08'59"N 1°57'18"W
				43°09'00"N 1°57'19"W
				43°09'00"N 1°57'19"W

^a Laginak kaka-leku artifizialetan bildu diren (Bai) edo ez (Ez) adierazten du.

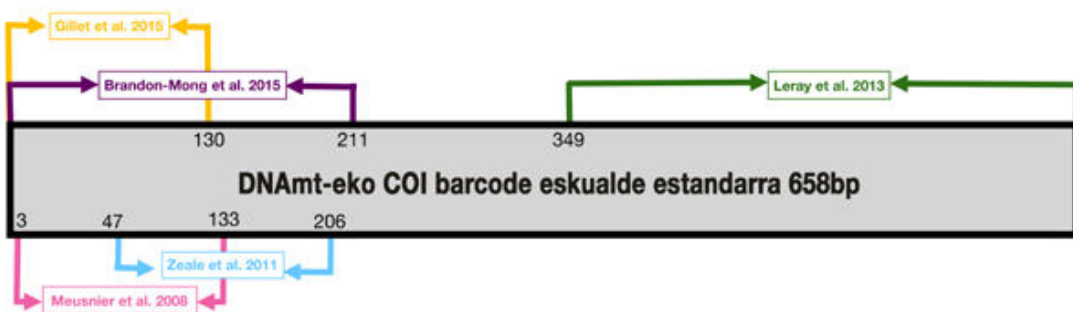
^b Laginak zehazki zein lekutan bildu diren adierazten du.

Ikerketa honek dieta-azterketetan DNA metabarcoding-a erabiltzerakoan hasleen etekinaren diferentzien inguruko informazio baliotsua eskaintzen du, hasle bakoitzaz eta euren arteko konbinazioaz lortutako estaldura taxonomikoaren inguruko laburpena eginez. Bestalde, muturluzearen ekologiari dagokionean, argitaratutako ikerketen (adb. Biffi eta kideak, 2017a; Hawlitschek eta kideak, 2018) emaitzak hobeto ulertu eta konparatzeko, zein galzorian dagoen espezie honen habitat-beharrizanak ezagutarazteko erabilgarri izango dira emaitzak. Informazio hau ezinbestekoa da bere kontserbaziorako berriarazko kudeaketa ekintzak diseinatu eta egikaritzeko.

2. Taula. Ikerketa honetan erabilitako bost hasleen xehetasunak.

Izen arrunta	Hasleen izenak	Aurreranzko haslea(k) (5'-3')	Atzeranzko haslea(k) (5'-3')	Luzera (bp) ^a	Jatorria
Brandon-Mong	F:LepF1	ATTCAACCAATCATAAAGA	CGTGGAAWGCTATATC	218	Brandon-Mong <i>et al.</i> , 2015
	R:MLepF1_Rev	TATTGG (25bp)	WGGTG (21bp)		
Gillet	F:LepF1 (modified)	ATTCHACDAAYCAYAARGA	ACTATAAAAARAAAATYTD	133	Gillet <i>et al.</i> , 2015
	R:EPT-long-univR (modified)	YATYGG (25 bp)	AYAAADGCRTG (29 bp)		
Leray	F:mICOlintF	GGWACWGGWTGAACWG	TAIACYTCIGGRTGICCR	313	Leray <i>et al.</i> , 2013
	R:jgHCO2198	TWTAYCCYCC (26 bp)	ARAAYCA (26 bp)		
Meusnier	F:Uni-MinibarF1	TCCACTAATCACAARGATAT	GAAAATCATAATGAAGGC	130	Meusnier <i>et al.</i> , 2008
	R:Uni-MinibarR1	TGGTAC (26 bp)	ATGAGC (24 bp)		
Zeale	F:ZBJ-ArtF1c	AGATATTGGAACWTTATATT	WACTAATCAATTWCCAAA	157	Zeale <i>et al.</i> , 2011
	R:ZBJ-ArtR2c	TTATTTTTGG (30bp)	TCCTCC (24bp)		

^a Hasleak kenduta aplikioiak duen neurria (bp = base pare).



2. Irudia. Hasleen kokapenak. COI mitondrialean markatzaileen kokapenen irudikapena (Brandon-Mong eta kideen (2015) lanetik moldatua).

Material eta metodoak

Lagin-bilketa

Pirinioetako muturluzearen gorotzak Urumea eta Oria ibaien arroetan bildu genituen, Euskal Herrian (Iberiar Penintsularen iparraldean), 2015eko azaroa eta 2016ko apirila artean, arroketako zuloetan eta sustraietan zuzenean bilatuz edo bereziki espezie honentzako diseinatutako kaka-leku artifizialak (1. Eranskina; González-Esteban eta kideak, 2018) eraikiz (1. taula). Denera 24 gorotz gorde ziren %98-eko etanolean eta -80°C -tan izoztu ziren.

Ikerketa hau Pirinioetako muturluzearen ekologia espazial eta trofikoaren ikerketa zabalago baten parte da, lekuko betebeharrak juridikoak bete zituen eta Euskal Herriko Unibertsitateko Animalien Ongizaterako Etika Batzordeak onartua izan zena (erref. CEBA/M20/2016/022). Ikerketa honetan egindako jarduerak ez zuten baimen zehatzik eskatzen, laginak modu ez inbasorean bildu zirelako eta ez zelako galzorian edo babestuta dagoen espezierik manipulatuta.

Hasle unibertsalen hautaketa

Harrapakin espezie potentzialen estaldura taxonomiko ahalik eta zabalena helburu izanik, bost hasle hautatu genituen (2. taula). Hasle horiek, egun ongi dokumentatutako erreferentziazko datu-basea duen eta animalien barkode eskualde estandarra den COI-aren baitako luzera desberdineko zatiak aplikatzen zituzten (2. irudia).

DNA-erazketa, liburutegiaren prestaketa eta HBS-sekuentziazioa (NGS)

DNA erazteko Quiagen Powerfecal DNA kit-a (Quiagen Iberia, S.L. Madril) erabili zen, fabrikatzailearen jarraibideak jarraituz. Ondoren, erazkinteko DNA PCR bidez aplikatu zen bost hasle erabiliz (2. taula), Euskal Herriko Unibertsitateko UPV/EHU Ikerkuntzarako Zerbitzu Orokorretan (SGIker). Laginak purifikatu, eta bigarren erreakzio bat egin zen Illumina Nextera v2 Kit-a erabiliz, aplikatutako produktu bakoitza indexatu eta Illuminako lotailuak eransteko. Zeale, Gillet, Leray eta Meusnier-en hasleekin egindako aplikazioak Quiagen Multiplex PCR Kit protokoloarekin egin ziren (Quiagen Iberia, S.L. Madril), $12,5\ \mu\text{L}$ Quiagen 2X (1X amaieran), $1,25\ \mu\text{L}$ aurreranzko hasle ($10\ \mu\text{M}$; $0,5\ \mu\text{M}$ amaieran), $1,25\ \mu\text{L}$ atzeranzko hasle ($10\ \mu\text{M}$; $0,5\ \mu\text{M}$ amaieran), $8\ \mu\text{L}$ H_2O eta $2\ \mu\text{L}$ DNA erabiliz,

amaierako 25 µL-ko bolumenetan. Brandon-Mong-en haslearekin egindako anplifikazioak aldiz 12,5 µL KAPA HIFI 2X (1X amaieran), 2,5 µL aurreranzko hasle (10 µM; 1 µM amaieran), 2,5 µL atzeranzko hasle (10 µM; 1 µM amaieran), 5,5 µL H₂O eta 2 µL DNA erabiliz egin ziren, amaierako 25 µL-ko bolumenetan. Hasle bakoitzak bere PCR-programa propioa zuen, erabilitako errektiboaren erreferentzia moldatuz, S1. taulan adierazi bezala. Anplifikatu ostean, PCR-z ekoiztatutako anplikoiak *MiSeq Reagent Kit v3 (600 ziklo)* erabiliz *Illumina MiSeq NGS plataforman* sekuentziatu ziren (parekatutako muturreko 2x300 bp-ko irakurketen sekuentziazioa), fabrikatzailearen jarraibideak jarraituz.

Analisi bioinformatikoak

USEARCH erabiliz (Edgar, 2010; Edgar eta Flyvbjerg, 2015), parekatutako muturreko sekuentzia-irakurketak bateratu ziren, eta ondoren hasleen bidez demultiplexatu, adaptadoreen eta hasleen sekuentziak ezabatu. CUTADAPT erabiliz (Martin, 2011) kalitatearen eta luzeraren arabera filtratu ziren sekuentzia-irakurketak. Ondoren, sekuentzia-irakurketa bakan edo *singletonak* ezabatu, eta gainontzeko sekuentziak USEARCH erabiliz miatu ziren kimerak topatzeko. Sekuentziak euren artean %97-ko antzekotasun-atalasearen arabera elkartu, eta OTU-ak (*Operational Taxonomic Units*) sortu ziren UPARSE algoritmoa (Edgar, 2013) erabiliz. Azkenik, BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) erabiliz, Genbank nt datubasea arakatu zen OTU-ei taxonomia esleitzeko. Espezie mailako esleipenak OTU-en sekuentziek erreferentziazko sekuentzietatikiko %98 baina parekako (*pairwise*) antzekotasun handiagoa eta %75 baina estaldura (*query cover*) handiagoa zutenean onartu ziren (Clare eta kideak, 2013). OTU-en sekuentziek datu-basean espezie batekin baina gehiagorekin bat egiten bazuten luzera handieneko parekatzea zuen emaitza hautatzen zen. Horrez gain, arau gisa, $1e^{-20}$ baina e-balio baxuagoko emaitzak soilik onartu ziren (Vesterinen eta kideak, 2013), parekatzea zoriz gertatu ez zela ziurtatzeko.

Ondorengo analisiak identifikatutako harrapakin-taxaren agerpenak (zenbat gorotzetan topatu zen taxon bat) (Shiel eta kideak, 1997) kontutan hartuz egin ziren. Hasleen emaitzetatik eraikitako OTU-ak ere aztertu ziren, horietako baten batek harraparia bera ere, hots Pirinioetako muturluzea, identifikatzen ote zuten ikusteko.

Datuen analisisia

Hasleek dietako espezieen konposizioaren aldakortasunean duten erabateko eragina aztertzeko, permutazio bidezko ANOVA multibariantea egin genuen

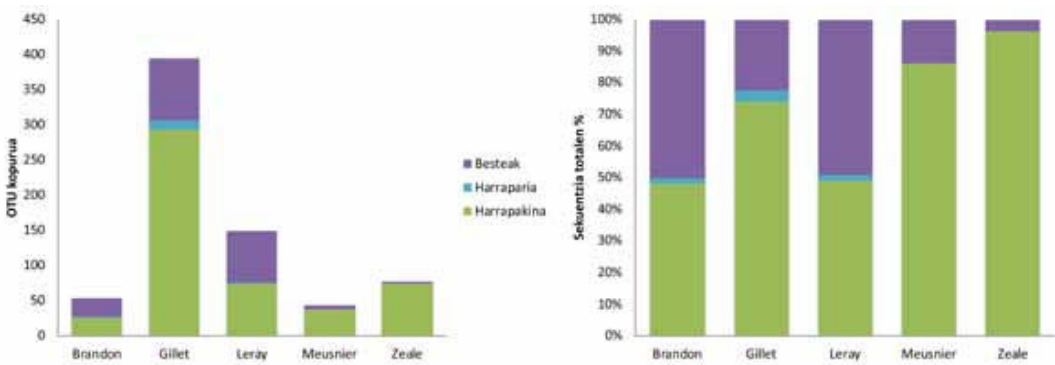
3.4.3 bertsioiko R-n (R Core Team, 2017) *vegan* 2.4–6 paketeko (Oksanen eta kideak, 2016) *adonis* erabiliz, azarezko 999 permutazioarekin. Laginen arteko diferentziak kalkulatzeko Jaccard distantzia-neurria erabili zen. Espezieen osaketan laginen artean zeuden desberdintasunak irudikatzeko NMDS bat egin genuen R-rako *vegan* 2.4–6 paketearekin. Espezieen osaketan hasleen artean zeuden parekako desberdintasunak ere aztertu ziren, R-rentzako *RVAideMemoire* 0.9-69-3 paketeko *pairwise.perm.manova* funtzioa erabiliz (Hervé, 2014). Hasleen baitako espezie osaketaren aldakortasuna (hots, homogeneousitasuna) 999-ko permutazioa erabiliz aztertu zen, R-rentzako *vegan* 2.4–6 paketeko (Oksanen eta kideak, 2016) *betadisper* eta *permutest* funtzioekin. Homogeneousitasunean hasleen arteko binakako desberdintasunak Tukey-ren HSD probarekin aztertu ziren, R-rentzako *vegan* 2.4–6 paketea erabiliz (Oksanen eta kideak, 2016).

Emaitzak

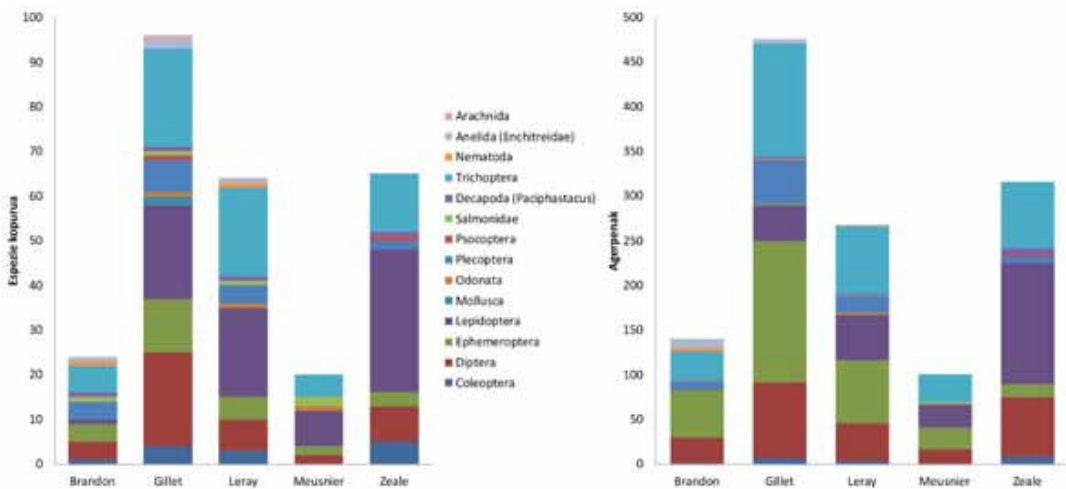
Hasleen artean sekuentziazioaren emaitzak nahiko desberdinak izan ziren, bai sekuentzien kopuruari zegokionean, baita OTU kopuru gordinari (osoari) zegokionean ere (3. irudia). Gillet hasleek lortu zuten sekuentzia eta OTU gordin kopuru altuena, eta ondoren Leray-renek (S2. taula). Brandon-Mong, Gillet eta Leray-ren hasleek muturluzea bera ere identifikatu zuten, baina Gillet eta Leray-renek soilik lortu zuten gorotz guztietan identifikatzea; ez zen muturluzea ez zen beste harraparirik identifikatu. Bost hasleek identifikatu zituzten harrapakin taxon arruntenak (hots, agerpen handienak zituzten *Baetis* sp., *Hydropsyche* sp., *Odontocerum* sp. eta *Psychoda* sp.), baina hain maiz ageri ez ziren harrapakin taldeei zegokienean ez zetozen guztiz bat (4. irudia eta S3. taula). Kuantitatiboki, Gillet-ek lortu zuen taxonomia esleitutako OTU zerrenda luzeena (OTU-en %19,15), eta baita agerpen balio altuenak ere. Zeale eta Leray-ren hasleek jarraitzen zuten aurrekoa, bai OTU gordinari eta esleipenei zegokienean ere (OTU-en %38,5 eta %7,81 esleituta, hurrenez hurren); aldiz, etekin baxuena Brandon-Mong eta Meusnier-en hasleek erakutsi zuten, OTU gordin gutxiagorekin eta OTU-en %15,79 eta %7,12 esleituta, hurrenez hurren. Esleitutako taxon kopurua kontutan izanik, Gillet-en hasleek lortu zuen Mollusca, Annelida eta Diptera, Ephemeroptera, Plecoptera, Trichoptera edo Arachnida bezalako artropodoen DNA anplifikatzen errendimendu altuena (S3 Table); Meusnier-en hasleek Salmonidoak anplifikatzen lortu zuen errendimendu altuena, eta Zeale-renak aldiz Coleoptera anplifikatzen izan zen hoberenak. Nahiz eta antzemandako agerpenak baxuagoak izan, Leray-ren hasleek Trichoptera eta Coleoptera anplifikatzen ere nahiko errendimendu altua lortu zuen. Gillet-en hasleen emaitzak Zeale-renekin alderatuta, Gillet-enek Mollusca eta Diptera, Ephemeroptera, Plecoptera, Trichoptera eta Arachnida bezalako

artropodoen inguruan Zeale-renek baino informazio gehiago eman zuen, Coleoptera, Psocoptera eta Decapodarekin kontrakoa gertatu zen bitartean. Bestalde, Gillet-en hasleen emaitzak Leray-renekin alderatuz, Leray-renek baina emaitza hobekia lortu zituen Gillet-enek Mollusca, Annelida eta Coleoptera, Diptera, Ephemeroptera, Plecoptera, Psocoptera, Trichoptera eta Arachnida bezalako artropodoak identifikatzean, Leray-renek Nematodarako balio pixka bat altuagoak lortu zituen bitartean.

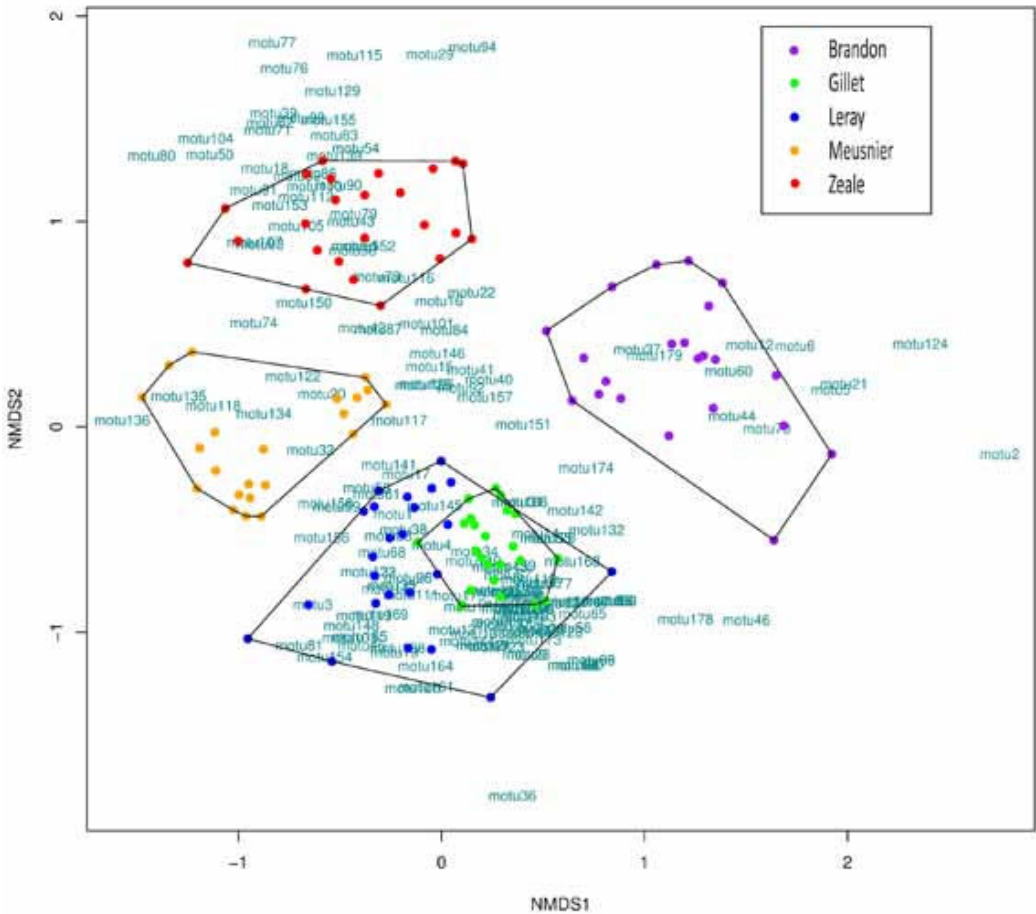
Hasleen hautaketak estatistikoki eragin esanguratsuak izan zituen dietaren azken osaketan ($F_{(4,115)} = 15,712$; $R^2 = 0,353$; $p = 0,001$). NMDS-az argi eta garbi adierazi zen hori (5. irudia), laginak lau talde nagusitan taldekatu zirelarik: Zeale, Meusnier, Brandon-Mong eta Leray+Gillet. Hasleak euren artean orokorrean eta pareka konparatuz ezberdintasun esanguratsuak lortu ziren espezieen osaketari zegokionean (proba orokorra: $F = 10,425$; $p < 0,001$;



3. Irudia. Hasle bakoitzarekin lortutako OTU-en espezie mailako esleipen taxonomikoak. OTU kopurua eta lortutako sekuentzia totalaren portzentaia.



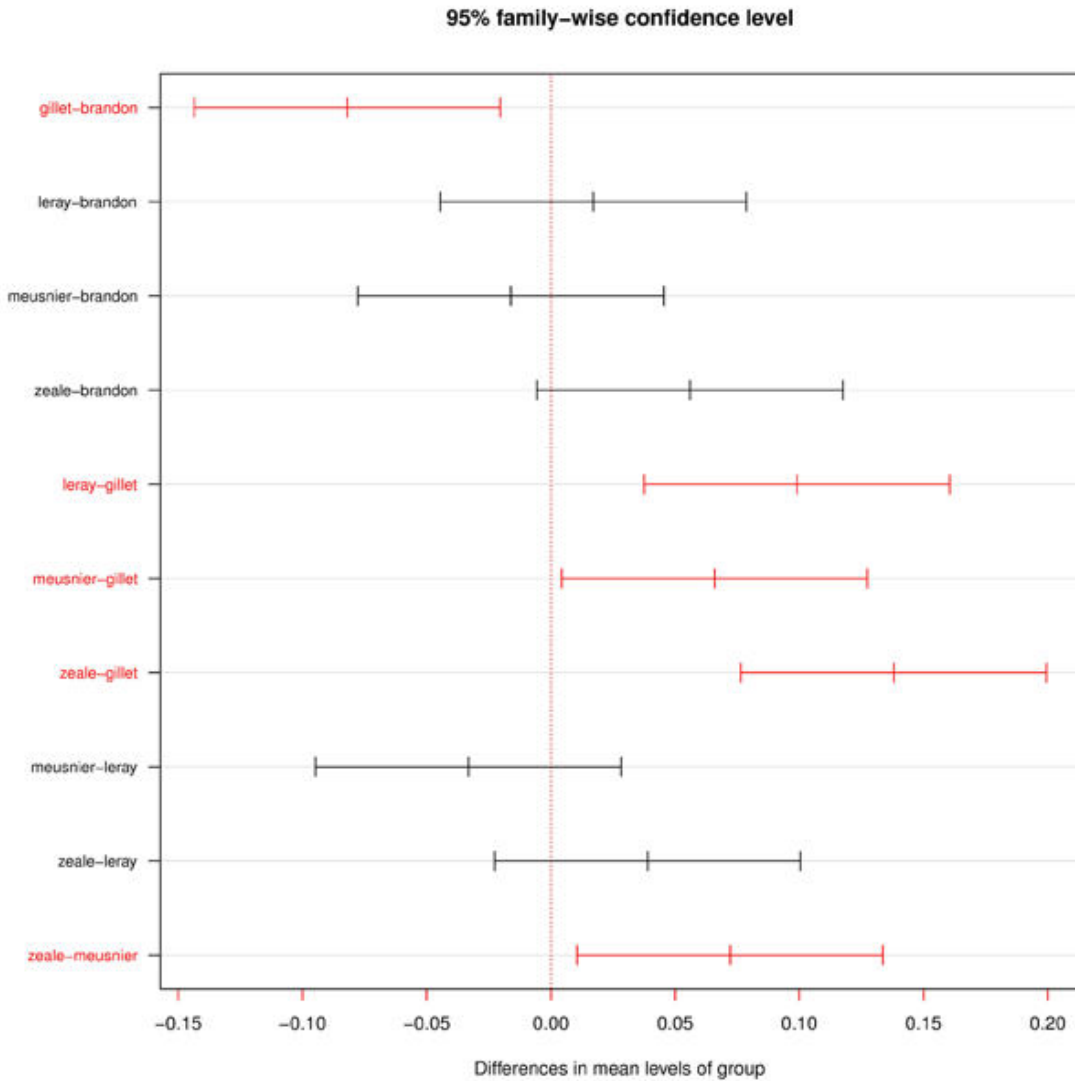
4. Irudia. Hasle bakoitzarekin espezieei esleitutako OTU kopuruak eta euren agerpenak. Maila taxonomiko altuak kontutan hartuta lortutako emaitzak.



5. Irudia. Laginen NMDS ordenazioa. Estresa = 0,193; $k = 2$; R^2 -aren doitze ez metrikoa = 0,963. Puntuek muturluzeen lagin bakoitza adierazten dute eta koloreek hasleak bereizten dituzte. OTU-ak letra berdeekin adierazi dira. Hurrunago dauden puntuek desberdinagoak diren harrapakin-konposizioak erakusten dituzte.

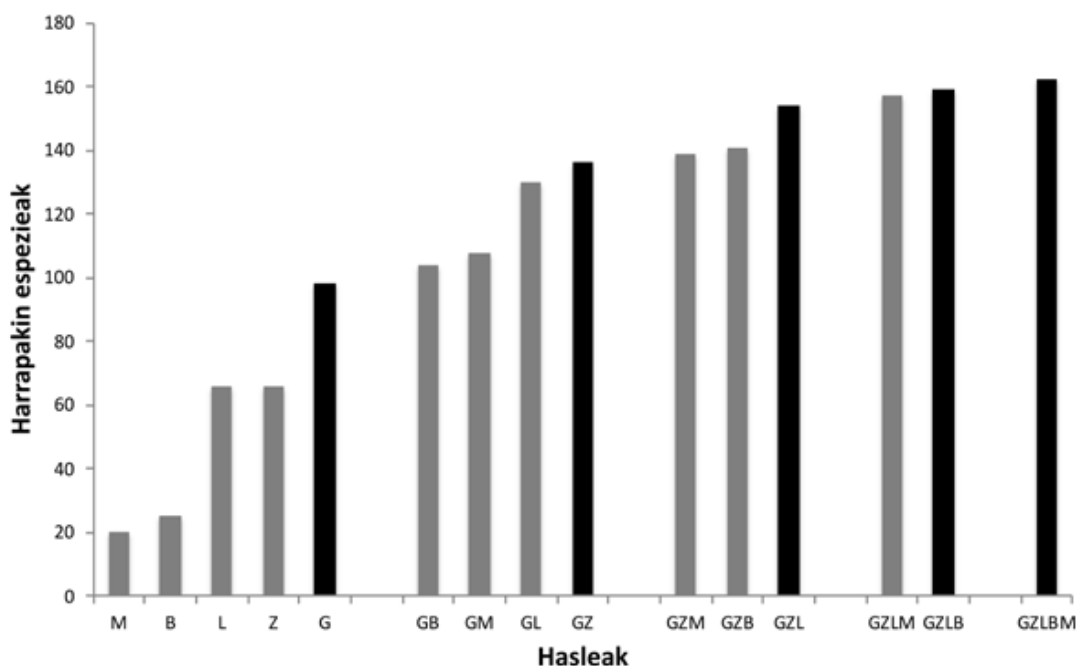
parekako proba: $p = 0,001$ guztietan), baina Zeale eta Leray-ren edo Gillet-en hasleekin anplifikatutako laginak izan ziren espezie osaketari zegokionean emaitza desberdinenak lortu zituztenak, Leray eta Gillet-en hasleek nahiko osaketa antzekoa erakutsi zuten bitartean, Leray-renek aniztasun handiagoa lortu zuela zirudien arren. Bestalde, espezieen osaketari zegokionean, Meusnier eta Gillet-en hasleen arteko diferentziak izan ziren baxuenak ($p = 0,029$), Zeale eta Meusnier-enez ($p = 0,013$) eta Gillet eta Brandon-Mong-enez ($p = 0,003$) jarraiki. Diferentziarik handienak aldiz, Zeale eta Gillet-enen artean topatu ziren ($p < 0,001$), Leray eta Gillet-enez ($p = 0,001$) jarraiki (6. irudia).

Bost hasleen anplifikazioen produktuak metatuz harrapakin espezie potentzialen identifikazioa optimizatu genuen. Gillet-enek lortu zituzten harrapakin identifikazio gehien (7. irudia). Gillet-en hasleak beste hasleekin konbinatuz, Zeale-renekin konbinatuz hazi zen gehien identifikatutako espezie kopurua



6. Irudia. Hasle guztien arteko konparaketentzako Tukey-ren HDS grafikoa. Kolore gorriak alde esanguratsuak adierazten ditu ($p < 0,05$). Grafikoa taldeen mailen arteko batzbesteko diferentziak erakusten ditu, non homogeneotasunari dagokionean Gillet eta Zeale-ren hasleen arteko diferentzia den handiena, Gillet eta Leray-ren artekoak jarraitzen duelarik.

(129 harrapakin espezie edo talde taxonomiko zehatz denera; Gillet-enak soilik erabiliz baina %37,2 gehiago). Ondorengo konbinazioetan, identifikatutako harrapakin espezieen kopurua Leray-renak gehituta hazi zen gehien, eta gero Brandon-Mong eta Meusnier-enak gehituta, hurrenkera horretan, pauso bakoitzean kuantitatiboki hazkuntza txikiagoa ematen zelarik.



7. Irudia. Hasle desberdinen konbinazio progresiboz lortutako harrapakin espezie kopuruaren metaketa. Hasleen mailakako konbinazioak (bat+bat, bi+bat, etabar) aurreko konbinazio-mailan metatutako espezieen kopuru altuenen arabera hautatu ziren (zutabe beltzak). B-k Brandon-Mong-en hasleak adierazten ditu, G-k Gillet-enak, L-k Leray-renak, M-k Meusnier-enak eta Z-k Zeale-renak.

Eztabaida

Gure ikerketak markatzaile molekularren aukeraketak —gure kasuan gene bereko gene desberdinak itu dituzten hasleak— dietaren karakterizazioan dezente eragiten duela erakutsi du. Hori filogenetikoki dieta zabala duten harrapari generalisten kasuan da bereziki garrantzitsua. Nahiz eta harrapakin taxon batzuk bost hasleak erabiliz erregulariki detektatu —maizen kontsumitutakoak, segur aski—, dietaren osaketaren azken argazkia erabilitako haslearen arabera izango da. Gure emaitzek Alberdi eta kideek (2018) saguzarretan lortutakoak baieztatu zituzten, bi markatzaile genetiko desberdin (COI-a eta 16S-a) itu zituzten lau hasle errendimendu ezberdina zutela behatu baituzten.

Hasleen errendimendua kuantitatiboki desberdina izateaz gain sekuentzia-irakurketen kopuru totalen, OTU gordinetan zein taxonomia esleitukoetan, eta identifikatutako ustezko harrapakin espezieen kopurutan, kualitatiboki ere desberdina izan zen, hasle bakoitzarekin lortutako talde filogenetiko nagusien proportzioan. Desberdintasun horiek taxon zehatzak anplifikatzeko hasle bakoitzak duen berariazko afinitatearen edo hasle bakoitzaren endekapen mailaren ondorio izan litezke.

Espresuki, DNA sekuentzia luzeagoek digestioa okerrago jasaten dutenez (Deagle eta kideak, 2006), DNA zati luzeagoak itu dituzten hasleek errendimendu baxuagoa izango dute gorotzetan harrapakinak identifikatzerako orduan. Luzera zehatzeko sekuentzia baten osotasuna ehunen arteko digestio-desberdintasuna, digestioan zeharreko denbora, eta erretentzio-denbora bezalako faktore askorekin erlazionatua legoke, eta desberdintasun hori aldakorra da harrapakin espezieen artean (Clare, 2014-n berrikusia). Hori hein batean bat dator gure ikerketarekin, izan ere Gillet-en hasleek, COI geneko ia zati laburrena itu dutenek (1. taula), lortu zituzten balio altuenak sekuentzia-irakurketei, OTU-ei eta detektatutako harrapakin potentzialei zegokienean. Leray-ren hasleek aldiz, taxonomikoki antzeko jomugak izan arren askoz aplikoi luzeagoak lortzen dituztenek, DNA zati gutxiago berreskuratutako zituzten eta ez ziren gai izan Gillet-en hasleek berreskuratutako zenbait taxon aplikatuzeko. Hala ere, ia zati luzera berdina jomuga zuten hasleek ere —Gillet eta Meusnier-enek, adibidez— emaitza desberdinak lortu zituzten, datu kuantitatiboek zein kualitatiboek zegokienean. Hori hasleen endekapen maila desberdinaren ondorio izan liteke (Elbrecht eta Leese, 2017). Izan ere, endekapen maila handiena duten Gillet eta Leray-ren hasleak (2. taula) izan ziren OTU gehien eman zituztenak, nahiz eta ondoren horietako asko taxon ezagunei esleitzea ezinezkoa izan (horietako %19.15 eta %7.8, hurrenez hurren); jomuga zuten COIaren zatitik haratago, beste DNA substratu asko aplikatuzeko ere gai liratekeela iradokitzen du honek. Endekapen maila baxuagoa zuten hasleek, aldiz —hala nola Zeale-renek— OTU kopuru askoz txikiagoa lortu zuten, nahiz eta horietatik ehuneko handiagoa izan taxon ezagunei esleitutakoak (%38,5), barkode-eskualdera lotura sendoagoa erakutsiz. Ondorioz, digestioaren ostean erlatiboki luzeak diren DNA-sekuentzien iraunkortasun aldakorrak eta horiek aplikatuzeko erabilitako hasleek eragina dute banakoen dietaren inguruan jasotako amaierako pertzepzioan. Hortaz, dieta-azterketetarako hasle egokien hautaketa ezinbestekoa da dietan aldakortasun handia espero den kasuetan (De Barba eta kideak, 2014).

Muturluzearen dieta aztertzeko ibilbide bat publikatu da berriki (Hawlitschek eta kideak, 2018), non “nested PCR”-en bidez gorotz zahar eta berrien DNA metabarcoding-a egiten den. Ekarpen metodologiko horrek aurre-aplikaziozko pauso bat gehitzen du, helburu diren taxari dagozkien sekuentzia-irakurketen kopuruak handitzeko. Autoreek, ordea, euren prozedurak aplikazioaren espezifikotasunean igoera eragin zezakeela azpimarratu zuten, hortaz, funtsezko espezie batzuen sekuentziak galduz. Gure emaitzek “nested PCR”-ak hasleen alborapena handiagotuko lukeela iradokitzen dute, erabilitako bi hasleen eragina biderkatuz. Hawlitschek eta kideek (2018) ere, etorkizunean ikerketen helburu dugun taldearen aplikazioaren arrakasta hobetzeko, hasle bat baino gehiagoren aplikazio produktuen hasle-koktel edo -konbinatuak

(*pools*) erabili beharko liratekeela proposatu zuten. Hori da, hain zuzen ere, lan honek aztertu eta egiaztatu duena.

Muturluzearen ekologia trofikoari dagokionean, berriki egin diren bi metabarcoding-ikerketek datu berriak eman dituzte: Biffi eta kideek (2017a), Gillet-en hasleak soilik erabiliz, Pirinioen iparraldean espeziearen udako dieta zabala batez ere Ephemeroptera eta Plecopteran oinarriturikoa zela ikusirik, muturluzea “lehenago uste zena baina generalistagoa” zela esan zuten; Hawlitschek eta kideek (2018), Meusnier-en hasleekin eta nested PCR-ekin lan eginez, Iberiar Penintsularen ipar-mendebaldean harrapakin talde ugariak Ephemeroptera eta Diptera zirela ikusi zuten. Nahiz eta eskualde desberdinean lan egin izan eta lagin tamainari dagokionean mugatuago egon, gure datuek ere taxon horiek identifikatu zituzten neguko muturluzeen dietaren beharrezko osagai gisa. Hala ere, espezieen kopurua eta euren agerpen-tasak erabilitako haslearen araberrakoa izan ziren nagusiki. Ikerketa horien emaitzek zein mailataraino erakusten dituzte alde metodologikoak, muturluzeen harrapakin-eskuragarritasunaren eskualde mailako desberdintasunak erakutsi beharrean? Ez da erraza esaten. Muturluzearen —edo beste edozein animaliaaren— dietaren karakterizazioa hasle-joko bakarra erabiliz eginez gero alborapen larriak izango dituela iradokitzen dute gure emaitzek. Horiek harrapari-harrapakin interakzioen edo harrapakin-hautespen patroien gisako ondorio ekologikoak ahulduko lituzkete, eta horiek gako izan daitezke kontserbazio-neurri egokiak ezartzeko.

Espezie bat generalista gisa deskribatzen denean, izan daiteke bai banako-mailako dieta espezialista ezberdin asko kontutan hartzen direlako (Araujo eta kideak, 2011), edo bai banakoak elikagai-dibertsitate zabalez bazkatzeko gai direlako (Clare eta kideak, 2011). Harrapari generalista baten dieta aztertzerakoan, haslearen aukeraketak bere ekologia trofikoaren eta espezializazioaren interpretazioa baldintzatuko du. Bazka-ohiturak hautemateko moduak komunitatearen dinamiken eta ekosistemen funtzionamenduaren interpretazioan eragiterik du (Bolnick eta kideak, 2011), eta hortaz, dietaren edozein deskribapen alboratuk kate trofikoaren egiturak gaizki ulertzera edo kontserbaziorako erabaki desegokiak hartzera eraman ditzake kudeatzaileak (Bolnick eta kideak, 2003; Devictor eta kideak, 2010). Adibidez, saguzarrentzako (Burgar eta kideak, 2013; Mata eta kideak, 2016; Krauel eta kideak, 2018), hegaztientzako (Gerwing eta kideak, 2016; Ando eta kideak, 2018), karraskarientzako (Lopes eta kideak, 2015) eta barraskiloak (Boyer eta kideak, 2013) bezalako ornogabeentzako —besteak beste— teknika molekularrak erabiliz deskribatu izan dira erlazio trofikoak. Espezie baten beharizan trofikoaren inguruko ezagutza zehatza jasotzeko funtsezkoa da ekologia trofiko eta espazialaren ikerketa zehatzetan inbertitzea. Harrapakin espezie potentzialen ahalik eta aukera zabalena detektatzeko, eta funtsezko informazioa galtzea

saihesteko, hasle desberdinak konbinatzearen garrantzia nabarmentzen dute gure emaitzek. Erabaki metodologikoez edozein animalia-eraren beharizan trofikoaren balorazioan eragiten dute, bai eta balorazio horretan oinarritutako kudeaketa neurrietan ere (Kohn eta Wayne, 1997).

Gainera, galzorian dauden edo iheskorak diren animaliekin lan egiterako orduan ezinbestekoa da bildutako laginen adskripzioa —gorotzen egilea, kasu— zuzenki identifikatzea. Oraingoz, tresna molekularrak erabilgarriak izan dira saguzarrak (Vesterinen eta kideak, 2013; Arrizabalaga-Escudero eta kideak, 2018), haragijaleak (Farrel eta kideak, 2000; Shehzad eta kideak, 2012), pinipedoak (Casper eta kideak, 2007), hegaztiak (Marchesi eta kideak, 2002) eta muturluzea bera (Biffi eta kideak, 2017a) bezalako harrapari sorta zabal baten gorotzetatik dieta-deskribapen zehatzak lortzeko. Ikerketa honetan probatutako hiru haslerekin, hots, Leray, Gillet eta Meusnier-enekin, identifikatu zen muturluzea gorotzetatik. Hala ere, Leray eta Gillet-en hasleek soilik identifikatu zuten muturluzea arrakastaz analizatutako gorotz lagin guztietan, Meusnier-enek kordatuak identifikatzeko errendimendu baxuagoa dutela baieztatuz (Meusnier eta kideak, 2008). Oro har, nahasgarriak diren gorotzak dituzten harrapari intsektiboroen dieta-azterketetan, aukeratutako hasleen artean hasle batek behintzat harrapariaren DNA anplifikatzeko gai izan behar du, identifikazio erroreak murrizteko.

Gure emaitzek Pirinioetako muturluzearen dieta hasle desberdinak konbinatuz karakterizatu beharko litzatekeela iradokitzen dute, baina hasleen hautaketak datuen zehaztasuna eta kostuak ere hartu behar ditu kontutan. Errentagarritasunari dagokionean, Gillet eta Zeale-ren hasleen arteko konbinazioa litzateke hoberena, harrapakinen aukera taxonomiko ahalik eta zabalena identifikatzeaz gain, muturluzea bera ere identifikatzeko. Konbinazio hori, mailaka aberaste altuenetik baxuenera, Leray, Brandon-Mong eta Meusnier-en hasleak gehituz hobetuko litzateke. Hala eta guztiz ere, hasleak konbinatzerakoan anplifikatutako sekuentzien luzerak euren artean oso desberdinak ez izatea ere kontutan izan behar da, HBS-sekuentziazio-prozedura batzuek aplikoi laburren alde dituzten alborapenak saihesteko. Hortaz, gure kasuan, Gillet eta Zeale-ren hasleen konbinazioak Gillet eta Leray-ren hasleen konbinazioak baino arazo gutxiago emango lituzke, Leray-renekin lortutako aplikoi zatiak tamainaz bikoitzak liratekeelako.

Oro har, ikerketa honek markatzaile zehatz batetan azpi-eskualde ezberdinak erabiltzeak emaitza kontrajarriak ematen dituela erakutsi du, eta harrapari generalista baten harrapakin-espektra karakterizatzeko hasle desberdinak kontutan hartzearen garrantzi ekologikoa azpimarratzen du. Azken batean, mehatxatutako espezieentzako kontserbazio-neurriak hartzerako orduan erabakigarria izan liteke prozedura metodologikoa. Adibidez, galzorian dagoen

animalia baten dietak bere paper ekologikoaren eta biologiaren inguruko funtsezko informazioa ematen du, berau babesteko ezinbestekoa izan litekeena. Beraz, metabarcoding bidezko dieta-azterketetan ezinbestekoa da hasle desberdinen konbinazioa probatu eta ebaluatzea. Baldintza trofikoak eta habitat-baldintzak faktore gakoak dira galzorian dauden espezie askoren kontserbaziorako (Sinclair eta kideak, 2006; Sutherland, 2000). Erabilitako hasleen konbinazioak dietaren emaitzetan hainbeste eragiten duenez gero, metabarcoding bitartezko ikerketetan hasleen arteko konbinazio desberdinak probatu eta ebaluatzea beharrezkoa da.

Eskerrak

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Informazio gehigarria

S1. Taula. Bost hasleen PCR baldintzen xehetasunak.

PCR pausoak	Zeale	Gillet	Leray	Meusnier	Brandon-Mong
Hasiera	95 °C, 10 min	95°C, 15 min	95°C, 10 min	95°C, 10 min	95°C, 2 min
Desnaturalizazioa	16 ziklo:	40 ziklo:	16 ziklo:	5 ziklo:	25 ziklo:
	95 °C, 30 seg	94 °C, 30 seg	95 °C, 10 seg	95 °C, 1 min	95 °C, 15 seg
	61 °C, 30 seg	45 °C, 45 seg	62 °C (-1 °C zikloko), 30 seg	46 °C, 1 min	45 °C, 30 seg
	72 °C, 30 seg	72 °C, 30 seg	72 °C, 60 seg	72 °C, 30 seg	72 °C, 3 min
Suberapena	24 ziklo:		25 ziklo:	35 ziklo:	
	95 °C, 30 seg		95 °C, 10 seg	95 °C, 1 min	
	53 °C, 30 seg		46 °C, 30 seg	53 °C, 1 min	
	72 °C, 30 seg		72 °C, 60 seg	72 °C, 30 seg	
Hedapena	72 °C, 10 min	72 °C, 10 min	72 °C, 10 min	72 °C, 10 min	72 °C, 10 min

S2. Taula. Hasle bakoitzarekin lortutako sekuentzia-irakurketa gordinak, bahetutako sekuentzia-irakurketa eta OTU kopurua.

	Brandon-Mong	Gillet	Leray	Meusnier	Zeale
Sekuentzia-irakurketa gordina	3754874	12667575	4364497	5050701	3110300
Bahetutako sekuentzia-irakurketak	557978	11788479	1668749	4120090	2231688
OTU kopuru gordina (osoa)	342	2063	1920	604	200
Taxonomia esleitutako OTU kopurua	54	395	150	43	77

S3. Taula. Hasle bakoitzarekin talde taxonomiko bakoitzean identifikatutako espezie (Esp.) eta agerpen kopuruak (Ager.).

Taldea taxonomikoa	Brandon-Mong		Gillet		Leray		Meusnier		Zeale	
	Esp.	Ager.	Esp.	Ager.	Esp.	Ager.	Esp.	Ager.	Esp.	Ager.
Annelida (Enchitreidae)	1	10	2	4	1	1	0	0	0	0
Arachnida	0	0	1	1	0	0	0	0	0	0
Coleoptera	1	1	4	7	3	3	0	0	5	8
Decapoda (Paciphastacus)	1	1	1	2	1	2	0	0	1	7
Diptera	4	29	21	84	7	43	2	17	8	67
Ephemeroptera	4	53	12	159	5	70	2	24	3	15
Lepidoptera	1	1	21	39	20	51	8	25	32	135
Mollusca	0	0	2	2	0	0	0	0	0	0
Nematoda	1	4	0	0	1	1	0	0	0	0
Odonata	0	0	1	2	1	2	1	1	0	0
Plecoptera	4	9	7	47	4	19	0	0	2	7
Psocoptera	0	0	1	1	0	0	0	0	1	2
Salmonidae	1	1	1	1	1	1	2	2	0	0
Trichoptera	6	31	22	127	20	75	5	31	13	75
Guztira	24	140	96	476	64	268	20	100	65	316

4. Kapituluia



Giroa da, ez menua.
Harrapakin eskuragarritasunak ez du
galzorian dagoen Pirinioetako muturluzearen
habitat hautespena eragiten

Argitalpena

Kapitulu honetan oinarrituriko artikulu bat aztertua izaten ari da *Aquatic Conservation: Marine and Freshwater Ecosystems* aldizkarian:

Esnaola, A., Mata, V., Arrizabalaga-Escudero, A., González-Esteban, J., Larrañaga, A., Rebelo, J., Elosegi, A., eta Aihartza, J. (2018). It is the ambience, not the menu. Prey availability does not drive habitat selection by the endangered Pyrenean desman. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Laburpena

1. Pirinioetako muturluzea (*Galemys pyrenaicus*) Iberiar Penintsula eta Pirinioetako erreketan endemikoa den eta galzorian dagoen intsektiboroa da. Ur-bare eta putzuen aldean urlasterrak ditu nahiago, baina oraindik ez dago argi habitat hautespen horrek harrapakin eskuragarritasuna edo bestelako faktoreren bat duen oinarri.
2. Ikerketa honetan muturluzearen dieta eta harrapakin-hautespena aztertu ziren, ia ukitu gabeko Elama errekan eta giza jardueraren eraginpean dagoen Leitzaran ibaian (Euskal Herria).
3. Kontsumitutako harrapakinak identifikatzeko 94 gorotz aztertu ziren metabarcoding bidez erreka bakoitzean eta harrapakin-eskuragarritasuna deskribatzeko, aldiz, 10 Surber lagin bildu ziren erreka bakoitzeko habitat mota bakoitzean (urlaster, ur-bare eta putzuetan). Ornogabeak sailkatu eta identifikatu egin ziren, taxon bakoitzaren biomasa kalkulatu zen eta, horrez gain, euren DNA erauzi zen, PCR bidez aplikatu eta sekuentziatuz erreferentziako datu-base bat eraikitzeko.
4. Harrapakin-eskuragarritasuna eta dieta ezberdinak izan ziren bi ibaietan. Muturluzeek zatitzaileak eta substratuaren gainean bizi diren ornogabeak hautatu zituzten positiboki, eta substratu finen barnean bizi diren taxa, oskol gogordunak eta oso mugikorak hautatu zituzten negatiboki. Muturluzeen dieta selektiboagoa izan zen Elaman, egoera ekologiko hobea duen errekan. Bazka-eskuragarritasunean ez zen alderik behatu habitaten artean, eta hortaz, honek ez du muturluzeen urlasterrekiko zaletasuna azaltzen: aitzitik, hautespen horren erantzuleak flotagarritasunari aurre egiteko muga mekanikoa eta habitat fisikoaren heterogeneotasuna izango zirela uste da.
5. Erreketan urlasterren sorrera bultzatzeak habitat degradatuetan bizi diren muturluzeen populazio batzuen egoera hobetu zezakeela iradokitzen dute emaitzek.

Hitz-gakoak

bazka, erreka, galzorian dauden espezieak, ibaia, jokabidea, metabarcoding, ornogabeak, ugaztunak

Sarrera

Galzorian dauden animalien ekologia trofikoak eta bazka-estrategiak ulertzea garrantzitsua da euren populazioen dinamikak iragarri eta kontserbazio-programa eraginkorrak garatzeko (Sinclair eta kideak, 2006). Bazka-jokabidea nagusiki bazka-baliabideen konposizio, ugaritasun eta banaketaren arabera denez (adb. Waggitt eta kideak, 2018), habitataren eta bazka-eskuragarritasunaren arteko erlazioa funtsezko gaia da kontserbazio-biologian (adb. Goiti eta kideak, 2008; Karanth eta kideak, 2004; Shuterland eta Green, 2004).

Pirinioetako muturluzea (*Galemys pyrenaicus*, É. Geoffroy Saint-Hilaire, 1811, Eulipotyphla, Talpidae, 1. Irudia) mendiko erreketan bizi den ugaztun intsektiboro generalista erdi-urtarra da. Endemikoa da Iberiar Penintsularen iparraldean eta Pirinioetan, baina bere banaketa-eremua gogorki murriztua izan da azken hamarkadetan, eta horrek Naturaren Kontserbaziorako Nazioarteko Batasunaren (IUCN) zerrenda gorrian Zaurgarri gisa sailkatzea ekarri du (Fernandes eta kideak, 2008). Zaletasun sendoa du jario bizkorreko urlasterrekiko, motelagoak diren ur-bare eta putzuen aldean (2. Kapituluak; Esnaola eta kideak, 2018b), baina ez dago argi hautespen hori harrapakin-eskuragarritasunaren edo bestelako faktoreen arabera den, hau da, giroaren (hots, ezaugarri fisikoak) edo menuaren (hots, harrapakin eskuragarriak) arabera hautatzen ote dituen bere bazka-lekuak (jatetxea). Muturluzearen ekologia trofikoaren inguruko informazio gabeziak espezie hau egokiro kudeatu ahal izatea zailtzen du (MAGRAMA, 2013; Némoz eta Bertrand, 2008).



1. Irudia. Pirinioetako muturluzea (*Galemys pyrenaicus*) Elama errekan. Argazkia: Jorge González-Esteban.

Muturluzeen gorotzen edo urdailen edukietako harrapakinen hondarren identifikazio bisualak (e.g. Bertrand, 1992; Casti n eta Gos lbez, 1995; Santamarina, 1993) ornogabe bentonikoen espektrora zabalaz bazkatzen direla erakutsi zuen. Berriki, gorotzen DNA metabarcoding-ak (Bohmann eta kideak, 2014) harrapakinak espezie mailaraino identifikatzea ahalbidetzen du, baita gorputz biguneko animalien kasuan ere. Asko izan dira metodo horiek muturluzeen gorotzetan aplikatu dituzten autoreak. Biffi (2017a) eta Biffi eta kideak (2017b), Pirinioen iparraldean, 91 familiatako eta 30 ordenetako 156 harrapakin genero identifikatu zituzten. Ondoren, Hawlitschek eta kideak (2018) 140 genero identifikatu zituzten denera Iberiar Penintsulan. Ikerketa horiek muturluzeak nagusiki Ephemeroptera, Plecoptera, Trichoptera eta Dipteraz bazkatzen direla baieztatu zuten, nahiz eta balitekeen hasleen alborapenaren (3. Kapituluua; Esnaola eta kideak, 2018a; Pi ol eta kideak, 2019) eta bestelako muga metodologiko batzuen eragina jaso izana (adb. Oehm eta kideak, 2011).

Muturluzearen urlasterrekiko zaletasuna bazka-eskuragarritasunaz edo bestelako faktoreez baldintzatua dagoen aztertzeke, bazka-eskuragarritasuna hiru mikrohabitatatetan (urlaster, ur-bare eta putzuetan) ezaugarritu eta Esnaola eta kideak (2018a; 3. Kapituluua) egindako irradi-telemetria bidezko habitat-hautespenaren ikerketaren garai berean bildutako muturluzeen gorotzetatik zehaztutako dietarekin konparatu zen. Muturluzeek urlasterrak bertako harrapakinen eskuragarritasuna handiagoa zelako (Dewson eta kideak, 2007) hautatzen zituztela izan zen hipotesi nagusia. Muturluzearen dietaren malgutasuna aztertzeke, egoera ekologiko desberdineko bi erreketan burutu zen ikerketa. Muturluzeak hautatzen dituen harrapakinak zein diren aztertzea ere helburu zuen ikerketak. Lan honen emaitzek kontserbazio-arduradunentzako informazio garrantzitsua eskaini eta mehatxatutako Pirinioetako muturluzearen populazioak hobeto babesteko kudeaketa-egitasmoak gidatu ditzakete etorkizunean.

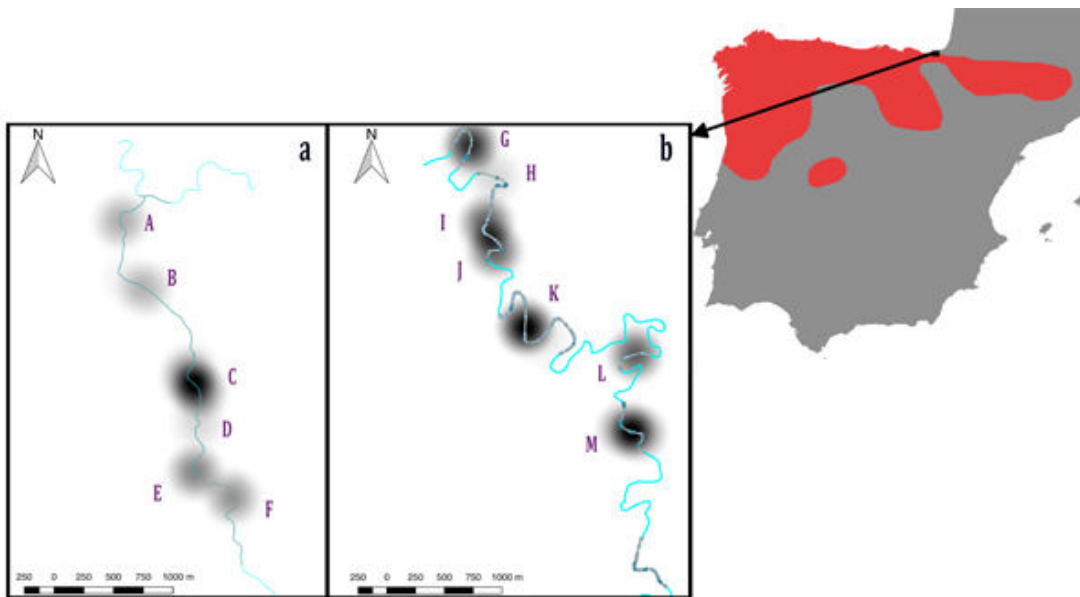
Material eta metodoak

Ikerketa-eremua

Ikerketa Iberiar Penintsularen iparraldeko mendiko bi erreketan egin zen (Euskal Herria; 2. Irudia). Europar Batasunaren Natura 2000 sarearen baitan babestuta daude bi erreka horiek, eta biek dute egoera ekologiko ona Uraren Europako Zuzentarauaren arabera (Council of the European Communities, 2000). Dena den, ingurumen-presio desberdinak pairatzen dituzte bi errekek. Elama bigarren ordenako mendiko erreka bat da, biztanlerik gabeko granito eta

eskistozko 1415 ha-ko arroa estaltzen duena, 1919-tik natura erreserba gisan zorrozki kudeatua izan dena, eta ondorioz pago eta haritzez osaturiko baso-estaldura zabala duena (Castro, 2009). Gaur egun Elamaren arroan ez dago baso-jarduerarik. Bestalde, Leitzarar laugarren ordenako ibaia da, kareharri, arbel eta hareharrizko 12 402 ha-ko arroa estaltzen duena. Elaman ez bezala, 3150 biztanle dituzten bi herri kokatzen dira Leitzararen arroaren goiko aldean, nahiz eta horren ostean errekek biztanlerik gabeko 25 km inguruko haran luzea zeharkatzen duen, non basogintza eta jarduera hidroelektrikoetarako ur-erazketak diren giza-eraginezko jarduera nagusiak (Izagirre eta kideak, 2013).

Ikerketa Esnaola eta kideek (2018b; 2. Kapituluak) argitaratutako irradi-telemetria laneko tarte eta garai beretan egin zen: Elama erreka 4 km-ko tartean ($1^{\circ}48'36''W$ $43^{\circ}12'40''N$ eta $1^{\circ}48'4''W$ $43^{\circ}11'14''N$ artean; batezbesteko altitudea, 330 m; batezbesteko zabalera, 7,08 m), eta Leitzarar ibaiko 10 km-ko tartean ($1^{\circ}57'26''W$ $43^{\circ}8'57''N$ eta $1^{\circ}56'9''W$ $43^{\circ}6'55''N$ artean; batezbesteko altitudea, 290 m; batezbesteko zabalera, 12,42 m). Tartean luzeren arteko aldeak Leitzarar ibaian muturluzeen dentsitate baxuagoari dagozkie.



2. Irudia. Ikerketa-eremua: (a) Elama eta (b) Leitzarar errekek. Gorotz laginak laginketa-tokietan multzokatu ziren (A-M) bilaketak egindako ibai tarteetako gorotzen agerpenen *heat map*-aren arabera. Iberiar Penintsulako Pirinioetako muturluzearen banaketa gorritz adierazita dago (Fernandes eta kideak 2008).

Muturluzearen dieta

Pirinioetako muturluzearen gorotzak bi erreketan bilatu ziren (2. Irudia) 2016ko irailean eta urrian, espezie honentzako bereziki diseinatutako kaka-leku

artifizialak eraikiz (1. Eranskina; González-Esteban eta kideak, 2018). Denera 188 gorotz (errekako 94) bildu eta hodiedtan gorde ziren, %98 etanolean, eta DNA erauzi arte -80°C-tan izoztu ziren.

Muturluzeen gorotz-laginetako DNA-ren erauzketa, PCR-anplifikazioa eta Errendimendu Altuko Sekuentziakzioa (EAS) Esnaola eta kideek (2018a; 3. Kapitulua) deskribatu bezala egin zen, Gillet (Gillet eta kideak, 2015) eta Zeale (Zeale eta kideak, 2011) hasleak konbinatuz, kontsumitutako harrapakin espezieen ahalik eta gama zabalena identifikatzeaz gain, harraparia bera ere identifikatzeko. Neurria eginiko datu-base bat eraikitzeke, bi erreketan bildutako ornogabeetatik (ikus beheerago) ere DNA erauzi zen. DNA erauzi, amplifikatu eta sekuentziatu, Euskal Herriko Unibertsitateko UPV/EHU Ikerkuntzarako Zerbitzu Orokorretan (SGIker) egin zen. Xehetasun metodologikoak 1. eta 2. informazio gehigarrietan topa daitezke (S1. Taula).

Dietaren emaitzak erreka bakoitzeko harrapakin taxa bakoitzaren agerpen-maiztasunen bitartez (*frequency of occurrence*, FO) eta agerpenen ehunekotan (*percentage of occurrence*, PO) adierazi ziren. Taxon baten FO-k taxon hori gorotzen zein proportziotan detektatzen zen adierazten du eta PO-k taxon bakoitzaren agerpen kopurua eta taxa guztien agerpen kopuru totalaren arteko ratioa adierazten zuen, ehunekotan (Deagle eta kideak, 2019).

Harrapakin-eskuragarritasuna

Makroornogabe urtarrak Surber sare batez (30 cm x 30 cm; 1 mm-ko sarea) lagindu ziren, aurrez egindako irrati-telemetrya ikerketan (2. Kapitulua; Esnaola eta kideak, 2018) identifikatutako hiru habitat motetan (Overton eta kideak, 1997): hots, “urlasterrak”, ura bizkor, zurrunbilotsu, azalera irregularrez eta zurixka duten lekuak; “ur-bareak”, ura ia laminarra eta sakonera erregularra dutenak; eta “putzuak”, ibilguaren sakonuneetan, emari motelekoak. Habitateko eta errekako 10 lagin bildu ziren, denera 60 lagin izanik. Lagin horiek plastikozko ontzietan gorde eta %97-ko etanoletan mantendu ziren identifikazioa burutu arte.

Laborategian, makroornogabeak 2mm-ko sareaz bahetu, sailkatu, zenbatu, neurtu eta disezio mikroskopioaz identifikatu ziren. Bereizmen taxonomikoa genero edo espezie mailan ezarri zen taxa gehienentzako, Diptera, Coleoptera, Oligochaeta, Hirudinidae eta Nematoda salbuespenak izanik, zeinak familia edo ordena mailan identifikatu ziren (2. Informazio gehigarria, S2. eta S3. Taulak). Harrapakin biomasa ($gAFDMm^{-2}$, edo metro karratuko errautsik gabeko masa lehorraren gramoak) tamaina-masa erregresioetatik kalkulatu zen (2. Informazio gehigarria, S4. Taula).

Analisi estatistikoak

Dietaren eta harrapakin-eskuragarritasunaren laginketen osotasuna 'iNEXT' online plataforma (Chao eta kideak, 2016) erabiliz ebaluatu zen, taxaren asetason-kurbak eraikiz.

Dieta-konposizioari zegozkion erreken arteko aldeak espezie zein familia mailan aztertu ziren Permutazio bidezko MANOVA (PerMANOVA; Anderson, 2001) erabiliz, eta alde horiek eragiten zituzten taxa Korrespondentzia-Analisiaz (CA) identifikatu ziren. PerMANOVA R-rako *geomorph* paketeko *procD.lm* funtzioa erabiliz egin zen (Adams eta kideak, 2017).

Erreken arteko harrapakin-eskuragarritasuna hiru mailatan konparatu zen: harrapakin-eskuragarritasun potentziala (lagindutako ornogabe guztiak barneratuz), kontsumitutako harrapakinen eskuragarritasuna (dietan agertzen ziren taxa soilik hartuz kontutan) eta positiboki hautatutako harrapakinen eskuragarritasuna (positiboki hautatzen ziren taxa soilik hartuz kontutan, hau da, dietan ornogabeen laginetan baina sarriago agertzen zirenak, %20-ko diferentziazko atalasearekin). Harrapakin potentzialen eskuragarritasunaren taxa aberastasunak ANOVA erabiliz konparatu ziren, espezie eta familia mailak kontutan hartuz. Eskuragarritasun neurketa bakoitzaren dentsitateak (banako m^{-2}), biomasak ($gAFDMm^{-2}$) eta batzbesteko gorputz-masak ($gAFDM$) ere ANOVA bidez konparatu ziren, \log_{10} transformazioaren bidez normalizatu ostean. Harrapakin-taxa bakoitzaren batzbesteko gorputz-masa ($gAFDM$) biomasa zati dentsitatea eginez kalkulatu zen (Flores eta kideak, 2011).

Harrapakin-hautespena ebaluatzeko harrapakin-eskuragarritasun potentziala, kontsumitutako harrapakinen eskuragarritasuna eta positiboki hautatutako harrapakinen eskuragarritasuna, taxon bakoitzaren dietako FO-ekin konparatu ziren. Harrapakin-eskuragarritasuna hiru eratan ezaugarritu zen horretarako: 1) harrapakin-eskuragarritasun totala (analisi ez-kontserbadoreena); 2) harrapakin-eskuragarritasun totala erreka, bakoitzeko muturluzeen bizi eremuko habitat eskuragarrien batzbesteko azaleraz zamatuta (Esnaola eta kideen, 2018b, irrati-telemetry lanean oinarritua; 2. Kapituluua); eta 3) harrapakin-eskuragarritasun totala, habitat mota eta erreka bakoitzeko muturluzeen batzbesteko jardueraz zamatuta (analisi kontserbadoreena; Esnaola eta kideen, 2018b, irrati-telemetry lanean oinarritua; 2. Kapituluua). Metodo horiek PerMANOVA erabiliz konparatu ziren, taxa espezie mailan zehaztuz. Harrapakin-hautespena erreka bakoitzerako zein aldi berean bi erreketarako estimatu zen, taxon bakoitzarentzako dietaren eta eskuragarritasunaren arteko aldeak FO-etan adieraziz. Horretarako, harrapakin-eskuragarritasuna ere erreka bakoitzeko harrapakin taxa bakoitzaren agerpen-maiztasunaz (FO) adierazi zen,

erreka bakoitzean harrapakin taxa bakoitza Surber laginen zein proportziotan agertzen zen erakutsiz. Taxaren hautespen positiboa edo negatiboa irizteko %20-ko diferentziako atalasea ezarri zen.

Positiboki hautatutako harrapakin-taxa harrapakin-eskuragarritasunaren hurbilpen kontserbadoreago bat lortzeko erabili ziren, muturluzearen ekologia espaziala (habitat lasterrekiko zaletasuna, Esnaola eta kideak, 2018b; 2. Kapituluua) azaldu zezaketen habitaten arteko diferentziak bilatzeko helburuz. Harrapakin eskuragarrien dentsitate, biomasa eta batazbesteko gorputz-masaren balioespenak bi erreketarako egin ziren eta balioak ANOVA bidez konparatu ziren.

Analisi guztiak $p < 0,05$ esangarritasun mailarekiko egin ziren. Analisi estatistikoak R 3.4.3 (R Core Team, 2014) erabiliz egin ziren.

Emaizak

DNA sekuentziak

Arrakastaz erauzi eta anplifikatu zen DNA lagin guztietatik, Leitzarango lagin baten salbuespenarekin, zeinak sekuentziazioan zehar ez zuen behar beste sekuentzia-irakurketa ekoitzi. Hori honela, Elamako 94 eta Leitzarango 93 gorotz aztertu ziren denera. Gillet haslearekin 4896 OTU lortu ziren denera eta Zeale haslearekin 844 OTU. Horietatik Gillet-en 64 eta Zeale-ren 75 OTU-k pasa zituzten dietaren azterketako iragazkiak, zeinak Gillet-ekin 41 taxari (23 familia) eta Zeale-rekin 46-ri (33 familia) esleitu zitzaizkien. Bi hasleek 13 familia partekatu zituzten. Gillet-ek muturluzeari berari zegozkion hainbat OTU anplifikatu zituen lagin guztietan, gorotzak espezie honi zegozkiola baieztatuz.

Dieta

Taxaren asetahun-kurbek asintotarantz jo zuten, eta laginen osotasuna %95 baina altuagoa izanik, erabilitako lagin tamaina (gorotz kopurua) Elama eta Leitzarango erreketako muturluzeen dieta deskribatzeko nahikoa zela baieztatzen (2. Informazio gehigarria, S1. Irudia).

Dietan Insecta eta Malacostraca izan ziren nagusi (FO %100 eta %93, hurrenez hurren), Ephemeroptera, Diptera, Amphipoda eta Trichoptera izanik ordena arruntenak (FO %99, %94, %92 eta %88, hurrenez hurren) (3. Irudia; 1. Taula;

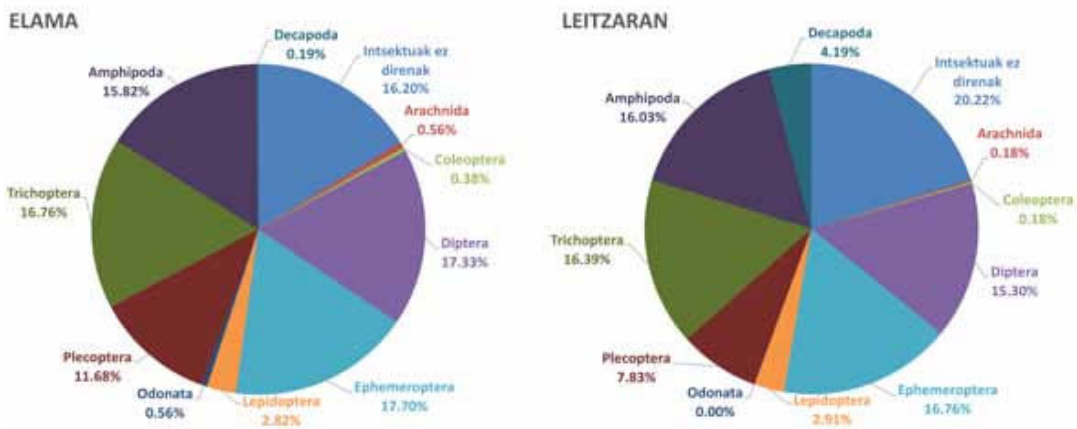
2. Informazio gehigarria, S5. Taula). Maizen kontsumitutako harrapakin-familiak Heptagenidae, Gammaridae, Baetidae eta Simuliidae izan ziren bi erreketan (FO %94, %92, %91 eta %90, hurrenez hurren), eta genero arruntenak (FO >%70) *Baetis*, *Ecdyonurus* eta *Echinogammarus* Elaman eta *Baetis*, *Echinogammarus* eta *Epeorus* Leitzararen.

Dietaren osaketa esangarriki desberdina izan zen bi erreketan, bai espezie (F = 58,24; a.g. = 1; p = 0,001) zein familia mailan (F = 16,88; a.g. = 1; p = 0,001). Odontoceridae, Perlidae, Leptophlebiidae eta Astacidae izan ziren diferentzia handienak eragin zituztenak (CA grafikoaren 1. dimentsioa, 2 Informazio gehigarria, S2. Irudia), lehenengo hiruak nagusiki Elaman eta azkena Leitzararen agertuz (4. Irudia).

Harrapakin-eskuragarritasuna

Ugaritasun zein presentzia-ausentzia datuetan taxaren asetasun-kurbek asintotarantz jo zuten eta laginen osotasuna %80 baina altuagoa zen, erabilitako lagin tamaina (Surber laginen kopurua) Elama eta Leitzararen erreketako muturluzeen harrapakin-eskuragarritasuna deskribatzeko nahikoa zela baieztatuz (2. Informazio gehigarria, S3. eta S4. Irudiak).

Ornogabe-taxaren aberastasuna altuagoa zen Leitzararen Elaman baino (5. Irudia), diferentziak espezie (F = 4,41; a.g. = 1; p = 0,041) zein familia mailan (F = 6,59; a.g. = 1; p = 0,013) izanik estatistikoki esangarri. Habitaten arteko eta erreken baitako habitaten arteko aldeak ez ziren esangarriak.



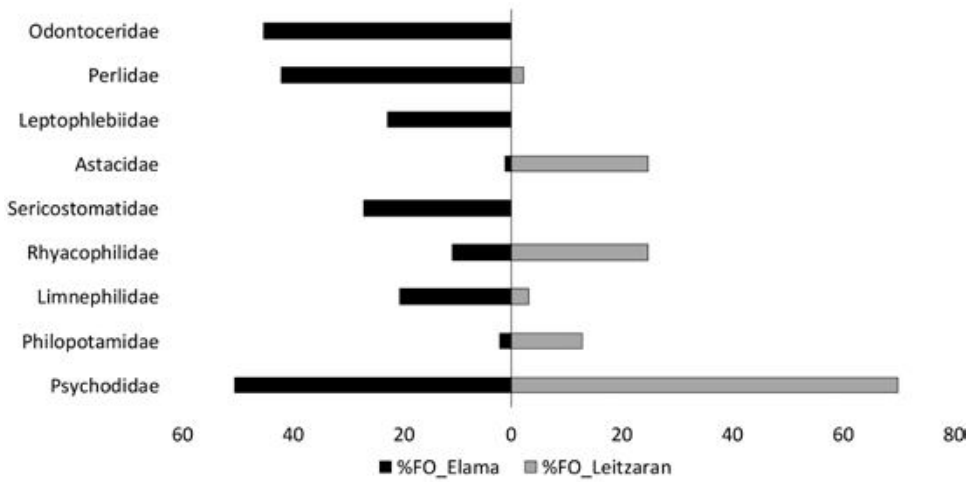
3. Irudia. Pirinioetako muturluzearen dieta-osaketaren sektore-diagramak. Harrapakin talde bakoitzaren agerpenen ehunekoak (PO), bi erreketan.

1. Taula. Dieta azterketa honen eta lehenagoko dieta azterketen arteko konparaketa. Emaizak FO-tan adierazi ziren. Ikerketa orotan gorotzen %5 baino gutxiagotan agertutako taxa ezabatuak izan ziren. Hasleak: G = Gillet eta kideak 2015; Z = Zeale eta kideak 2011; F = Folmer eta kideak 1994; M = Meusnier eta kideak 2008. Ikerketa honetan erabilitako datuak, hau da, Gillet eta Zeale hasleekin lortutako datuak, beltzez nabarmenduak izan dira.

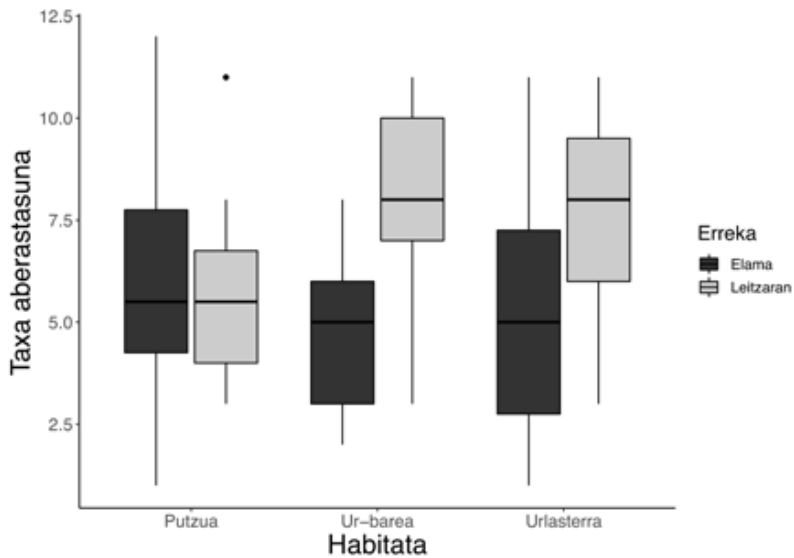
Klasea	Ordena	Familia	Ikerketa hau						Biffi	Biffi	Hawliits-	
			Elama			Leitzaran			eta kideak,	eta kideak,	check	
			Hasleak	G	Z	G+Z	G	Z	G+Z	2017	2017	eta kideak,
			Iberiar									Penintsilaren
									Pirinioen	Pirinioen	IE	
									iparraldea	iparraldea	F eta M	
Arachnida			0	3	3	1	0	1	2	8	0	
Clitellata	Haplotaxida	Lumbricidae	0	0	0	0	0	0	1	6	0	
Diplopoda			0	0	0	0	0	0	4	22	0	
	Glomerida		0	0	0	0	0	0	2	16	0	
		Doderiidae	0	0	0	0	0	0	0	8	0	
		Glomeridae	0	0	0	0	0	0	2	11	0	
	Polydesmida		0	0	0	0	0	0	1	7	0	
Insecta			100	100	100	100	100	100	100	93	100	
	Coleoptera		1	1	2	1	1	1	14	14	56	
		Carabidae	0	1	1	0	0	0	7	1	0	
		Scirtidae	1	0	1	0	0	0	6	8	0	
	Diptera		16	100	98	12	99	90	46	66	100	
		Anthomyiidae	0	0	0	0	0	0	3	5	0	
		Blephariceridae	0	0	0	0	0	0	1	7	0	
		Chironomidae	13	51	53	10	41	44	15	42	66	
		Empididae	1	3	3	0	9	9	0	2	0	
		Limoniidae	0	10	10	0	4	3	1	2	0	
		Mycetophilidae	0	2	2	0	6	5	0	4	0	
		Psychodidae	2	51	50	0	76	70	3	8	0	
		Simuliidae	1	96	94	2	94	86	15	18	0	
		Stratiomyidae	0	0	0	0	0	0	11	1	0	
		Tipulidae	0	7	6	0	0	0	5	4	0	
	Ephemeroptera		100	43	100	99	60	99	87	71	100	
		Baetidae	87	0	87	95	0	95	57	42	0	
		Ephemerellidae	2	1	3	0	0	0	37	2	0	
		Ephemeridae	4	0	4	0	0	0	0	8	0	
		Heptageniidae	98	41	98	90	60	90	59	61	0	
		Leptophlebiidae	22	2	22	0	0	0	5	1	0	
	Lepidoptera		0	16	16	0	19	17	1	3	0	
		Erebidae	0	7	6	0	7	6	0	1	0	

1. Taula. (jarraipena).

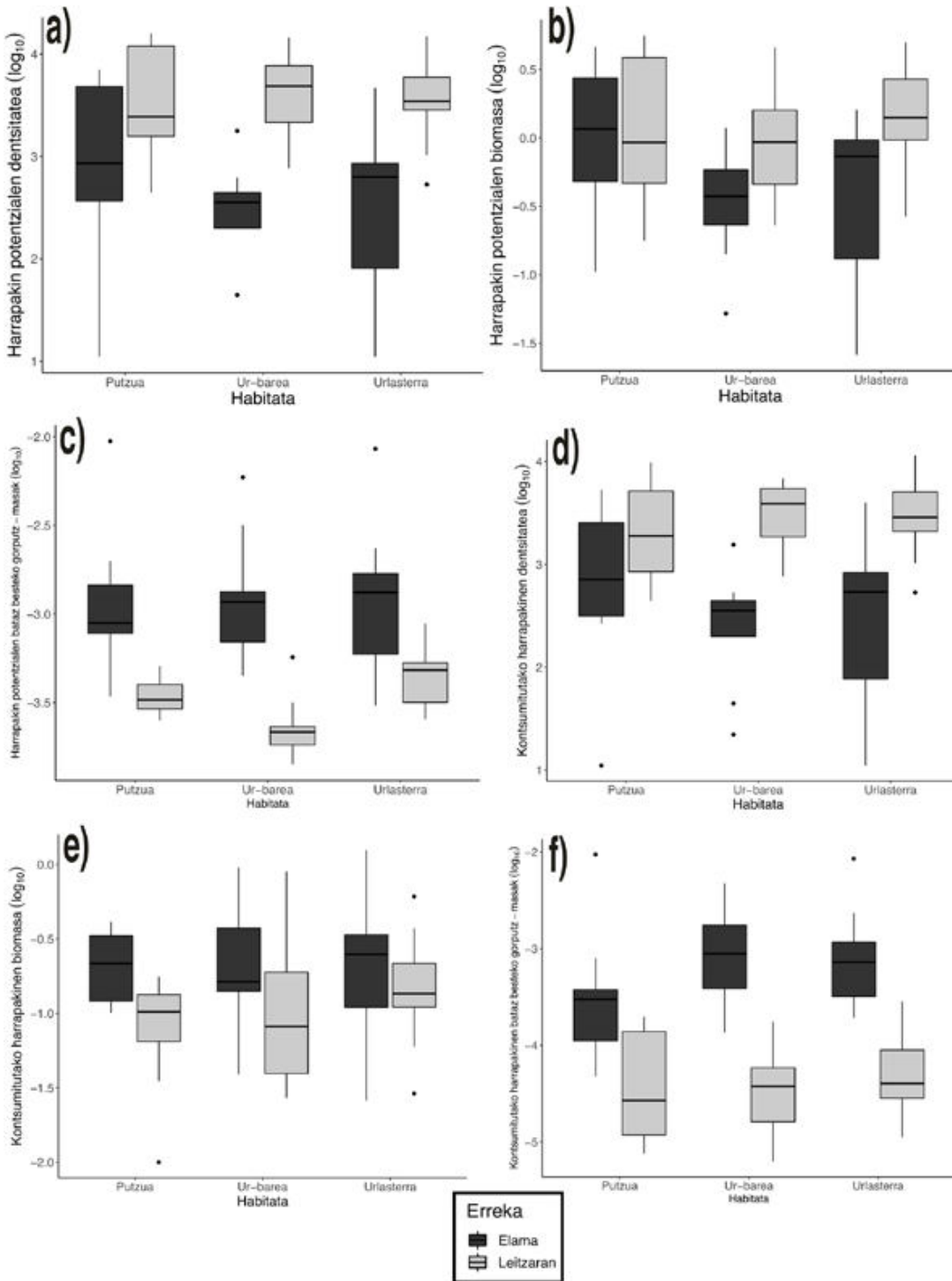
Klasea	Ordena	Familia	Ikerketa hau						Biffi	Biffi	Hawlits-	
									eta kideak,	eta kideak,	check	
									2017	2017	eta kideak,	
									2018			
Ikerketa-eremua			Elama			Leitzarar			Pirinioen	Pirinioen	Iberiar	
									iparraldea	iparraldea	Penintsilaren	
									G	G	IE	
			Hasleak	G	Z	G+Z	G	Z	G+Z	G	G	F eta M
		Geometridae	0	7	6	0	11	10	0	2	0	
	Neuropteroida		0	0	0	0	0	0	0	0	12	
	Orthoptera		0	0	0	0	0	0	7	0	0	
		Anostomatidae	0	0	0	0	0	0	7	0	0	
	Plecoptera		62	21	66	33	44	46	85	53	37	
		Leuctridae	22	21	28	30	44	45	30	9	0	
		Nemouridae	4	0	4	1	0	1	49	26	0	
		Notonemouridae	0	0	0	0	0	0	6	0	0	
		Perlidae	41	0	41	2	0	2	26	33	0	
		Perlodidae	0	0	0	0	0	0	33	5	0	
		Pteronarcyidae	0	0	0	0	0	0	5	6	0	
	Trichoptera		61	75	87	75	46	88	64	64	46	
		Hydropsychidae	48	27	51	73	7	73	47	54	0	
		Limnephilidae	2	20	20	0	4	3	19	15	0	
		Odontoceridae	0	46	45	0	0	0	3	12	0	
		Philopotamidae	0	2	2	6	13	13	0	4	0	
		Polycentropodidae	0	4	4	0	6	5	1	0	0	
		Rhyacophilidae	0	11	11	0	27	25	7	7	0	
		Sericostomatidae	27	0	27	0	0	0	2	5	0	
	Malacostraca		89	1	89	95	27	97	18	23	0	
	Amphipoda		89	0	89	95	0	95	17	21	0	
		Gammaridae	89	0	89	95	0	95	17	21	0	
	Decapoda	Astacidae	0	1	1	0	27	25	0	1	0	
		Lagin kopurua	94	92	94	93	85	93	287	184	41	



4. Irudia. Bi erreken arteko dietaren diferentzietan gehien eragin zuten harrapakin familien agerpen-maiztasunak (FO). Taxa horiek %1 baino gehiago eragin zuten CA-ren 1. dimentsioan, erreka bakoitzari dagozkion tokiak bereiziz. Taxa ekarpen-balio altuenetik (goian) baxuenera (behean) ordenatu ziren.



5. Irudia. Habitat eta erreka bakoitzeko harrapakin potentzialen aberastasuna. Konfiantza-mugak “1.5 legearekin” adierazi ziren: biboteak muturreko datuaren punturaino luzatzen dira, zeina inoiz ez den kaxaren kanporantz kaxaren luzeraren [1.5] baino luzeagoa inoiz izaten.



6. Irudia. Habitat eta erreka bakoitzeko harrapakin potentzialen eta kontsumitutako harrapakin dentsitatea (a, d), biomasa (b, e) eta batzbesteko gorputz-masak (c, f). Konfiantza-mugak “1.5 legearekin” adierazi ziren: biboteak muturreko datuaren punturaino luzatzen dira, zeina inoiz ez den kaxaren kanporantz kaxaren luzeraren [1.5] baino luzeagoa inoiz izaten.

Harrapakin potentzialen dentsitatea ($F = 28,60$; a.g. = 1; $p < 0,001$), biomasa ($F = 7,62$; a.g. = 1; $p = 0,008$) eta batzbesteko gorputz-masa ($F = 52,31$; a.g. = 1; $p < 0,001$) esangarriki desberdinak izan ziren erreken artean, Leitzarane dentsitatea eta biomasa altuagoak eta Elaman batzbesteko gorputz-masa handiagoa izanik (6. Irudia, a-c). Habitaten arteko eta erreken baitako habitaten arteko aldeak ez ziren esangarriak. Bestalde, kontsumitutako harrapakinen dentsitatea ($F = 28,6$; a.g. = 1; $p < 0,001$), biomasa ($F = 7,81$; a.g. = 1; $p = 0,007$) eta batzbesteko gorputz-masa ($F = 73,85$; a.g. = 1; $p < 0,001$) ere esangarriki desberdinak ziren erreken artean, dentsitatea Leitzarane altuagoa eta biomasa eta batzbesteko gorputz-masa Elaman altuagoak izanik (6. Irudia, d-f). Berriz ere, habitaten arteko eta erreken baitako habitaten arteko aldeak ez ziren esangarriak.

Harrapakin-hautespena

Harrapakin-eskuragarritasunean ez zen alde esangarririk topatu erabilitako metodoetako bakar batekin ere, hots, eskuragarritasun totala muturluzeen bizi-eremuko habitat eskuragarrien batzbesteko azalera zamaturako eskuragarritasunarekin eta habitat mota eta erreka bakoitzeko muturluzeen batzbesteko jardueraz zamaturako eskuragarritasunarekin konparatuta ($F = 0,07$; a.g. = 2; $p = 0,932$), beraz muturluzeen jardueraz zamaturako datuak erabili ziren harrapakin-hautespena aztertzeko.

Bi errekak konbinatuz, muturluzeez Simuliidae eta Psychodidae (Diptera), Baetidae eta Heptageniidae (Ephemeroptera), Odontoceridae (Trichoptera) eta Gammaridae (Crustacea) positiboki hautatu zituzten (2. Taula). Bestalde, Planorbidae (Gastropoda), Goeridae (Trichoptera), Perlidae (Plecoptera) eta Athericidae (Diptera) negatiboki hautatu zituzten. Horrez gainera, Hydropsychidae (Trichoptera), Limnephilidae (Trichoptera), Leptophlebiidae (Ephemeroptera), Chironomidae (Diptera) eta Leuctridae (Plecoptera), ere positiboki hautatu zituzten Elaman, Rhyacophilidae (Trichoptera) eta Limoniidae (Diptera) negatiboki hautatu zituzten bitartean. Leitzarane, Rhyacophilidae (Trichoptera) ere positiboki hautatu zuten, Naididae eta Lumbricidae (Oligochaeta) eta Polycentropodidae (Trichoptera) negatiboki hautatu zituzten bitartean.

Positiboki, oportunistikoki eta negatiboki hautaturako taxaren dietako FO balioak batuz (2. Taula), batzbeste %4,49, %2,83, eta %0,25 izan ziren, hurrenez hurren. Ehunekoetan, 6,37, 0,88 eta 0,57 izan ziren Elaman, eta 2,71, 4,43 eta 0,08 Leitzarane.

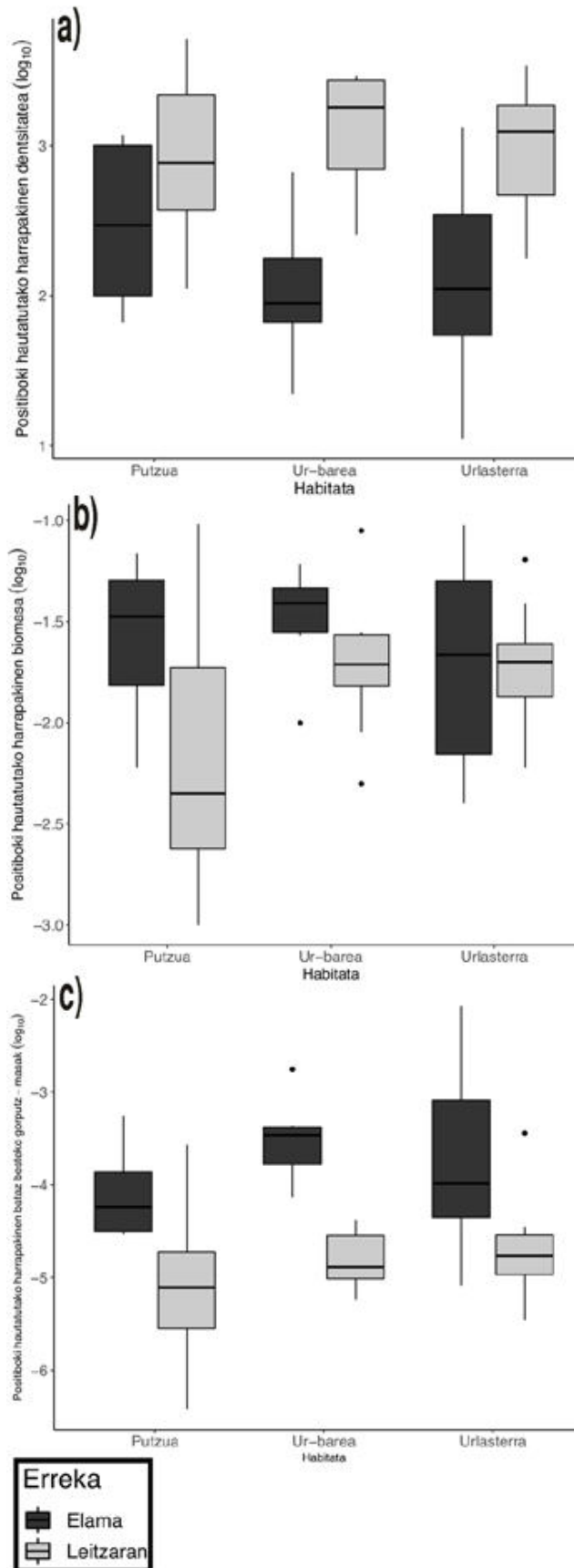
2. Taula. Dieta hautespena familia mailan, erreka bakoitzerako zein aldi berean bi erreketarako. Dieta agerpen-maiztasunetan (FO) adierazi zen. Taxon bakoitzaren batzbesteko eskuragarritasuna (Eskur.) muturluzeen batzbesteko jarduerarekin zamatuta (ikusitako azalpena testuan) eta hau ere FO-tan adierazi zen. Dif-ek bi metriken arteko aldea adierazten du. Hautespen positiboa eta negatiboa beltzez nabarmendu dira.

Familia	Elama			Leitzaran			Gutzira		
	Dieta (FO)	Eskur. (FO)	Dif	Dieta (FO)	Eskur. (FO)	Dif	Dieta (FO)	Eskur. (FO)	Dif
Simuliidae	0,936	0,064	0,87	0,860	0,115	0,75	0,898	0,089	0,81
Psychodidae	0,500	0,000	0,50	0,699	0,000	0,70	0,599	0,000	0,60
Baetidae	0,872	0,064	0,81	0,946	0,804	0,14	0,909	0,434	0,48
Heptageniidae	0,979	0,679	0,30	0,903	0,678	0,23	0,941	0,679	0,26
Odontoceridae	0,447	0,000	0,45	0,000	0,000	0,00	0,223	0,000	0,22
Gammaridae	0,894	0,445	0,45	0,946	0,960	-0,01	0,920	0,702	0,22
Hydropsychidae	0,511	0,241	0,27	0,731	0,716	0,02	0,621	0,478	0,14
Limnephilidae	0,202	0,000	0,20	0,032	0,000	0,03	0,117	0,000	0,12
Leptophlebiidae	0,223	0,000	0,22	0,000	0,015	-0,01	0,112	0,007	0,10
Geometridae	0,064	0,000	0,06	0,097	0,000	0,10	0,080	0,000	0,08
Astacidae	0,011	0,000	0,01	0,247	0,099	0,15	0,129	0,049	0,08
Chironomidae	0,532	0,335	0,20	0,441	0,498	-0,06	0,486	0,416	0,07
Erebidae	0,064	0,000	0,06	0,065	0,000	0,06	0,064	0,000	0,06
Leuctridae	0,277	0,073	0,20	0,452	0,547	-0,10	0,364	0,310	0,05
Mycetophilidae	0,021	0,000	0,02	0,054	0,000	0,05	0,038	0,000	0,04
Noctuidae	0,043	0,000	0,04	0,022	0,000	0,02	0,032	0,000	0,03
Empididae	0,032	0,064	-0,03	0,086	0,000	0,09	0,059	0,032	0,03
Syrphidae	0,000	0,000	0,00	0,043	0,000	0,04	0,022	0,000	0,02
Lonchopteridae	0,011	0,000	0,01	0,032	0,000	0,03	0,021	0,000	0,02
Scatopsidae	0,000	0,000	0,00	0,032	0,000	0,03	0,016	0,000	0,02
Linyphiidae	0,032	0,000	0,03	0,000	0,000	0,00	0,016	0,000	0,02
Cordulegastridae	0,032	0,003	0,03	0,000	0,000	0,00	0,016	0,002	0,01
Aturidae	0,000	0,000	0,00	0,011	0,000	0,01	0,005	0,000	0,01
Diptera	0,000	0,000	0,00	0,011	0,000	0,01	0,005	0,000	0,01
Gyrinidae	0,000	0,000	0,00	0,011	0,000	0,01	0,005	0,000	0,01
Carabidae	0,011	0,000	0,01	0,000	0,000	0,00	0,005	0,000	0,01
Chloropidae	0,011	0,000	0,01	0,000	0,000	0,00	0,005	0,000	0,01
Oniscidae	0,011	0,000	0,01	0,000	0,000	0,00	0,005	0,000	0,01
Pediciidae	0,011	0,000	0,01	0,000	0,000	0,00	0,005	0,000	0,01
Scirtidae	0,011	0,000	0,01	0,000	0,000	0,00	0,005	0,000	0,01
Glossosomatidae	0,011	0,003	0,01	0,000	0,000	0,00	0,005	0,002	0,00
Philopotamidae	0,021	0,064	-0,04	0,129	0,084	0,05	0,075	0,074	0,00
Rhyacophilidae	0,106	0,322	-0,22	0,247	0,030	0,22	0,177	0,176	0,00
Lumbriculidae	0,000	0,000	0,00	0,000	0,001	0,00	0,000	0,001	0,00
Dytiscidae	0,000	0,000	0,00	0,000	0,003	0,00	0,000	0,001	0,00

2. Taula. (jarraipena).

Familia	Elama			Leitzaran			Guztira		
	Dieta (FO)	Eskur. (FO)	Dif	Dieta (FO)	Eskur. (FO)	Dif	Dieta (FO)	Eskur. (FO)	Dif
Asellidae	0,000	0,003	0,00	0,000	0,000	0,00	0,000	0,002	0,00
Leptoceridae	0,000	0,003	0,00	0,000	0,000	0,00	0,000	0,002	0,00
Ephemerelellidae	0,032	0,040	-0,01	0,000	0,000	0,00	0,016	0,020	0,00
Nemouridae	0,043	0,067	-0,02	0,011	0,000	0,01	0,027	0,033	-0,01
Cucurlionidae	0,000	0,000	0,00	0,000	0,015	-0,01	0,000	0,007	-0,01
Sphaeriidae	0,000	0,003	0,00	0,000	0,015	-0,01	0,000	0,009	-0,01
Rhagionidae	0,000	0,040	-0,04	0,000	0,001	0,00	0,000	0,021	-0,02
Sericostomatidae	0,266	0,316	-0,05	0,000	0,000	0,00	0,133	0,158	-0,03
Nematoda	0,000	0,064	-0,06	0,000	0,000	0,00	0,000	0,032	-0,03
Tipulidae	0,064	0,131	-0,07	0,000	0,000	0,00	0,032	0,065	-0,03
Lepidostomatidae	0,000	0,073	-0,07	0,000	0,000	0,00	0,000	0,037	-0,04
Planariidae	0,000	0,000	0,00	0,000	0,084	-0,08	0,000	0,042	-0,04
Hirudinidae	0,000	0,000	0,00	0,000	0,099	-0,10	0,000	0,049	-0,05
Hydrobiidae	0,000	0,000	0,00	0,000	0,100	-0,10	0,000	0,050	-0,05
Ephemeridae	0,043	0,006	0,04	0,000	0,149	-0,15	0,021	0,078	-0,06
Elmidae	0,000	0,000	0,00	0,000	0,115	-0,11	0,000	0,057	-0,06
Hydrophilidae	0,000	0,131	-0,13	0,000	0,000	0,00	0,000	0,065	-0,07
Naididae	0,000	0,000	0,00	0,000	0,196	-0,20	0,000	0,098	-0,10
Lumbricidae	0,000	0,040	-0,04	0,000	0,296	-0,30	0,000	0,168	-0,17
Polycentropodidae	0,043	0,006	0,04	0,054	0,446	-0,39	0,048	0,226	-0,18
Limoniidae	0,096	0,391	-0,30	0,032	0,099	-0,07	0,064	0,245	-0,18
Planorbidae	0,000	0,090	-0,09	0,000	0,354	-0,35	0,000	0,222	-0,22
Goeridae	0,021	0,381	-0,36	0,000	0,197	-0,20	0,011	0,289	-0,28
Perlidae	0,415	0,616	-0,20	0,022	0,418	-0,40	0,218	0,517	-0,30
Athericidae	0,043	0,292	-0,25	0,000	0,548	-0,55	0,021	0,420	-0,40

Positiboki hautatutako harrapakin-taxan, desberdintasunak esangarriak ziren erreken artean dentsitateari ($F = 33,1$; a.g. = 1; $p < 0,001$), biomasari ($F = 4,80$; a.g. = 1; $p = 0,033$) eta batazbesteko gorputz-masari zegokienean ($F = 38,14$; a.g. = 1; $p < 0,001$). Dentsitate eskuragarria altuagoa zen Leitzaranen, baina biomasa eta batazbesteko gorputz-masa eskuragarria altuagoak ziren Elaman (7. Irudia, a-c). Habitaten eta erreken baitako habitaten arteko aldeak ez ziren esangarriak.



7. Irudia. Habitat eta erreka bakoitzeko positiboki hautatutako harrapakinen dentsitatea (a), biomasa (b) eta batzbesteko goputz-masak (c). Konfiantza-mugak “1.5 legearekin” adierazi ziren: biboteak muturreko datuaren punturaino luzatzen dira, zeina inoiz ez den kaxaren kanporantz kaxaren luzeraren [1.5] baino luzeagoa inoiz izaten.

Eztabaida

Ikerketa honek lehenengoz aztertzen ditu aldi berean Pirinioetako muturluzearen bi populaziotako ekologia espaziala (2. Kapituluua; Esnaola, eta kideak, 2018b), dieta eta bazka-eremuetako harrapakin eskuragarritasuna, muturluzeen beharrian trofikoaren inguruko lehen ikerketa osatua burutuz. Ikerketaren aurkikuntza nagusia harrapakin-eskuragarritasunak ez duela habitat-hautespena azaltzen izan zen.

Dieta

Muturluzeak urlasterren aldeko zaletasun sendoa duten (2. Kapituluua; Esnaola, eta kideak, 2018b) harrapari erreofilo espezialistak dira. Bestalde, emaitzek generalista gisa definitzen dituzte muturluzeak, aldeztatik deskribatu izan den bezala, askotariko harrapakinez bazkatzen direlarik: euren dietan 23 familia eta 9 ordenetako 41 artropodo espezie identifikatu ziren.

Heste, urdail zein gorotzetako harrapakinen hondarren morfologian oinarrituriko dieta azterketa tradizionalak muturluzeek nagusiki Trichoptera, Ephemeroptera, Plecoptera, Crustacea (Amphipoda) eta Diptera (Bertrand, 1992; Castián eta Gosálbez, 1995; Santamarina eta Guitian, 1988), eta gutxiagotan Coleoptera, Annelida eta Odonata (Puisségur, 1935; Richard eta Viillard, 1969; Santamarina, 1992) jaten dituztela erakutsi zuten. Era berean, lan honetan Ephemeroptera –Heptagenidae eta Baetidae–, Amphipoda –Gammaridae–, Diptera –Simuliidae– eta Plecopteraz bazkatu ziren batez ere muturluzeak, hautespena desberdina izanik erreken artean –bereziki Rhyacophilidae trikopteroei zegokienean–. Metodo molekularrei esker lortutako kontsumitutako harrapakinen espezie mailako identifikazioari esker, muturluzearen dietaren karakterizazio zehatzagoa lortu zen. Nabarmentzekoa da metodo molekularrek gorputz biguneko harrapakin taldeen garrantzi txikia baieztatu zutela, zeinak oharkabean pasa zitezkeen lan morfologikoetan. Honela, Clitellata, Nematoda eta Anfibioen gisako harrapakin potentzialak oso frekuentzia baxuetan agertu ziren (Biffi eta kideak, 2017a; 3. Kapituluua; Esnaola eta kideak, 2018a). Bestalde, ibai-karramarroa (*Pacifastacus*), ordura arte muturluzearen harrapakin gisa identifikatua izan ez zena, ohiko harrapakina izan zen Leitzaranean.

DNA metabarcoding-a erabiliz muturluzearen dietaren inguruan berriki argitaratu diren bost lanei dagokienez (Biffi eta kideak, 2017a,b; 3. Kapituluua; Esnaola eta kideak, 2018a; Gillet eta kideak, 2015; Hawlitschek eta kideak, 2018), diferentzia metodologikoen konparaketa sendoak mugatzen badituzte ere (Alberdi eta kideak, 2019), badira kontuan hartu beharreko alderdi interesgarri

batzuk. Lehenik eta behin, Hawlitschek eta kideek (2018), Biffi eta kideek (2017a) eta Biffi eta kideek (2017b) baino harrapakin taxa kopuru txikiagoa topatu zen, segur aski lan honetako prozedura bioinformatikoak askoz kontserbadoreagoak izan zirelako, hain zuzen ere, harraparitza sekundarioa, positibo faltsuak eta kimerak ekiditeko (Alberdi eta kideak, 2018, 2019; De Barba eta kideak, 2014; Sheppard eta kideak, 2005) sekuentzia-irakurketen %1 baino gutxiago zuten OTU-ak baztertu zirelako (hots, Gillet-en OTUen %98,7 eta Zeale-ren OTUen %91). Hortaz, Biffi eta kideek (2017a) eta Biffi eta kideek (2017b) detektatutako taxa arraro gehienak iragaziak izango ziren ziur aski lan honen prozedurarekin. Hare gehiago, litekeena da Biffi eta kideek (2017a) eta Biffi eta kideek (2017b) lan honetan ohikoenak ziren harrapakin talde batzuk (FOO>%25) gutxietsi izana, hasleen alborapenaren ondorioz: lan honetan Zeale haslearekin identifikatu ziren nagusiki Psychodidae, Simuliidae, eta beharbada Chironomidae dipteroak, Odontoceridae, Phylopotamidae, Polycentropodidae edo Rhyacophilidae triopteroak, Leuctridae plekopteroak, eta Astacidae edo Lepidoptera (1. Taula), metabarcoding-lanetan hasleen hautaketak duen garrantzia baieztatuz (Elbrecht eta Leese, 2017; 3. Kapituluak; Esnaola eta kideak, 2018a). Bestalde, dietak zenbait alde erakutsi zituen ikerketa-eremuaren arabera, eta baita Biffi eta kideen (2017a), Biffi eta kideen (2017b) eta Hawlitschek eta kideen (2018) lanekin alderatuz ere, zeinek eremu zabalak ikertu zituzten (1. Taula). Baliteke kontraste horiek baldintza lokalen diferentzien ondoriozko faunaren osaketaren aldeak adieraztea, baina hori ezin da ebaluatu, gainontzeko autoreek ez baitzuten harrapakin-eskuragarritasuna deskribatu. Diferentzien arrazoiak edozein izanik ere, nabarmentzekoa da Elamako dietak Leitzarangoak baino antza handiagoa duela Biffi eta kideek (2017a) eta Biffi eta kideek (2017b) Pirinioen iparraldean deskribatu zutenarekiko. Espekulazioak egin litezke alde horren inguruan, baina litekeena da baso-landaketa handien eta jarduera hidroeletrikorako ur erauzketen ondorioz (CHC, 2018) Leitzaran ibaiaren egoera pobregoa islatzea.

Harrapakin-hautespena

Kalkulatutako harrapakin-hautespenaren patroia orokorrean, alde txikiak izan arren, bat egiten dute aurreko ikerketekin. Lehenik eta behin, badirudi muturluzeek Simuliidae, Baetidae eta Heptageniidae bezalako substratuen gainean agerian bizi diren ornogabeak positiboki hautatzen dituztela. Ildo honetan, Bertrand-ek (1992) ere muturluzeek harrapakin bentiko eta erreofiloak positiboki hautatuko lituzketela espekulatu zuen. Bigarrenik, badirudi muturluzeek Gammaridae, Odontoceridae, Leptophlebiidae, Limnephilidae eta Leuctridae bezalako materia organiko lodiari loturiko ornogabe zatitzaileak positiboki hautatzen dituztela. Castián eta Gosálbez-ek (1995) Gammaridoekiko hautespen negatiboa topatu

zuten eta euren tegumentu esklerifikatuari egotzi zioten, baina ikerketa honen emaitzak kontraesanean daude euren behaketekin. Litekeena da horrek muturluzeek orbel-pilaketekiko duten zaletasuna islatzea, substratu mota honetan ornogabeen dentsitatea, bereziki zatitzaile handiena, gainontzekoetan baino handiagoa izaten delako (Flores eta kideak, 2017). Hirugarrenik, muturluzeek negatiboki hautatzen dituzte Athericidae, Limoniidae, Naididae eta Lumbricidae bezalako sedimentu finen barnean bizi diren gorputz biguneko taxa. Laugarrenik, negatiboki hautatzen dituzte Goeridae edo Planorbidae bezalako oskol gogorreko ornogabeak, agian prozesatzen zailak diren bazka-aleekiko interes txikiagoaren isla. Moluskuak ere negatiboki hautatuak zirela esan zuen Santamarina-k (1992). Bostgarrenik, muturluzeek negatiboki hautatu zituzten Perilidae, Polycentropodidae eta Rhyacophilidae bezalako harrapariak. Hautespen negatibo horren arrazoia ez dago batere argi, baina baliteke harrapari hauen defentsa/ihesaldi estrategiekin lotuta egotea. Hala eta guztiz ere, hemen aurkeztutakoak behin behineko interpretazioak dira eta ezin dira orokortu; izan ere antzeko ezaugarriak dituzten zenbait harrapakin (adb. Psychodidae) positiboki hautatuak izan dira. Harrigarria zera zen, hautespena kokapenari lotuta zegoela, Rhyacophilidae Elaman negatiboki hautatzen zelako eta Leitzaranen aldiz positiboki. Ezaugarri (*trait*) biologiko eta ekologikoen ikerketak hautespen patroik hauek argitzen lagundu lezake.

Kokapena vs hautespena

Bazirudien Elaman muturluzeek materia organiko lodiari loturiko taxa (2. taldekoak) positiboki hautatzen zituztela, Leitzaranen ez bezala. Gure iradokizuna zera zen, Elamako habitataren konplexutasunak materia organiko lodiaren pilaketak faboratzen zituela; bertan ornogabeentzako habitataren egonkortasuna, azalera eta bazka-baliabideak handiagoak ziren, harrapakin-biomasa handiagoa metatzen zen (Flores eta kideak, 2013), eta muturluzeek batzbesteko gorputz-masa handiagoko harrapakinak —bazka profitagarriagoa— zuten eskura. Harrapakin nagusien profitagarritasuna atalase kritiko batetatik behera erortzen den unean, harrapari generalista batek bere hautua profitagarriago diren harrapakin alternatiboetara aldatzea espero da (Křivan, 1996). Hortaz, badirudi Leitzaran ibaian muturluzeek hobetsitako harrapakinen gabeziaren aurrean, bestelako batzuen aldeko hautua egiten zutela, eskuragarritasun pobregoari aurre egiteko euren nitxo trofikoa zabalduz (Araujo eta kideak, 2011).

Jatetxea hautatzea: giroa edo menua?

Aurretiaz muturluzearen bazka-ekologiaz egindako ikerketa batean (2. Kapituluua; Esnaola eta kideak, 2018b), aztertutako bi eremuetan urlasterrak

hobetsitako habitata zirela plazaratu zen, eta hautespen hori bertako harrapakin-eskuragarritasuna altuagoa zelako gertatuko zela hipotetizatu zen. Alabaina, lan honek ez du harrapakin-eskuragarritasunean alde esangarririk erakutsi habitaten artean, ezta kontsumitutako, ez positiboki hautatutako harrapakinei zegokienean ere. Eraitza horiek galdera bat mahaigaineratzen dute: zergatik bazkatzen ote diren muturluzeak lehentasunez urlasterretan. Seguruenik habitat horretako jario bizkorreko urek erraztu egiten diete muturluzeei euren flotagarritasun naturalari aurre egin eta, euren atzapar sendoez helduz, hondoan ibiltzea (Richard, 1986).

Ondorioak

Ikerketa honek muturluzearen bazka-ohituren inguruko informazio berria eskaintzen du, zeina gakoa den kudeaketa-politika egokietarako. Lan honetako emaitzek muturluzeak habitat-espezialistak baina harrapari generalistak direla erakutsi dute, harrapakin gama zabalaz bazkatzen direnak, eta tokiko eskuragarritasunaren aldaketen arabera euren lehentasunak aldatzen dituztenak. Muturluzeei nahiago dituzte pilaketa organikoetan agertzen diren harrapakinak eta oportunistagoak bihurtzen dira baldintzak hain mesedegarriak ez diren egoeretan. Badirudi egur hilaren ugaritasuna handituz ibilguaren heterogeneotasuna areagotu eta materia organikoaren metaketa handitzea (Flores eta kideak, 2013) kudeaketa-ekintza positiboa izan daitekeela muturluzeen habitatak hobetzeko. Hauen urlasterrekiko zaletasun sendoak arrazoi mekanikoei erantzuten diela dirudi, habitaten artean harrapakin-eskuragarritasunean ez baita alderik topatu. Hortaz, erreketako emariaren kudeaketa eta horrek urlasterren eskuragarritasunean duen eragina muturluzearen kontserbaziorako gakoak diren gaiak dira.

Eskerrak

Ikerketa hau LIFE IREKIBAI EU proiektuak (LIFE14 NAT/ES/000186), Gipuzkoako Foru Aldundiak, Donostiako Udaletxeak, Eusko Jaurlaritzak [156P/2016 eta IT754-13] eta Euskal Herriko Unibertsitateak UPV/EHU finantzatua izan da. AEs-k Eusko Jaurlaritzako doktoretza aurreko beka bat jaso zuen [bekaren zenbakia PRE_2015_1_0408]. Iñigo Mendiola eta Aitor Lekuona (Gipuzkoako Foru Aldundia), eta Asunción Yarzabal eta Iñaki Uranga (Donostiako Udaletxea) eskertu nahi ditugu lan hau egin ahal izateko eman ziguten laguntzarengatik; Mafalda Galhardo (CIBIO-InBIO, Universidade do Porto) analisi bioinformatikoekin laguntzeagatik; eta Sekuentziazio eta Genotipo

Azterketen Unitatea —Genomic Facilities— SGIker (UPV/EHU/ERDF, EU) eman digun babes tekniko eta gizatiarrarengatik. DESMA Estudios Ambientales-ek JGE-en lana soldata moduan finantzatu du, baina ez du ikerketaren diseinuan, datuen bilketan eta analisisian, publikatzeko erabakian, edo idazlanaren prestaketan bestelako eginkizunik izan. Autoreek interes-gatazkarik ez dagoela aitortzen dute.

Informazio gehigarria

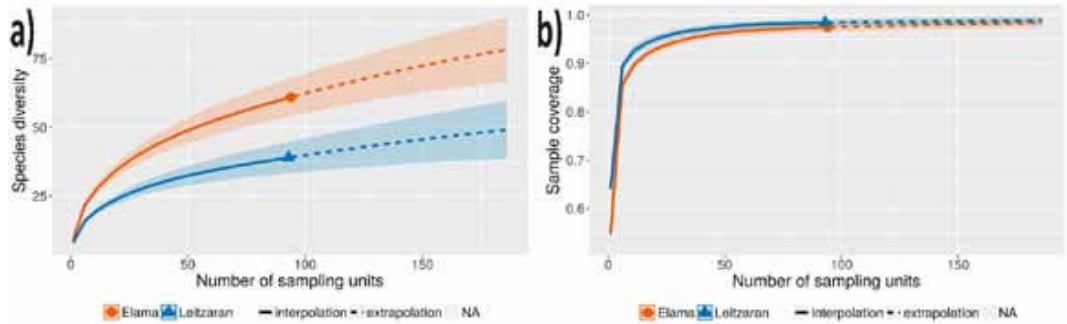
1. Informazio gehigarria

USEARCH erabiliz (Edgar, 2010, 2013; Edgar eta Flyvbjerg, 2015). Laburki, parekatutako muturreko sekuentzia-irakurketak bateratu eta hasleen bidez demultiplexeatu ziren, adaptadoreen eta hasleen sekuentziak ezabatu ziren, eta azkenik haplotipoetan bateratu ziren sekuentziak. Sekuentzia-irakurketa bakanak (edo *singletonak*), kimerak eta PCR akatsak 'unnoise3' komandoa erabiliz ezabatu ziren. Azkenik, OTU taula bat sortu zen sekuentzia gordinak %100-ean antzekoak ziren haplotipoekin bateratuz. Prozesu hau modu independentean egin zen hasle bakoitzarentzako.

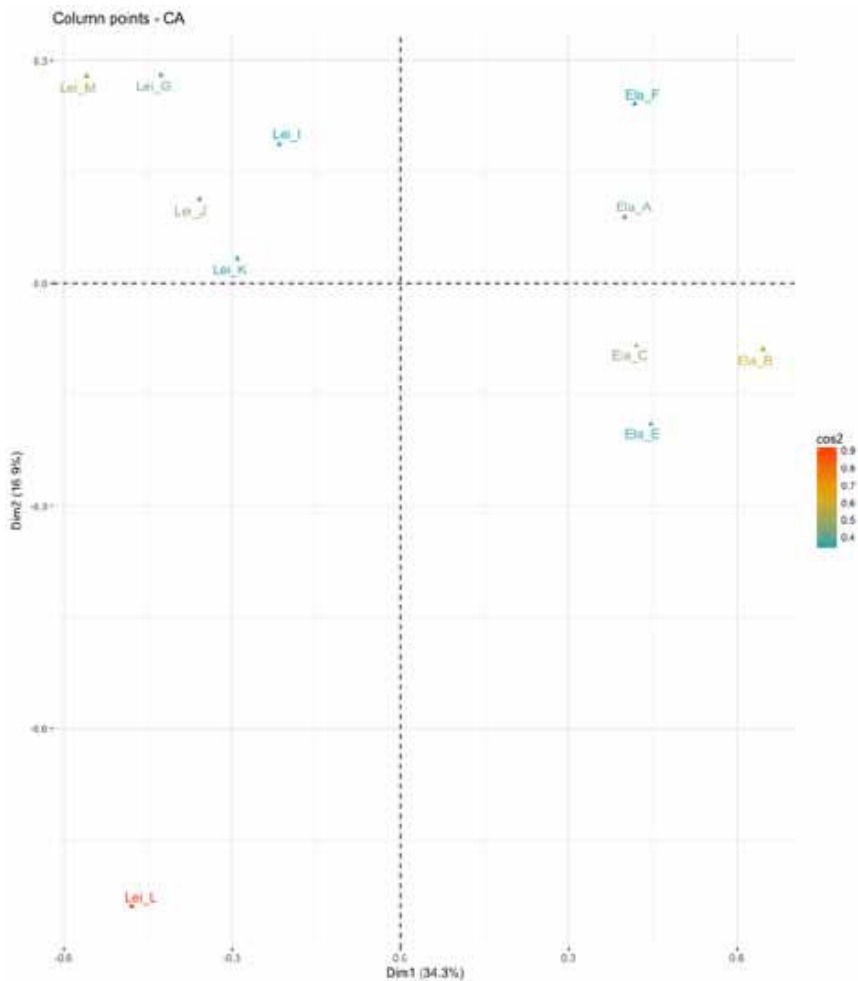
Azkenik, Genbank nt (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), BOLD (<http://www.boldsystems.org/>) eta datu-base pertsonalak erabil ziren OTU-ei taxonomia esleitzeko. Clare eta kideei (2013) jarraiki, espezie mailako esleipenak OTU-en sekuentziek erreferentziazko sekuentzietatikiko %98 baina parekako (*pairwise*) antzekotasun handiagoa eta %75 baina estaldura (*query cover*) handiagoa zutenean onartu ziren. Horrez gain, arau gisa, $1e-20$ baina e-balio baxuagoko emaitzak soilik onartu ziren (Vesterinen eta kideak, 2013), parekatzea zoriz gertatu ez zela ziurtatzeko. Positibo faltsuak ekiditeko helburuarekin 3 lagin baino gutxiagotan agertutako OTUak, lagin bakoitzaren baitan %1 baino gutxiago adierazten zutenak (Mata eta kideak, 2016), edo muturluzearen bazka izaterik ez zuten taxari zegozkionak (Brose eta kideak, 2006) analisisietatik baztertu ziren. Identifikatutako harrapakin taxaren agerpen-maiztasunak (*frequency of occurrence*, FO; taxon bat gorotzen zein proportziotan detektatzen zen; Shiel eta kideak, 1997) hartu ziren kontutan ondorengo analisiak egiteko. Sekuentzia-irakurketak ikuskatuz gorotz-laginetan harrapariaren beraren, hots Pirinioetako muturluzearen, DNA ere identifikatzen ote zen aztertu zen.

Intsektuen DNA isopropanol bidezko eskuzko erauzketa-metodoa erabiliz erauzi zen, Gilbert eta kideen (2007), Pfeiffer eta kideen (2004) eta Aljanabi eta Martinezez (1997) lanetan oinarrituz. Erauzitako DNA Gillet eta Zealeren hasleekin anplifikatu ziren, dieta aztertzeke erabilitako baldintza berdinak erabiliz. Purifikatu, kuantifikatu eta liburutegia prestatu ostean DNA aplikoiak *Illumina MiSeq NGS platforman* sekuentziatu ziren. Intsektuen DNA-sekuentziak lerrokatu eta eskuz editatu ziren *Geneious 8.0.5 software*arekin (Kearse eta kideak, 2012). Lerrokatutako sekuentziak Genbank nt eta BOLD datubaseetan bilatu ziren.

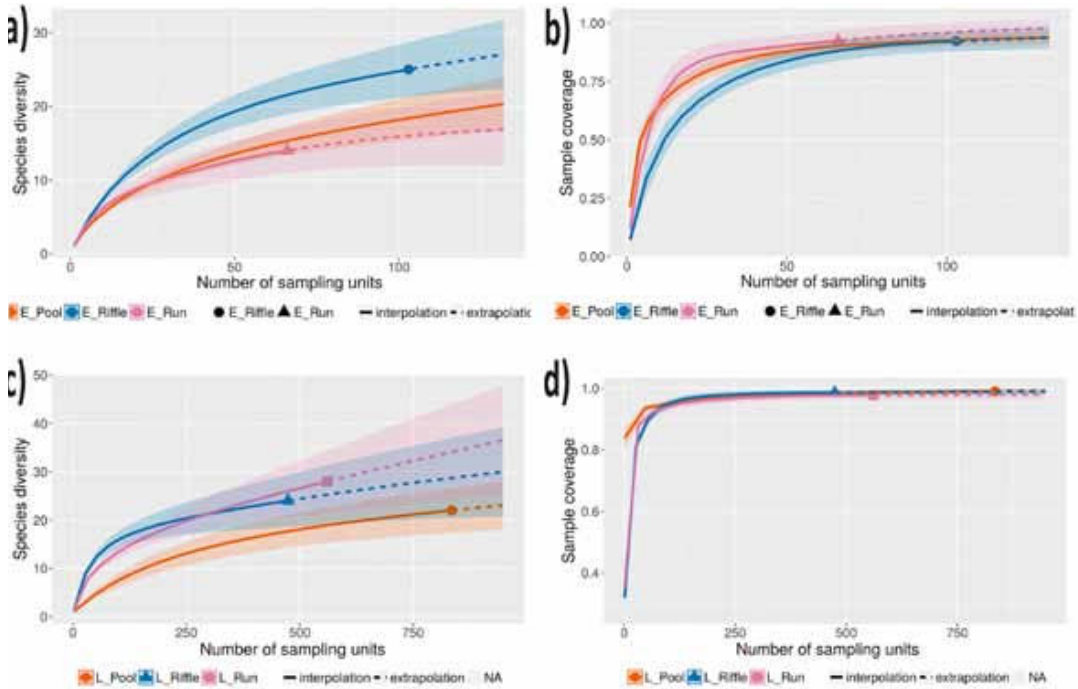
2. Informazio gehigarria



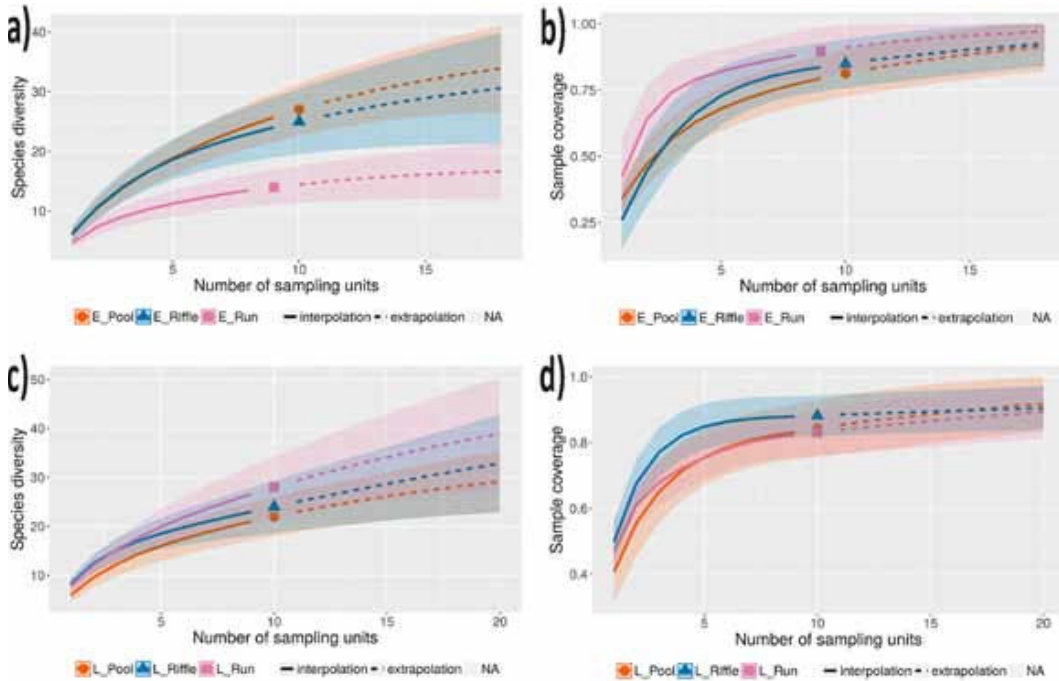
S1. Irdia. Muturluzeen dieta-datueta espezieen asetasun-kurbak: espezieen aberastasunaren (“*species diversity*”; a) eta laginen osotasunaren (“*sample coverage*”; b) Elama eta Leitzarango grafikoak.



S2. Irdia. Familia mailako muturluzeen dietaren analisiaren CA grafikoa. Ela puntuek Elamari egiten diote erreferentzia eta Lei puntuek Leitzarani. D eta H laginketa-tokiak ezabatu egin ziren Ca analisitik, lagin kopuru baxua zutelako. 1. dimentsioan erreken arteko diferentziak behatzen ziren.



S3. Irudia. Muturluzeen harrapakin eskuragarrien ugaritasun-datuetao espezieen asetasun-kurbak: espezieen aberastasunaren (“*species diversity*”; a, c) eta laginen osotasunaren (“*sample coverage*”; b, d) Elama (a, b) eta Leitzarango (c, d) grafikoak. *Riffle* = urlasterra; *Run* = ur-barea; *Pool* = putzua.



S4. Irudia. Muturluzeen harrapakin eskuragarrien presentzia-ausentzia-datuetao espezieen asetasun-kurbak: espezieen aberastasunaren (“*species diversity*”; a, c) eta laginen osotasunaren (“*sample coverage*”; b, d) Elama (a, b) eta Leitzarango (c, d) grafikoak. *Riffle* = urlasterra; *Run* = ur-barea; *Pool* = putzua.

S1. Taula. Ikerketa honetan erabilitako hasleen xehetasunak eta bakoitzaren PCR baldintzak. "Luzerak (bp)" haslerik gabeko aplikoiaren tamainari egiten dio erreferentzia.

Izen arrunta	Hslearen izena	Aurreranzko haslea(k) (5'-3')	Atzeranzko haslea(k) (5'-3')	Luzera (bp)*	Iturria	PCR pausoak
Gillet	F:LepF1	ATTCHACDAAYCAYAAR	ACTATAAAAPAAAAATYTD	133	Gillet eta	Hasiera: 95 °C, 15 min
	(modified)	GAYATYGG (25 bp)	AYAAADGCRTG (29 bp)		kideak,	
	R:EPT-long-univR				2015	Desnaturalizazioa (40 ziklo): 94 °C, 30 seg 45 °C, 45 seg 72 °C, 30 seg
	(modified)					Hedapena: 72 °C, 10 min
Zeale	F:ZBJ-ArtF1c	AGATAITGGACWTTAT	WACTAATCAATTWCCAA	157	Zeale eta	Hasiera: 95 °C, 10 min
	R:ZBJ-ArtR2c	ATTTTATTTTGG (30bp)	ATCCTCC (24bp)		kideak,	Desnaturalizazioa (16 ziklo): 95 °C, 30 seg 61 °C, 30 seg 72 °C, 30 seg
					2011	Suberapena (24 ziklo): 95 °C, 30 seg 53 °C, 30 seg 72 °C, 30 seg
						Hedapena: 72 °C, 10 min

S2. Taula. Elamako harrapakin-eskuragaritasun totala: habitataz bakoitzeko lagin bakoitzeko ugartasunak.

Taxa	Urasterra										Ur-barea										Putzua																			
	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	01	02	03	04	05	06	07	08	09	10											
<i>Ancylius fluviatilis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aselus aquaticus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix ibix</i>	0	0	0	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i> sp	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Berosus</i> sp	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	0	0	0	1	0	0	9	1	8	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chrysopilus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Climocerinae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dinocras cephalotes</i>	0	4	1	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ecdyonurus</i> sp	1	0	0	0	0	0	0	1	0	1	0	1	1	2	4	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Echinogammarus cf aquilifer</i>	1	2	0	0	1	0	0	1	2	0	0	0	0	4	1	0	0	2	0	0	0	0	0	2	16	0	0	3	4	2	0	0	0	0	0	0	0	0	0	0
<i>Eiseniella tetraedra</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epeorus</i> sp	0	0	1	3	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera danica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera ignita</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma boltoni</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydropsyche dinarica</i>	0	0	1	0	0	0	4	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydropsyche instabilis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepidostoma hirtum</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leuctra</i> sp	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limoniidae	0	0	0	1	0	0	1	0	2	0	1	0	2	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nematoda	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oecetis testacea</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

S2. Taula. (jarraipena)

Taxa	Uriasterra										Ur-barea										Putzua									
	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10
<i>Oescimus monedula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	6	0	0	4	0	0	0	0
<i>Perla</i> sp	0	2	4	2	0	4	1	1	0	0	1	3	4	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	2	0
<i>Philopotamus</i> sp	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
<i>Protonemura pirenaica</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Protonemura vandeli</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Rhithrogena</i> sp	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila adjuncta</i>	0	1	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila meridionalis</i>	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila obelix</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila tristis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Sericostoma</i> sp	0	0	0	0	0	0	1	0	0	0	0	0	3	1	1	4	1	4	0	8	4	32	1	1	17	21	18	3	8	0
<i>Silo</i> sp	0	1	0	0	0	1	0	0	1	0	1	1	1	2	0	0	0	1	0	1	0	3	0	0	0	0	0	0	0	0
Simuliidae	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sphaerium</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tipulidae	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

S3. Taula. Leitizarango harrapakin-eskuragaritasun totala: habitiat bakoitzeko lagin bakoitzeko ugartitasunak.

Taxa	Urtasterra										Ur-barea										Putzua									
	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10
<i>Ancylus fluviatilis</i>	2	0	0	0	2	0	0	1	2	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	3	0	0	0	0
<i>Atherix ibix</i>	0	3	0	1	0	1	1	5	3	0	0	0	0	1	0	0	0	1	0	2	1	0	0	0	0	0	0	0	0	
<i>Aulodrilus pluriseta</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
<i>Baetis</i> sp	4	7	10	1	0	5	2	3	11	3	2	0	0	6	0	3	0	0	0	0	0	1	1	1	0	1	0	0	0	
Chironomidae	4	5	0	0	0	0	11	0	0	2	51	44	21	3	35	4	94	8	32	25	6	81	100	12	18	167	33	103	13	231
<i>Chrysopilus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cucurionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Ecdyonurus</i> sp	0	0	0	2	0	0	0	1	2	0	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	2	0	0	0	
<i>Echinoogammarus longisetosus</i>	17	38	17	2	39	25	25	19	59	19	9	2	0	0	9	11	1	4	2	25	0	0	0	0	0	1	0	2	1	0
<i>Eiseniella tetraedra velutinus</i>	0	0	2	0	0	0	1	2	0	0	4	0	0	0	0	0	2	0	0	6	0	0	0	0	0	0	0	0	0	
<i>Embolocephalus velutinus</i>	0	0	0	0	0	0	0	0	0	1	0	1	0	4	0	4	1	4	4	3	0	0	0	1	0	0	0	0	2	
<i>Epeorus</i> sp	0	1	0	0	1	0	1	1	6	7	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
<i>Ephemera danica</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	1	2	1	0	1	0	1	0	0	0	0	1	
<i>Goera pilosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Hirudinae	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Hydropsyche dinarica</i>	0	1	2	0	0	0	3	4	3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Hydropsyche siltalai</i>	0	4	1	1	2	0	0	4	5	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Leuctra</i> sp	4	7	0	0	0	0	1	1	5	0	14	13	0	9	10	4	5	0	1	14	2	1	4	2	0	1	0	1	0	
<i>Limnius</i> sp	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
<i>Limnodrilus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
Limoniidae	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lumbriculidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

S3. Taula. (jarraipena)

Taxa	Uriasterra										Ur-barea										Putzua									
	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10
<i>Oulimnius</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pacifastacus leniusculus</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Perla</i> sp	1	4	3	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Philopotamus</i> sp	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Planaria</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Platambus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i> sp	1	9	0	0	1	0	0	0	1	0	6	1	0	1	1	4	0	0	0	3	4	0	0	0	1	2	1	3	1	0
<i>Potamopyrgus antipodarum</i>	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila meridionalis</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila relicta</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Silo</i> sp	0	0	1	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simuliidae	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	1	0
<i>Sphaerium</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Stylodrilus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2
Tubificinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0

S4. Taula. Taxa desberdinen biomasaren (gAFDMm⁻²) kalkulurako erabilitako erreferentziak.

Erreferentzia	Taxa
Burgherr eta Meyer (1997)	Cucurlionidae, Dytiscidae, Elmidae (<i>Limnius</i> sp.), Hydropsychidae
Larrañaga eta kideak (2009)	Gammaridae
Meyer (1989)	Ancyliidae, Athericidae, Baetidae, Chironomidae, Elmidae (<i>Oulimnius</i> sp.), Empididae, Ephemerellidae, Glossosomatidae, Goeridae, Heptagenidae, Leuctridae, Limoniidae, Nemouridae, Perlidae, Rhagionidae, Rhyacophilidae, Sericostomatidae, Simuliidae
Benke eta kideak (1999)	Cordulegastridae, Ephemeridae, Lepidostomatidae, Leptoceridae, Leptophlebiidae, Philopotamidae, Planariidae, Polycentropodidae, Sphaeriidae, Tipulidae
Stoffels eta kideak (2003)	Hydrobiidae, Oligochaeta
Baumgärtner eta Rothhaupt (2003)	Asellidae

S5. Taula. Dietaren taula: muturluzeen dietako taxa eta haien agerpenak erreka bakoitzean (hasle bakoitzarekin bere aldetik-G = Gillet, Z = Zeale- eta bi hasleekin batera-T = totala-). Gorriz, laginen %50 baino gehiagotan agertzen ziren taxa, eta laranja laginen %30 baino gehiagotan agertzen ziren taxa.

Filuma	Klasea	Ordena	Familia	Espeziea	Elama			Leitzaran		
					G	Z	T	G	Z	T
Arthropoda	Crustacea	Malacostraca	Oniscidae	<i>Oniscus asellus</i>	1	0	1	0	0	0
Arthropoda	Arachnida	Araneae	Aturidae	<i>Aturidae</i> sp	0	0	0	1	0	1
Arthropoda	Arachnida	Trombidiformes	Linyphiidae	-	0	3	3	0	0	0
Arthropoda	Insecta	Coleoptera	Carabidae	-	0	1	1	0	0	0
Arthropoda	Insecta	Coleoptera	Gyrinidae	-	0	0	0	0	1	1
Arthropoda	Insecta	Coleoptera	Gyrinidae	<i>Orectochilus villosus</i>	0	0	0	1	1	1
Arthropoda	Insecta	Coleoptera	Scirtidae	<i>Elodes marginata</i>	1	0	1	0	0	0
Arthropoda	Insecta	Diptera	-	-	0	0	0	0	1	1
Arthropoda	Insecta	Diptera	Athericidae	<i>Atherix ibix</i>	0	4	4	0	0	0
Arthropoda	Insecta	Diptera	Chironomidae	-	12	47	50	8	31	36
Arthropoda	Insecta	Diptera	Chironomidae	<i>Barbadocladius</i> sp	0	0	0	0	5	5
Arthropoda	Insecta	Diptera	Chironomidae	<i>Brillia bifida</i>	0	0	0	1	0	1
Arthropoda	Insecta	Diptera	Chloropidae	-	0	1	1	0	0	0
Arthropoda	Insecta	Diptera	Empididae	<i>Wiedemannia rhychops</i>	1	0	1	0	0	0
Arthropoda	Insecta	Diptera	Empididae	<i>Wiedemannia zetterstedti</i>	0	3	3	0	8	8
Arthropoda	Insecta	Diptera	Limoniidae	-	0	1	1	0	3	3
Arthropoda	Insecta	Diptera	Limoniidae	<i>Eloeophila</i> sp	0	8	8	0	0	0
Arthropoda	Insecta	Diptera	Lonchopteridae	-	0	1	1	0	3	3
Arthropoda	Insecta	Diptera	Mycetophilidae	-	0	0	0	0	5	5
Arthropoda	Insecta	Diptera	Mycetophilidae	<i>Gnoriste apicalis</i>	0	2	2	0	0	0
Arthropoda	Insecta	Diptera	Pediciidae	-	0	1	1	0	0	0
Arthropoda	Insecta	Diptera	Psychodidae	<i>Psychoda erminea</i>	2	47	47	0	65	65
Arthropoda	Insecta	Diptera	Scatopsidae	-	0	0	0	0	3	3
Arthropoda	Insecta	Diptera	Simuliidae	-	1	88	88	2	80	80
Arthropoda	Insecta	Diptera	Syrphidae	<i>Heringia</i> sp	0	0	0	0	4	4

S5. Taula. (jarraipena - 1)

Filuma	Klasea	Ordena	Familia	Espeziea	Elama			Leitzarari		
					G	Z	T	G	Z	T
Arthropoda	Insecta	Diptera	Tipulidae	-	0	6	6	0	0	0
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Alainites muticus</i>	51	0	51	0	0	0
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Baetis</i> sp	70	0	70	88	0	88
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Centroptilum luteolum</i>	1	0	1	0	0	0
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Procloeon pennulatum</i>	0	0	0	1	0	1
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	<i>Ephemerella ignita</i>	0	1	1	0	0	0
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	<i>Torleya major</i>	2	0	2	0	0	0
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	<i>Ephemera danica</i>	4	0	4	0	0	0
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	-	0	1	1	0	0	0
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Ecdyonurus</i> sp	91	18	91	56	0	56
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Electrogena lateralis</i>	7	0	7	0	0	0
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Epeorus</i> sp	47	26	51	79	51	79
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Rhithrogena</i> sp	9	0	9	0	0	0
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	<i>Habrophlebia eldae</i>	20	0	20	0	0	0
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	<i>Habrophlebia lauta</i>	4	2	4	0	0	0
Arthropoda	Insecta	Lepidoptera	Erebidae	<i>Herminia tarsicrinalis</i>	0	6	6	0	6	6
Arthropoda	Insecta	Lepidoptera	Geometridae	-	0	1	1	0	1	1
Arthropoda	Insecta	Lepidoptera	Geometridae	<i>Idaea degeneraria</i>	0	1	1	0	1	1
Arthropoda	Insecta	Lepidoptera	Geometridae	<i>Parectropis similaria</i>	0	2	2	0	0	0
Arthropoda	Insecta	Lepidoptera	Geometridae	<i>Trichopteryx carpinata</i>	0	4	4	0	7	7
Arthropoda	Insecta	Lepidoptera	Noctuidae	<i>Deltote pygarga</i>	0	4	4	0	1	1
Arthropoda	Insecta	Lepidoptera	Noctuidae	<i>Spodoptera exigua</i>	0	0	0	0	1	1
Arthropoda	Insecta	Odonata	Cordulegastridae	<i>Cordulegaster</i> sp	3	0	3	0	0	0
Arthropoda	Insecta	Plecoptera	Leuctridae	<i>Leuctra</i> sp	21	19	26	28	37	42
Arthropoda	Insecta	Plecoptera	Nemouridae	<i>Protonemura meyeri</i>	0	0	0	1	0	1
Arthropoda	Insecta	Plecoptera	Nemouridae	<i>Protonemura pirenaica</i>	1	0	1	0	0	0
Arthropoda	Insecta	Plecoptera	Nemouridae	<i>Protonemura vandeli</i>	3	0	3	0	0	0
Arthropoda	Insecta	Plecoptera	Perlidae	-	1	1	1	0	0	0
Arthropoda	Insecta	Plecoptera	Perlidae	<i>Dinocras cephalotes</i>	5	0	5	0	0	0
Arthropoda	Insecta	Plecoptera	Perlidae	<i>Perla</i> sp	36	0	36	2	0	2
Arthropoda	Insecta	Trichoptera	Glossosomatidae	<i>Glossosoma boltoni</i>	1	1	1	0	0	0
Arthropoda	Insecta	Trichoptera	Goeridae	<i>Silo</i> sp	0	2	2	0	0	0
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche dinarica</i>	45	25	48	16	6	19
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche instabilis</i>	1	0	1	1	0	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche siltalai</i>	0	0	0	65	0	65
Arthropoda	Insecta	Trichoptera	Limnephilidae	-	0	17	17	0	3	3
Arthropoda	Insecta	Trichoptera	Limnephilidae	<i>Chaetopteryx villosa x fusca</i>	1	0	1	0	0	0
Arthropoda	Insecta	Trichoptera	Limnephilidae	<i>Halesus radiatus</i>	1	1	1	0	0	0
Arthropoda	Insecta	Trichoptera	Odontoceridae	<i>Odontocerum albicorne</i>	0	42	42	0	0	0
Arthropoda	Insecta	Trichoptera	Philopotamidae	<i>Chimarra marginata</i>	0	0	0	0	3	3
Arthropoda	Insecta	Trichoptera	Philopotamidae	<i>Philopotamus</i> sp	0	2	2	6	8	9
Arthropoda	Insecta	Trichoptera	Polycentropodidae	<i>Polycentropus</i> sp	0	4	4	0	5	5

S5. Taula. (jarraipena - 2)

Filuma	Klasea	Ordena	Familia	Espeziea	Elama			Leitzaran		
					G	Z	T	G	Z	T
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	<i>Rhyacophila fasciata</i>	0	1	1	0	0	0
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	<i>Rhyacophila meridionalis</i>	0	9	9	0	0	0
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	<i>Rhyacophila relictata</i>	0	0	0	0	23	23
Arthropoda	Insecta	Trichoptera	Sericostomatidae	<i>Sericostoma</i> sp	25	0	25	0	0	0
Arthropoda	Malacostraca	Amphipoda	Gammaridae	-	0	0	0	2	2	2
Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Echinogammarus cf aquilifer</i>	84	0	84	0	0	0
Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Echinogammarus longisetosus</i>	1	0	1	88	0	88
Arthropoda	Malacostraca	Decapoda	Astacidae	<i>Pacifastacus leniusculus</i>	0	1	1	0	23	23
Laginak guztira					93	92	94	94	85	93

5. Kapitulua



Pirinioetako muturluzearen
(*Galemys pyrenaicus*, Insectivora, Talpidae)
dietaren karakterizazioa
harrapakinen ezaugarri bereizgarriak
(*traitak*) erabiliz

Laburpena

Tradizionalki, kontsumitutako *versus* eskuragarri dagoen bazkaren osaketa taxonomikoaren konparaketa egin ez balu, ez da dietaren hautespena. Esleipen taxonomikoak, ordea, ez du garrantziarik harrapariengan, euren hautespena bestelako ezaugarrietan oinarritzen dutelako, zeinaren inguruko informazioa harrapakinen ezaugarri —edo *trait*— biologikoetan topa daitekeen. Larriki galzorian dagoen Pirinioetako muturluzearen (*Galemys pyrenaicus*) bazka-hobespenak eragiten dituen irizpideak ulertze aldera, *trait*etan oinarritutako harrapakin-hautespen analisia egin genuen bi erreka desberdinetan, bata kontserbazio-egoera bikainean, eta bestea jarduera hidroelektrikorako ur-erazketen eraginpean egonik. Horretarako, erreka bakoitzean 94 muturluzegorotz lagindu eta metabarcoding bidez aztertu ziren, kontsumitutako harrapakinak identifikatzeko. Harrapakin-eskuragarritasuna hiru habitat mota nagusietan —putzuetan, ur-bareetan eta hobetsitako urlasterretan— jasotako Surber laginetatik neurtu zen. *Trait*en zaletasuna inferitzeko eskuragarri zeuden ornogabeak kontsumitutakoekin konparatu genituen bederatzi *trait* biologiko aztertuz. Muturluzeen dieta eta harrapakin-hautespen patroia nagusia oso antzekoa izan zen bi erreketan, erreken zein habitaten artean harrapakin eskuragarritasunean aldeak egon arren. Muturluzeez 2-8mm-ko tamaina potentzialeko harrapakinak, bentosaren *gainazalean* bizi diren harrapakinak, harrapakin *biziak*, *gorputz-malgutasun pixka bat* duten harrapakinak eta *taldekatuta* bizi diren harrapakinak hautatu zituzten positiboki. Hobetsitako gainontzeko *traitak* (*ur-fluxu bizkorra*, *harritzar-tamainako substratua*, eta abar) urlasterrei loturik zeuden. Positiboki hautatutako *trait*-kategoria ia guztiak ugariagoak ziren urlasterretan. Orokorrean, gure datuek urlasterrek Pirinioetako muturluzearen bazka-habitat gisa duten garrantzia nabarmendu zuten, nahiz eta ez dagoen argi horrek hobetsitako harrapakinen eskuragarritasun altuagoa edo euren ehiza-habitatetan dituzten muga mekanikoak islatzen dituen.

Hitz-gakoak

bazka-jokabidea, DNA metabarcoding, kudeaketa, makroornogabeak, *trait* funtzionalak, ugaztun urtarra

Sarrera

Galzorian dauden espezieen kontserbazioa euren biologiaren inguruko ezagutza zehatzaren gabeziak mugatuta egoten da maiz, euren banaketa, habitata edo dieta-eskakizunak zehazten dituzten faktoreak barne-bilduz (Morrison eta kideak, 2006). Tradizionalki, dieta-azterketak harraparien gorotz edo hesteetako harrapakinen hondarren identifikazioaren menpe egon dira, baina DNA metabarcoding-a bezalako tresna molekular berriez ordeztuak izaten ari dira (Bohmann eta kideak, 2014; Sheppard eta Harwood, 2005; Taberlet eta kideak, 1999). Teknika edozein izanik ere, ikerketa gehienek konposizio taxonomikoaren ikuspuntutik aztertzen dute dieta, nahiz eta esleipen taxonomikoak ez garrantziarik izan harrapariengan, seguruenik harrapakinen hautespena euren ugaritasuna, detektagarritasuna, harrapagarritasuna, profitagarritasuna edo kalitate nutrizionala bezalako bestelako ezaugarrietan oinarritzen dutelako (Almenar eta kideak, 2013; Symondson, 2002). Hautespena harrapakin-konsumoa eskuragarritasunarekiko esangarriki desberdina denean inferitzen da (Manly eta kideak, 2002), nahiz eta azken hori neurtzen zaila izan, ez baita harrapakinen zenbaketaren baliokidea, bestelako faktore batzuek harrapakinen detektagarritasunean, harrapagarritasunean, eta bar, eragiten dutelako (Backwell eta kideak, 1998; Holmes eta Schultz, 1988). Harraparien zentzumen-gaitasunek ere (Siemers eta Guttinger, 2006), harrapakinen harrapagarritasuna, iheserako edo defentsarako jarrera eta zaporea bezalako bestelako faktoreez jarraituz (De Crespín eta Usseglio-Polatera, 2002; Spitz eta kideak, 2014; Stephens eta Krebs, 1986), “harrapakin-hautespen aktiboaz” hautatu daitekeen harrapakin potentzialen gama mugatzen dute, harrapariaren bazka-estrategian eraginez. Biologikoki esanguratsuak diren ezaugarri horietako batzuk ezaugarri bereizgarri edo *trait* funtzionaletan islatzen dira (Schmitz, 2017), zeinak harrapariaren bazkarekiko hobespenetan eragiten duten faktoreen inguruko funtsezko ideiak ematerik dituzten.

Nahiz eta oso gutxitan erabili izan diren ugaztun intsektiboroekin, *trait*etan oinarritutako hurbilpenek, ikerketa taxonomikoetan oinarrituz era desegokian oportunistak gisa definitutako espezieen harrapakin-hautespena antzematea (Spitz eta kideak, 2014), edo alderantziz, lehendik harrapakin-espezialista hertsizat hartzen zen espezie batean espektro trofiko funtzional zabala ezagutaraztea (Arrizabalaga-Escudero eta kideak, 2019) ahalbidetu diete ikertzaileei. Hortaz, *trait*etan oinarritutako hurbilpenek ikaragarri hobetu dezakete espezieen eta euren ingurumenaren arteko erlazioaren inguruan dugun ezagutza (Luck eta kideak, 2012), eta bazka-ekologian haustura bat ere markatzerik badute gainera (Spitz eta kideak, 2014). Harrapakinen *trait*etan oinarritutako ikerketek muga batzuk badituzte ere (Cesar eta Frid, 2012; Kremer eta kideak, 2017; Poff eta kideak, 2006), harrapariaren ikuspegitik seguruenik

egokiagoa izango da *traitek* harrapakin komunitateak ezaugarritzen dituzten modua esleipen taxonomikoa baino.

Pirinioetako muturluzea (*Galemys pyrenaicus*, É. Geoffroy Saint-Hilaire, 1811, Insectivora, Talpidae) mendiko erreka hotz eta garbietan bizi den eta dieta generalista duen ugaztun intsektiboro erdi-urtarra da. Endemikoa da Iberiar Penintsularen iparraldean eta Pirinioetan, baina bere banaketa-eremua gogorki murriztua izan da azken hamarkadetan, eta horrek Naturaren Kontserbaziorako Nazioarteko Batasunaren (IUCN) zerrenda gorriaren kategorietan Zaugarri gisa sailkatzea ekarri du (Fernandes eta kideak, 2008). Zaletasun sendoa du fluxu bizkorreko urlasterrekiko, motelagoak diren ur-bare eta putzuen aldean (2. Kapituluua; Esnaola eta kideak, 2018b), nahiz eta muturluzearen harrapakin nagusi diren ornogabe bentikoak ez ugariagoak izan bertan (4. Kapituluua). Horrek galdera mahaigaineratzen du muturluzeek erakusten duten urlasterrekiko zaletasun sendo horren zergatiei buruz. Azalpen potentzial bat zera da: ugaritasun orokorra antzekoa izan arren, eurek hobetsitako harrapakin motak nabarmenki ugariagoak direla bertan. Galdera horrek muturluzeen zaletasunak ezagutarazteko *traitetan* oinarritutako hurbilpen bat eskatzen du, kontserbazio-neurri eraginkorrak hartu ahal izateko informazio gakoa eskain lezakeena.

Hemen, errekaen kontserbazio-egoerarekin harrapakin-hautespena aldatzen ote den aztertzen duen ikerketa baten emaitzak aurkezten ditugu, eta bertan DNA metabarcoding bidez muturluzearen dietak aztertu eta habitat mailako harrapakin eskuragarritasunekin konparatu dira bi erreka desberdinetan. Gure ikerketaren helburuak (1) Pirinioetako muturluzearen dieta-hautespena eragiten duten harrapakin-*traitak* identifikatzea, (2) *traitek*iko zaletasunak muturluzeen habitat-hautespena azal ote dezakeen aztertzea, eta (3) hobetsitako *traitak* bi erreken artean desberdinak ote ziren egiaztatzea izan dira. Hipotesi nagusiaren arabera, muturluzeek errekaen hondoa azalean bizi diren harrapakin handiak, taldekatuak, motelak, eta defentsa mekanismo gabeak hobetsiko lituzke. Bigarren hipotesiaren arabera, hobespen horiek muturluzeen habitat-erabilera eragingo lukete, eta kontserbazio-egoera okerragoa duen erreka urlasterrekiko zaletasun handiagoa azalduko lukete.

Material eta metodoak

Ikerketa-eremua

Ikerketa hau 2016an egin zen, irrati-telemetry lan batekin batera (2. Kapituluua; Esnaola eta kideak, 2018b), Iberiar Penintsularen iparraldeko bi erreketan

(Euskal Herrian). Elama bigarren ordenako mendiko erreka da, Artikutzako lursailean biztanlerik gabeko granito eta eskistozko 1.415 ha-ko arroa estaltzen duena, 1919-tik natura erreserba gisa zorrozki kudeatua izan dena, egun baso-jarduerarik gabekoa, eta ondorioz pago eta haritzez osaturiko baso estaldura zabala duena (Castro, 2009). Urasterren eta ur-bareen eskuragarritasuna antzekoa da ikerketa eremuan (%45 bakoitza), eta putzuak dira habitat urriena bertan (2. Kapituluak; Esnaola eta kideak, 2018b). Leitzaranean, berriz, laugarren ordenako ibaia da, kareharri, arbel eta hareharrizko 12.402 ha-ko arroa estaltzen duena. Elaman ez bezala, 3.150 biztanle dituzten bi herri kokatzen dira Leitzaranean arroaren goiko aldean, nahiz eta horren ostean errekek biztanlerik gabeko 25 km inguruko haran luzea zeharkatzen duen, non basogintza eta jarduera hidroelektrikoetarako ur-erazketak diren giza-eraginezko jarduera nagusiak (Izagirre eta kideak, 2013). Leitzaranean ur-bareak dira nagusi (%60) eta putzuak dira berriro ere habitat urrienak (2. Kapituluak; Esnaola eta kideak, 2018b). Erreka hauek ingurumen-presio desberdinak pairatzen dituzte eta, Elamak kontserbazio-egoera hobea duen arren, biek dute egoera ekologiko ona Uraren Europako Zuzentarauaren arabera (Council of the European Communities, 2000) eta biak daude Europar Batasunaren Natura 2000 sarearen baitan babestuta (Council of the European Communities, 1992).

Laginketa Esnaola eta kideek (2018; 2. Kapituluak) argitaratutako irrati-telemetria laneko tarte eta garai beretan egin zen, Elama erreka 4 km-ko tartean (43°12'40"N 1°48'36"W eta 43°11'14"N 1°48'4"W artean; batazbesteko altitudea, 330 m; batazbesteko zabalera 7,08 m), eta Leizaranean ibaiko 10 km-ko tartean (43°8'57"N 1°57'26"W eta 43°6'55"N 1°56'9"W artean; batazbesteko altitudea, 290 m; batazbesteko zabalera, 12,42 m). Tartean luzeren arteko aldeak Leizaranean ibaian muturluzeak harrapatzeko zailtasun handiagoari dagozkio.

Muturluzearen dieta

Pirinioetako muturluzearen gorotzak bi erreketan bilatu ziren 2016ko irailean eta urrian, espezie honentzako bereziki diseinatutako babesleku artifizialak eraikiz (1. Eranskina; González-Esteban eta kideak, 2018). Denera 188 gorotz (erreka bakoitzeko 94) bildu eta hodietan gorde ziren, %98 etanolean, eta DNA erauzi arte -80°C-tan izoztu ziren.

Muturluzeen gorotz-laginetako DNAREN erazketa, PCR-anplifikazioa eta Errendimendu Altuko Sekuentziazioa (HTS) Esnaola eta kideek (2018a; 3. Kapituluak) deskribatu bezala egin zen. Laburbilduz, *DNA Qiagen Powerfecal DNA kit*-a (Qiagen Iberia, S.L. Madrid) erabiliz erauzi zen gorotzetatik, fabrikatzailearen jarraibideak jarraituz, eta jarraian PCR bidez amplifikatu ziren

COI barkode guneko zatiak, LepF1/EPT-long-univR (Gillet eta kideek, 2015, bezala moldatua) eta ZBJ-ArtF1c/ZBJ-ArtR2c (Zeale eta kideak, 2011) hasle pareak erabiliz. Laginak purifikatu eta bigarren erreakzio bat egin zen Illumina Nextera v2 kit-a erabiliz, anplifikatutako produktu bakoitza idexatu eta Illuminako lotailuak eransteke. Sekuentziazioa *Illumina MiSeq NGS plataforman* (parekatutako muturreko 2x300 bp-ko irakurketen sekuentziazioa) egin zen *MiSeq® Reagent Kit v3 (600 ziklo)* erabiliz, fabrikatzailearen jarraibideak jarraituz. Sekuentzien prozesamendu orokorra USEARCH erabiliz (Edgar, 2010; Edgar eta Flyvbjerg, 2015) egin zen. Genbank nt (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), BOLD (<http://www.boldsystems.org/>) eta datu-base pertsonala erabili ziren BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) erabiliz OTU-ei (*Operational Taxonomic Units*) taxonomia esleitzeko. Lan hau Euskal Herriko Unibertsitateko UPV/EHU Ikerkuntzarako Zerbitzu Orokorretan (SGIker) egin zen. Emaizak agerpen-maiztasunen bitartez (FO, taxon hori gorotzen zein proportziotan detektatzen zen) adierazi ziren.

Harrapakin-eskuragarritasuna

Makroornogabe urtarrak Surber sare batekin (30 cm x 30 cm; 1 mm-ko sarea) lagindu ziren 2016ko iralean eta urrian hiru habitat motetan (Overton eta kideak, 1997): “urlasterrak”, ura bizkor, zurrunbilotsu, azalera irregularrekoa eta zurixka duten lekuak; “ur-bareak”, ura ia laminarra eta sakonera erregularrekoa dutenak; eta “putzuak”, ibilguaren sakonuneetan, emari motelekoak. Habitat eta erreka bakoitzeko 10 lagin bildu ziren zoriz erabakitako kokapenetan, denera 60 lagin izanik. Lagin horiek plastikozko ontzietan gorde eta %70-eko etanoletan mantendu ziren identifikazioa burutu arte.

Laborategian, makroornogabeak 2mm-ko sareaz bahetu, sailkatu, zenbatu eta disekzio-mikroskopioaz identifikatu ziren. 2mm-ko sarea hautatu izanaren arrazoa zera zen, Brose eta kideek (2006) deskribatutako harrapari-harrapakin gorputz tamainaren erlazioaren arabera ez zela muturluzeek 2 mm baino txikiagoak ziren harrapakinak jaterik espero. Bereizmen taxonomikoa genero edo espezie mailan ezarri zen taxa gehienentzako, Diptera, Coleoptera, Oligochaeta, Hirudinidae eta Nematodaren salbuespenekin, zeinak familia edo ordena mailan identifikatu ziren.

Datuen analisisa

Dietaren eta harrapakin-eskuragarritasunaren laginketen osotasuna ‘iNEXT’ online plataforma (Chao eta kideak, 2016) erabiliz ebaluatu zen, taxaren asetasun-kurbak eraikiz. Horiek asintota lortu zuten eta laginen osotasuna

%95 baina altuagoa izanik, erabilitako lagin tamaina bi erreketako muturluzeen dieta deskribatzeko egokia zela baieztatu zen (ikusi 4. Kapitulua).

Traiten hautespena muturluzeen harrapakin-hautespenean potentzialki eragiten zuten 9 *trait* erabiliz ondorioztatu zen. Horietako 4 (sakoneraren zaletasuna, korrontearen abiaduraren zaletasuna, substratuaren zaletasuna eta ur-fluxuarekiko esposizioaren zaletasuna; 1. Taula) habitataren (urlasterra, ur-barea edo putzua) eraginpean leudeke seguruenik, eta beste bostak (substratuarekiko mugikortasun/lotura mota, arintasuna, agregazio-joera, gorputzaren tamaina potentzial maximoa eta gorputzaren malgutasuna; 1.

1. Taula. Muturluzeen harrapakinak ezaugarritzeko erabilitako *traitak* eta beren kategoriak. Jatorrizko *trait*-kategoriak De Crespín eta Usseglio-Polatera-k (2002), deskribatuak izan ziren, eta hemen agertzen den moduan berrizendatuak eta ber-sailkatuak izan dira. Datu-bilduma: Sánchez-Hernández (2014). Ingelesezko laburdurak mantendu dira.

<i>Traita</i>	Laburdura	Kategoriak	Sánchez-Hernández-en jatorrizko kategoriak
Sakonera	DEP1	<i>Hiporreikoa</i>	'Zulatzaille' hiporreikoa+ Hiporreiko 'interstiziala'
	DEP2	<i>Epibentikoa</i>	Higadurazko epibentikoa + deposizional epibentikoa
	DEP3	<i>Ur-zutabean</i>	Ur-zutabean
Korrontearen abiadura	CUV1	<i>Motela</i>	Geldia / Motela (0-25 cm/s)
	CUV2	<i>Ertaina</i>	Ertaina (25-75 cm/s)
	CUV3	<i>Bizkorra</i>	Bizkorra (>75 cm/s)
Substratua	SUS1	<i>Blokeak</i>	Blokeak (>256 mm)
	SUS2	<i>Txintxarra-Uharria</i>	Txintxar fina / Txintxarra-Uharria (2-256 mm)
	SUS3	<i>Sedimentu finak</i>	Lohia (0.001-0.2 mm) + Harea-Lohia (0.2-2 mm) + Lokatza
	SUS4	<i>Makrofitoak-Sustraiak</i>	Briofitoak + Bestelako makrofitoak + Sustraiak
	SUS5	<i>Orbela</i>	Orbela, detritu organikoa
Ur-fluxuarekiko esposizioa	FLE1	<i>Gordeta</i>	Gordeta
	FLE2	<i>Agerian</i>	Agerian
Substratuarekiko mugikortasuna/lotura	MAS1	<i>Igerilaria</i>	Igerilaria
	MAS2	<i>Harrestaria</i>	Herrestari / ibiltaria + Herrestari / irristaria
	MAS3	<i>Lotua</i>	Betiko lotua + Denboraldi batez lotua
Arintasuna	AGI1	<i>Ezer ez</i>	Ezer ez
	AGI2	<i>Ahula</i>	Ahula
	AGI3	<i>Handia</i>	Handia
Agregazio-joera	AGT1	<i>Handia</i>	Handia
	AGT2	<i>Ahula</i>	Ahula
Tamaina potentziala	POS1	≤ 2	≤ 2 mm
	POS2	2-8	> 2-4 mm + > 4-8 mm
	POS3	8-32	> 8-16 mm + > 16-32 mm
	POS4	> 32	> 32 mm
Gorputzaren malgutasuna (zorroak/tutuak barne)	BOF1	<i>Ezer ez</i>	Ezer ez (<10°)
	BOF2	<i>Ahula</i>	Ahula (10-45°)
		<i>Handia</i>	Handia (> 45°)

Taula) independente gisa uler daitezke. *Trait* bakoitzak 2-5 kategoria zituen, eta taxon bakoitzaren kategoria bakoitzarekiko afinitate-balioak (Otik 5era) *fuzzy coding* bidez (Chevenet eta kideak, 1994; Sánchez-Hernández, 2014; S1. Taula) adierazi ziren.

Afinitateak afinitate erlatiboetara transformatu ziren, balio bakoitza taxon bakoitzaren *trait* bakoitzaren afinitatearen baturaz zatituz. *Trait* daturik ez zuten maila taxonomikoei (adb. Chironomidae) maila taxonomiko baxuagoko balioen batazbestekoa atxiki zitzaien (ikus Sánchez-Hernández, 2014; S1. Taula). Daturik ez zuten taxak, aldiz, (anelidoak, lepidopteroak eta zenbait koleoptero lurtar, guztiak oso gutxitan kontsumituak; ikusi 4. Kapituluua) analisietatik baztertuak izan ziren (S1. Taula).

Analisietarako “taxa x *traitak*” matrizea eraiki zen dietarako eta eskuragarritasunerako, zeina agerpen-maiztasunekin (FO) zamatua izan zen dietarako, eta banakoen ugaritasunarekin bentoseko eskuragarritasunerako. Balio horiek batu eta proportzio erlatiboetara transformatu ziren dietan erreka bakoitzerako (“erreka x *traitak*” matrizea eraikiz), eta eskuragarritasunean lagin kategoria bakoitzerako (“lagin kategoria x *traitak*” matrizea eraikiz, non lagin-kategoriak honakoak ziren: Elama urlasterra, Elama ur-barea, Elama putzua, Leitzaran urlasterra, Leitzaran ur-barea eta Leitzaran putzua).

Harrapakin-eskuragarritasuneko eta muturluzeen dietako *traitak* bi erreketarako deskribatu ziren (eskuragarritasunean habitat mota bakoitzerako ere bai), *trait*-kategoria bakoitzaren proportzioekin. Harrapakin-eskuragarritasunean erreken eta habitaten artean zamatutako *trait*-kategorien balioetan zeuden aldeak permutazio bidezko ANOVA erabiliz aztertu ziren, R-rako *ImPerm* paketeko *Imp* funtzioa erabiliz (Wheeler eta Torchiano, 2016). Permutazio bidezko modeloei erreka, habitata, *trait*-kategoria eta bigarren mailako interakzioak doitu genizkien.

Dietaren eta eskuragarritasunaren datuen arteko, zein erreken eta habitaten arteko antzekotasunak bilatzeko analisi multibariantea egin zen. Horretarako, Osagai Nagusien fuzzy Analisia egin zen (FPCA; R-rako *ade4* paketeko *prep.fuzzy.var* eta *dudi.fpca* funtzioak erabiliz), *trait* guztietako kategoria guztiak aldi berean kontutan hartuz. FPCA Osagai Nagusien Analisiaren aldaera sendo bat da (Cundari eta kideak, 2002). Azkenik, Erredundantzia Anlisi bat erabili zen (RDA, Hellinger-en distantzia matrizea eta R-rako *vegan* paketeko *rda* eta *anova.cca* funtzioak erabiliz) errekek, habitatak eta dieta *vs* eskuragarritasunak *trait*-kategorien arteko aldakortasuna azaltzeko zuten esangarritasuna lortzeko. *Trait* bakoitzak bi erreken, hiru habitaten eta dieta eta eskuragarritasunaren arteko alde orokorrak azaltzeko duen garrantzi erlatiboa, inertzia-balioen bidez erakutsi dugu.

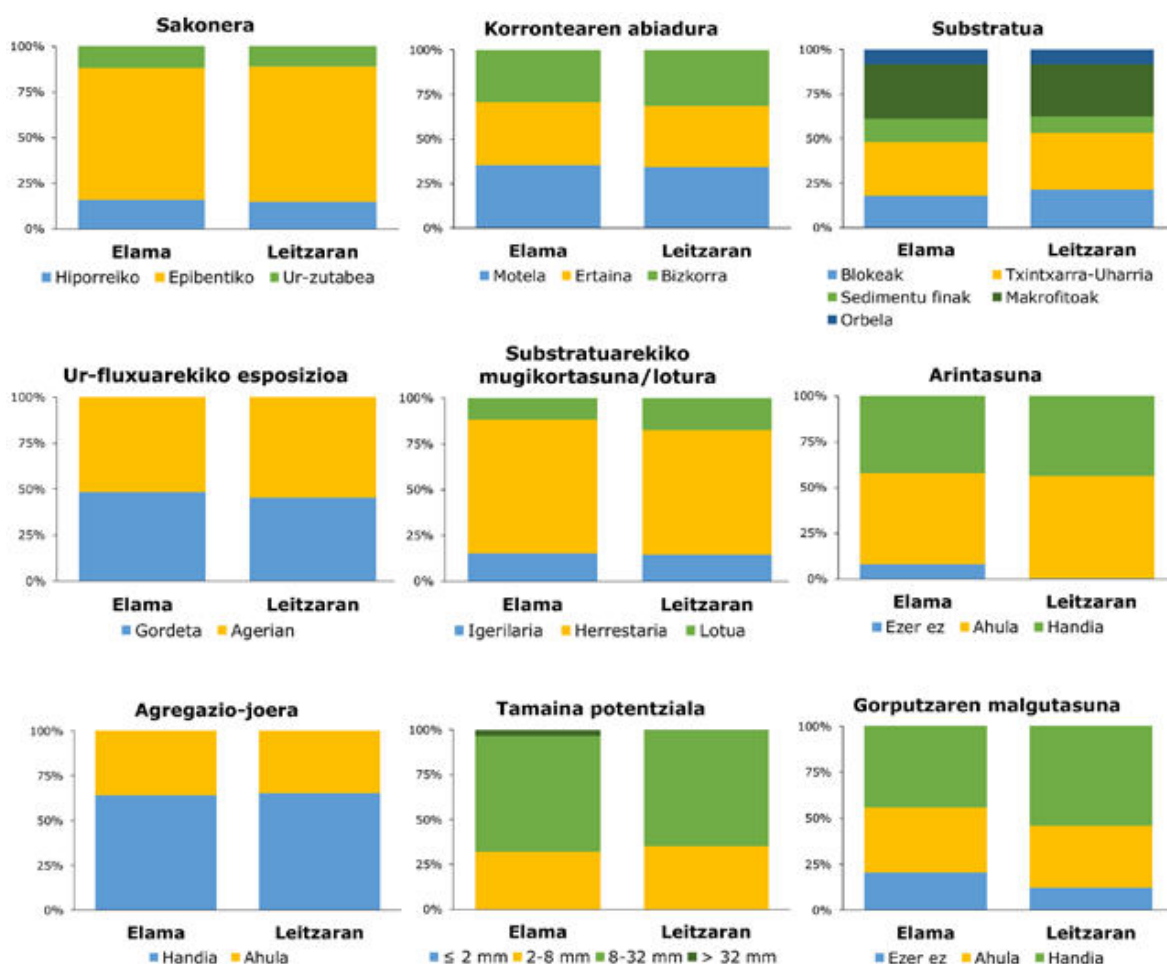
Ondoren, muturluzeen harrapakin-hautespena dieta eta eskuragarritasunaren arteko proportzioak erabiliz aztertu zen. Muturluzeen gorotzak ezin zirenez habitat zehatzekin lotu, habitat bakoitza ez zen bere aldetik aztertu ahal izan ikerketaren zati honetan. Horren orde, eskuragarritasuneko datuen “*taxa x traitak*” matrizea alde aurretik erreka bakoitzean ikertutako tartean habitatek zuten azalera erlatiboarekin zamaturiko taxaren ugaritasun-balioez bidertu zen. Bentoseko ugaritasuna habitataren azalera zamaturuz, habitatak ikertutako tartetako guztizko harrapakin-eskuragarritasunean duen ekarpena hartzen dugu kontutan. Horren ostean, $\log_{10}(\text{dieta/eskuragarritasuna})$ proportzioa kalkulatu zen *trait*-kategoria eta lagin bakoitzerako, erreka bakoitzeko dietaren balioa eskuragarritasuneko erreplika bakoitzaren balioarekin zatituz. Proportzioak kalkulatzeko eskuragarritasuneko zeroek sortutako arazoa konpontzeko, proportzioa kalkulatu baino lehen konstante bat (datu-baseko balio minimoaren erdia) gehitu genien balio guztiei. Azkenik, erreka bakoitzeko batazbesteko proportzioa eta bere konfidantza-tartea kalkulatu ziren. Proportzio horrek muturluzeak harrapakin *trait*-kategoria zehatzak (positiboki edo negatiboki) hautatzen ote zituzten aztertzeko balio izan zuen. *Trait*-kategorien hautespena erreka mendeko zegoen aztertzeko permutazio bidezko ANOVA bat egin zen, R-ko *ImPerm* paketeko *Imp* funtzioa erabiliz (Wheeler eta Torchiano, 2016), bi erreka arteko *trait*-kategorien afinitateetako antzekotasunak aztertzeko. Hemen ere, permutazio bidezko modeloei erreka, habitata, *trait*-kategoria eta bigarren mailako interakzioak gehitu genizkien. Analisi guztien esangarritasun maila $p < 0,05$ -n ezarri zen. Analisi guztiak R 3.4.3 (R Core Team, 2014) erabiliz egin ziren.

Results

Traitak dietan

Trait-kategoria ugari muturluzeen dietan zuten ekarpen erlatiboa oso antzekoa zen bi erreketan (1. Irudia). Sakonera *traitari* zegokionean, muturluzeek harrapakin *epibentikoak* kontsumitu zituzten nagusiki (batazbeste %73,3), *hiporreikoek* (%15,3) eta *ur-zutabeko* harrapakinek (%11,3) jarraituta. Aldeak hutsalak ziren *korrontearen abiadurari* loturiko *traitaren* kasuan (%35,0 *ertaina*, %34,8 *motela*, %30,2 *bizkorra*). Substratuaren kategoriei zegokienean, muturluzeek *txintzar-uharri* (%31,1) eta *makrofito-sustrai*ei (%29,8) loturiko harrapakinak jan zituzten nagusiki, *blokeek* (%19,5), *sedimentu finek* (%11,1) eta azkenik *orbelak* (%8,4) jarraituta. Ur-fluxuarekiko esposizioa *traitak* alde txikiak erakutsi zituen (%53,2% *agerian vs.* %46,8 *gordeta*), substratuarekiko mugikortasuna/loturari zegokionean *herrestari* harrapakinak beste kategoriak

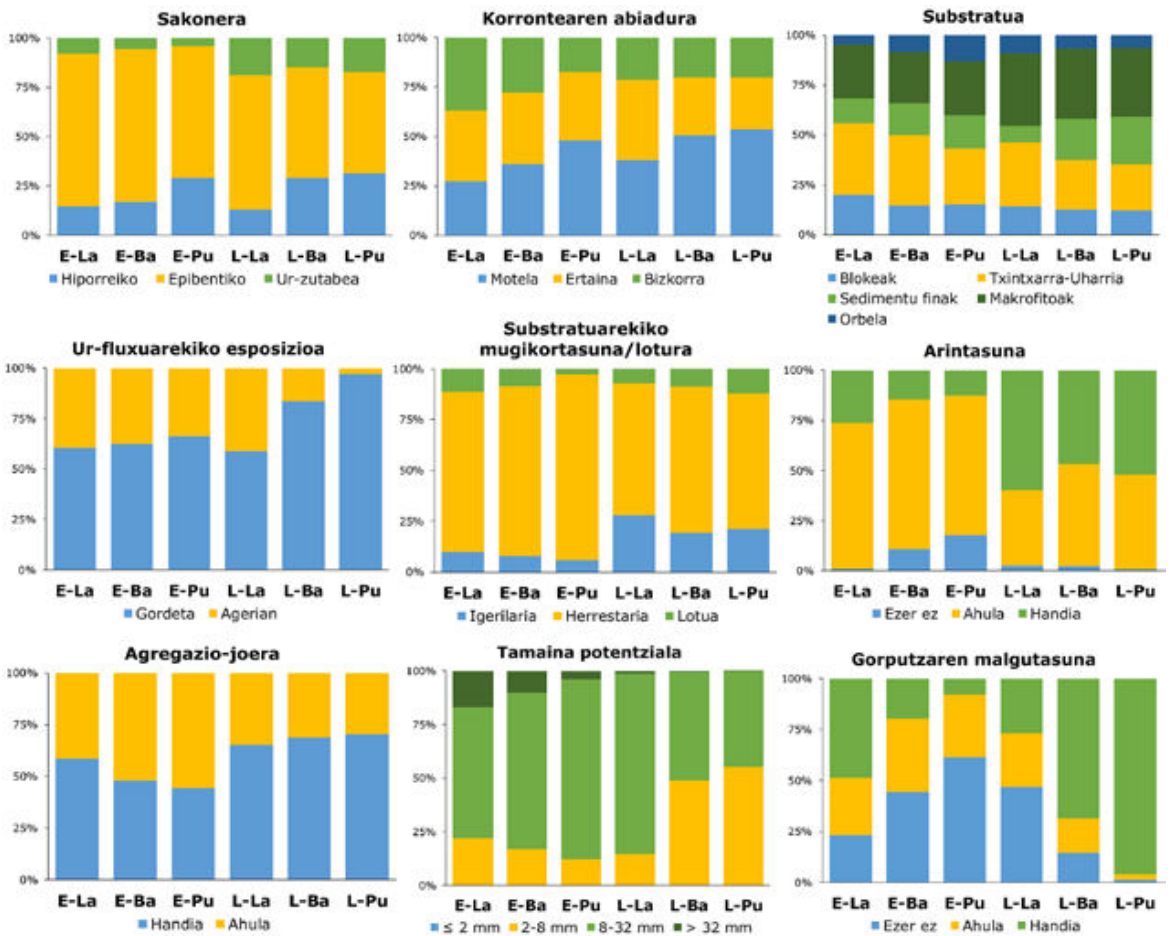
baino askoz maizago (%70,5) jaten zituzten bitartean. *Arintasunik gabeko* harrapakinak besteak baino askoz maiztasun gutxiagoz jaten zituzten (%4,2 vs. %52,9 *arintasun ahulekoak* eta %42,8 *arintasun handikoak*, hurrenez hurren), dietan *agregazio-joera handiko* harrapakinak (%64,6) *joera ahulekoak* baino (%35,4) ohikoagoak ziren bitartean. Tamaina potentzialari zegokionean, *tamaina ertaineko* harrapakinak jan zituzten nagusiki muturluzeek (%64,3 8-32 mm, %33,6 2-8 mm-z jarraituz), oso gutxitan jan zituzten *tamaina* potentzial *handiko* harrapakinak (> 32 mm, %2,1) eta ez zuten ia inoiz jan ≤ 2 mm tamaina potentzialeko harrapakinik. Azkenik, gorputzaren malgutasuna *traitak malgutasun handirako* erakutsi zuen ekarpen erlatibo altuena (%49,0), *ahula* (%34,7) eta *ezer ez* (%16,2) kategoriek jarraituta.



1. Irudia. *Trait*-kategoria bakoitzak Elama eta Leitzaran erreketako muturluzeen dietan duen ekarpen erlatiboa.

Harrapakin-eskuragarritasuna

Trait-kategorien arabera sailkatutako ornogabeen ugartasunei (eskuragarritasuna) zegokienean, alde interesgarriak zeuden habitaten eta ibaien artean (2. Irudia). Fauna *epibentikoa* ugariagoa zen habitat eta erreka guztietan (batazbeste %66,3), fauna *hiporreikoak* (%22,3) eta *ur-zutabe* faunak (%11,4) jarraituta; sakonera-zaletasunaren proportzioa esangarriki aldatzen zen ikertutako habitaten artean (sasi-F = 28,25, a.g. = 4, $p < 0,001$), estatistikoki fauna *epibentikoa* urlasterretan ugariena izanik, fauna *hiporreikoak* putzuetan ugariena zen bitartean. *Trait*-kategorien eta erreken arteko interakzioa esangarria zen (sasi-F = 57,67, a.g. = 2, $p < 0,001$), Elaman fauna *epibentikoa* Leitzaranean baino ugariagoa izanik.



2. Irudia. *Trait*-kategoria bakoitzak hiru habitatetako (La = urlasterrak, Ba = ur-bareak eta Pu = putzuak) eta bi erreketako (E = Elama eta L = Leitzaranean) harrapakin-eskuragarritasunaren erlatiboa.

Korrontearen abiadura *traitaren* kategorien eskuragarritasun orokorra *motela* kategoriarentzako izan zen altuena (%42,3), *ertainak* (%33,8) eta *bizkorrak* (%23,9) jarraitzen zutela. Hori nabarmenki aldatzen zen habitaten artean (sasi-F = 23,42, a.g. = 4, $p < 0,001$), putzuetatik ur-bare eta urlasterretara *bizkorra* kategoriak gora eginez, *motela* kategoriak kontrakoa egiten zuen bitartean (2. Irudia). *Trait*-kategorien eta erreken arteko interakzioa ere esangarria zen (sasi-F = 18,90, a.g. = 2, $p < 0,001$), *motela* kategoria ugariagoa izanik Leitzaranen, urlasterrak ugaritasun gutxieneko habitatak diren errekan.

Orokorrean, eskuragarri zeuden ornogabe gehienak *makrofito-sustrai*ei loturik zeuden (%30,9), *txintzar-uharriek* (%29,9), *sedimentu finek* (%16,4), *blokeek* (%14,7) eta *orbelak* (%8,1) jarraitzen zutela. *Trait*-kategorien eta erreken arteko interakzioa esangarria zen (sasi-F = 29,52, a.g. = 4, $p < 0,001$), *sedimentu finak* eta *makrofitoak-sustraiak* Leitzaranen altuagoak izanik. Habitat mailan, *trait*-kategorien eta habitaten arteko interakzioa esangarria zen (sasi-F = 15,28, a.g. = 8, $p < 0,001$), urlasterretan *txintzar-uharri*ei eta *bloke*ei loturiko fauna izanik ugariena (%33,9 eta %17,1, hurrenez hurren), putzuetan ugariena *sedimentu finei* eta *orbelari* loturikoak ziren bitartean (%20,2 eta %9,6, hurrenez hurren).

Ur-fluxuarekiko esposizioari zegokionean, *gordeta* kategoria izan zen ugariena habitat eta erreka guztietan (batazbeste %71,3). Kasu honetan alde esangarriak zeuden erreken artean (sasi-F = 51,04, a.g. = 1, $p < 0,001$), *gordetako* ornogabeak ugariagoak izanik Leitzaranen, eta *trait*-kategorien eta habitaten arteko interakzioa ere esangarria zen (sasi-F = 27,69, a.g. = 2, $p = 0,006$). *Agerian* kategoriak urlasterretan izan zituen balio altuenak (%40,4) eta putzuetan baxuenak (%18,5).

Substratuarekiko mugikortasuna/loturari zegokionez, *herrestaria* kategoriak izan zituen ugaritasun balio altuenak (batazbeste %76,3), *igerilaria* (%15,3) eta *lotua* (%8,4) kategoriez jarraiki. *Trait*-kategorien eta erreken arteko interakzioa esangarria zen (sasi-F = 77,46, a.g. = 2, $p < 0,001$), *herrestaria* kategoria Elaman ugariagoa izanik. *Trait*-kategorien eta habitaten arteko interakzioa ere esangarria zen (sasi-F = 8,96, a.g. = 4, $p = 0,043$), *herrestaria* eta *igerilaria* kategorien gorakada eta *lotua* kategoriaren beherakada leunak erakutsiz urlasterretatik ur-bare eta putzuetara.

Ornogabe gehienek (batazbeste %58,8) *arintasun ahula* zuten, *handia* (%35,3) eta *ezer ez* (%5,9) kategoriek jarraiki. *Trait*-kategorien eta erreken arteko interakzioa esangarria zen (sasi-F = 157,90, a.g. = 2, $p < 0,001$), Leitzaranen *arintasun handiak* balio altuagoak izanik. *Trait*-kategorien eta habitaten arteko interakzioa ere esangarria zen (sasi-F = 12,67, a.g. = 4, $p < 0,001$), *handia* kategoriak urlasterretan izanik balio altuenak (%42,9), *ahulak* ur-bareetan (%62,7) eta *ezer ez* kategoriak putzuetan (%9,2).

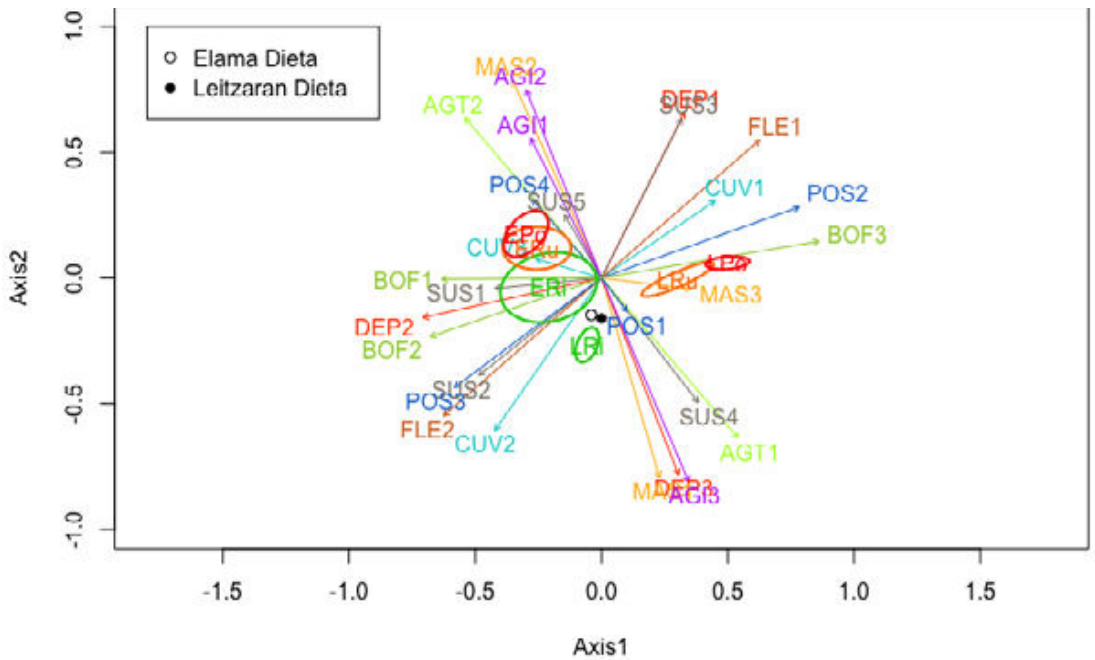
Agregazio-joera handiko ornogabeak ziren habitat eta erreka guztietan ugariena (batazbeste %59,2). *Trait*-kategorien eta erreken arteko interakzioa estatistikoki esangarria zen (sasi-F = 184,44, a.g. = 1, $p < 0,001$), Leitzaranean *agregazio-joera handia* ugariagoa izanik. *Trait*-kategorien eta habitataren arteko interakzioa ordea ez zen esangarria (sasi-F = 2,60, a.g. = 2, $p = 0,086$).

Tamaina potentzialari zegokionean, **8-32 mm** tamaina potentzialaren kategoria izan zen habitat eta erreka guztietan ugariena (batazbeste %66,1), **2-8 mm** (%28,3), **> 32 mm** (%5,5) eta **≤ 2 mm** (%0,0) kategoriek jarraitzen zutela. *Trait*-kategorien eta erreken arteko interakzioa estatistikoki esangarria zen (sasi-F = 37,62, a.g. = 3, $p < 0,001$), Leitzaranean **8 mm baino txikiagoak** ziren ornogabeak ugariagoak izanik. *Trait*-kategorien eta habitataren arteko interakzioa ere esangarria zen (sasi-F = 9,79, a.g. = 6, $p < 0,001$), **8 mm baino txikiagoak** ziren ornogabeen gorakada eta **8 mm baino handiagoak** ziren ornogabeen beherakada behatu baitzen urlasterretatik ur-bare eta putzuetara.

Azkenik, gorputzaren malgutasunari zegokionean, eskuragarritasun orokorra *handia* kategoriarentzako izan zen altuena (%44,5), *ezer ez* (%32,0) eta *ahula* (%23,5) kategoriek jarraitzen zutela. *Trait*-kategorien eta erreken arteko interakzioa esangarria zen (sasi-F = 45,27, a.g. = 2, $p < 0,001$), *malgutasun handiko* ornogabeek balio altuagoak izan baitzituzten Leitzaranean. *Trait*-kategorien eta habitataren arteko interakzioa ere esangarria zen (sasi-F = 5,10, a.g. = 4, $p < 0,001$), *handia* kategoria putzuetan izanik ugariena (%51,9) eta *ezer ez* eta *ahula* kategoriak urlasterretan (%35,1 eta %31,7, hurrenez hurren).

Traiten hautespena

Datu guztiekin (dieta eta eskuragarritasuna, 3. Irudia) egindako *fuzzy* PCA-n gorputzaren malgutasuna, ur-fluxuarekiko esposizioa eta arintasuna *traitek* erakutsi zituzten inertzia-balio altuenak ($> 0,020$; S2. Taula), lehenengo ardatzak guztizko bariantzaren %58,4 azalduz, eta bigarrenak %16,5. Erreken arteko aldeak eskuragarritasunarentzako baino txikiagoak ziren dietarentzako. FPCA-k erreken (Axis1) eta habitataren (Axis2) arteko aldeak erakusten zituen, batez ere. Nabarmena izan zen muturluzearen bi erreketako dietak elkarren ondoan agertu zirela, urlasterretako eskuragarritasunetik gertu eta putzuetako eskuragarritasunetik urrun. Habitat-mailako eskuragarritasuna estaltzen zuten Elamako elipseak Leitzarangoak baino handiagoak eta gainjarriagoak ziren, hobekien kontserbaturiko errekan habitataren baitako eskuragarritasunaren heterogeneotasun handiagoa baina habitataren arteko alde txikiagoa erakutsiz. Erredundantzia analisi batek erreken ($F = 34,16$; a.g. = 1; $p = 0,001$; bariantzaren %32,8 azalduta) zein habitataren ($F = 6,48$, a.g. = 2, $p = 0,001$; %12,4) arteko



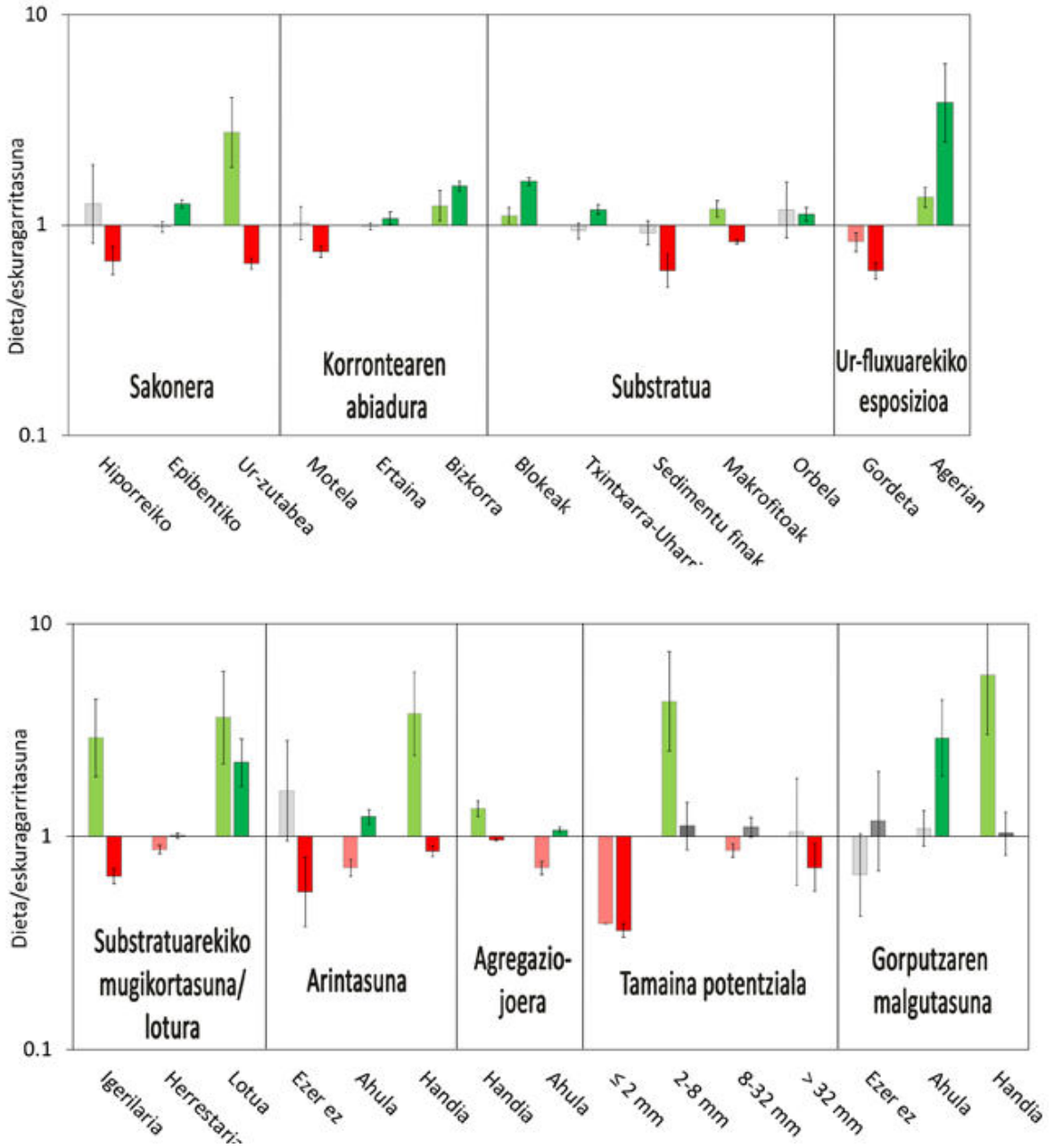
3. Irudia. Dietako eta erreko eskuragarritasuneko ornogabeen *traiten* FPCA. Puntuak dieta adierazten dute, elipseek habitat bakoitzeko eskuragarritasuna (ERi = Elamako urlasterrak; ERu = Elamako ur-bareak; EPo = Elamako putzuak; LRi = Leitzarango urlasterrak; LRu = Leitzarango ur-bareak; LPo = Leitzarango putzuak; urlasterrak berdez adierazi dira, ur-bareak laranja eta putzuak gorri) eta geziek *trait*-kategoria (ikusi laburdurak 1. Taulan). *Trait* bakoitza kolore desberdinez adierazi da. Ohartu Elamako elipseak Leitzarangoak baino handiagoak direla eta gainjartze handiagoa dutela. *Axis* = ardatza.

aldeak esangarriak zirela, baina eskuragarritasunaren artekoak esangarriak ez zirela ($F = 1,01$, a.g. = 1, $p = 0,348$; %1,0) erakutsi zuen (S3. Taula).

Harrapakin-hautespenari zegokionean, dieta eta eskuragarritasunaren arteko proportzioek erakutsi zuten muturluzeek positiboki hautatzen zituztela *ur-zutabe*ari loturiko harrapakinak eta harrapakin *epibentiko*ak, *ur bizkorrei* (> 75 cm/s), *blokeei* edo ur-fluxuarekiko *agerian* dauden habitatei loturiko harrapakinak (4. Irudia). Harrapakinen ezaugarri zegoenean, *2-8 mm* luze ziren harrapakinak, *gorputz mugikortasun pixka bat* (*ahula* edo *handia*) zuten harrapakinak, *igerilariak* zein *substratuari lotzeko mekanismo*ak zituztenak, *bizitasun handiko* harrapakinak eta *agregazioak eratzeko joera sendoa* zuten harrapakinak hautatu zituzten positiboki.

Bestalde, muturluzeek negatiboki hautatu zituzten *ur-abiadura motel*ei loturiko harrapakinak, eta *substratu finei* zein ur-fluxuarekiko *gordetako* guneei loturiko harrapakinak (4. Irudia), *2 mm baino txikiago*ak ziren harrapakinak, *herrestariak*, eta *agregazio-joerarik ez* zuten harrapakinak.

Bi erreketan patroi orokorra berdina izan zen arren (4. Irudia), hautespen-proportzioen erreken arteko aldeak esangarriak izan ziren sakonera ($p < 0,001$), ur-fluxuarekiko esposizioa ($p = 0,004$), substratuarekiko mugikortasuna/lotura ($p < 0,001$), arintasuna ($p < 0,001$) eta tamaina potentziala ($p < 0,001$) *trait*entzako. *Trait*-kategorien eta erreken arteko hautespen-proportzioen interakzioak ere esangarriak ziren *trait* guztientzako (p guztiak $< 0,001$).



4. Irudia. Trait-kategorien hautespena: positiboa (berdea), negatiboa (gorria) eta hautespenik eza (grisa). Koloreak argiagoko dira Elamarako eta ilunagoak Leitzaranerako.

Eztabaida

Ikerketa hau eskusiba bat da harrapakinen *traite*n analisiak Pirinioetako muturluzea intsektiboro generalistaren harrapari-harrapakin harremanak eragiten dituzten mekanismoak ulertzeko hurbilpen funtzional gisa. Hemen ikertutako *traitek* muturluzearen bazka-erabakiak harrapakinen esleipen taxonomikoei baino hobeto azaltzen laguntzen dute.

Traitak dietan

Muturluzeak urlasterren aldeko zaletasun sendoa duten (2. Kapituluua; Esnaola eta kideak, 2018b) harrapari erreofilo espezialistak dira. Beren dietaren inguruan egindako ikerketa taxonomikoei Trichoptera, Ephemeroptera, Plecoptera, Crustacea (Amphipoda) eta Diptera (Bertrand, 1992; Castián eta Gosálbez, 1995; Santamarina eta Guitian, 1988), eta gutxiagotan Coleoptera, Annelida eta Odonata (Puisségur, 1935; Richard eta Viallard, 1969; Santamarina, 1992) jaten dituztela erakutsi dute. Horietako autore batzuek (adb. Santamarina, 1993) muturluzeen dieta urte-sasoien arabera aldatzen dela ikusi zuten, baina beraietako gehienak bat zetozen muturluzeak harrapakin ugariz elikatzen diren harrapari generalista gisa definitzean (e.g. Biffi eta kideak, 2017a,b; Hawlitschek eta kideak, 2018). Ikerketen arteko aldeak lagin kopuruari, eskualde geografikoari edo metodologiari dagozkion ezberdintasunetatik eratorriak izan daitezke.

Aurretiaz eginiko gure hurbilpen taxonomikoan (4. Kapituluua), muturluzeek Elama eta Leitzaran erreketan aldeztuak deskribatutako dietaren antzekoa zutela topatu genuen. *Traitetan* oinarritutako gure ikerketaren arabera, muturluzeek gehien kontsumitu zituzten ehizakiak hauek ziren: harrapakin *herrestariak*, harrapakin *epibentikoak* —Psychodidae, Baetidae, Heptageniidae, Rhyacophilidae eta Gammaridae—, *txintzar-uharri* edo *makrofito-sustrai* substratuetan bizi diren harrapakinak —Simuliidae, Baetidae, Heptageniidae eta Gammaridae— (nahiz eta Elama eta Leitzaran erreketan ez ziren ia goroldioak ez ziren makrofitoak agertzen), *8-32 mm* tamaina potentzialeko harrapakinak — Heptageniidae, Odontoceridae, Rhyacophilidae eta Gammaridae —, eta maizago *agregazio-joera baxua* baino *handia* zuten harrapakinak —Simuliidae, Baetidae, Odontoceridae eta Gammaridae— (S1. Taula). Horrez gain, muturluzeen harrapakinek ez zuten ia alderik erakutsi korrontearen abiadurarekin lotutako *traitei* zegokionean, eta *ur-fluxuarekiko esposizio pixka bat* zuten harrapakinak jan zituzten, *gorputz-malgutasunik gabeko* (Limnephilidae bezalako) harrapakin gutxi jan zituzten eta ez zuten ia *arintasunik gabeko* (moluskuen gisako) harrapakinik jan. Gorputzaren

malgutasuna *traitari* zegokionean, muturluzeek nolabaiteko plastikotasuna erakutsi zuten, *gorputz-malgutasun handiko* —gehien bat Psychodidae, Simulidae, Baetidae—, ahuleko —adb. Heptageniidae—, edo *malgutasunik gabeko* —adb. Gammaridae— harrapakinak janez.

Traitetan oinarritutako dietaren deskribapena eta egindako analisi multibariantea (FPCA) bat zetozen ikertutako bi erreketan eta, hortaz, muturluzeen dietek errekarren kontserbazio-egoerak eragiten ez dion patroia argia erakutsi zuten. Emaizta horiek, beraz, animalia honen bazka-jokabidearen konsistentzia eta harrapakinak ezaugarritzeko erabilitako metodologiaren (taxonomia *vs. trait* biologikoak) garrantzia azpimarratzen dituzte. *Traiten* erabilerak, esate baterako, talde taxonomiko desberdinetako harrapakinak partekatzen dituzten ezaugarriak azaleratzea ahalbidetzen du, *trait* desberdinak erabiliz talde taxonomiko berdinean taldekatutako espezieak bereizteaz batera.

Traiten hautespena

Dieta agerpen-maiztasunen (FO) bitartez neurtzeak gutxi baino maiz jaten diren harrapakinak gehiegi balioestea eta gutxitan baino kantitate handitan jaten diren beste batzuk gutxiestea eragin dezake (Deagle eta kideak, 2019). Arazo metodologiko hori kontutan hartuta, laginketen alborapena ahalik eta gehien ekiditeko lagin-kopuru handia biltzen saiatu ginen (Mata eta kideak, 2019).

Muturluzeek *ur-zutabearekiko* eta *gune epibentikoarekiko* zaletasuna zuten harrapakinak, *gune bizkorretan* agertu eta substratu gisa *blokeak* zituzten harrapakinak, ur-fluxuarekiko *agerian* bizi ziren harrapakinak, *2-8 mm*-ko tamaina potentziala zuten harrapakinak, *arintasun handiko* harrapakinak, *gorputz-malgutasun pixka bat* zuten harrapakinak, *taldekatutako* harrapakinak, eta *igerilariak* zein *substratuari lotzeko mekanismoak* zituzten harrapakinak hobesten zituzten. *Alanites*, *Baetis* edo Simuliidae (S1. Taula) bezala maiz jandako taxak *trait* horietako asko konpartitzen dituzte. Aldiz, muturluzeek negatiboki hautatu zituzten *abiadura moteleko* uretan, *substratu finetan*, ur-fluxuarekiko *gordeta* bizi ziren harrapakinak, tamaina potentziala *2 mm baino txikiagoa* zuten harrapakinak, *agregazio-joerarik ez* zuten harrapakinak, eta *herrestariak*. Nahiz eta harrapakin taxa bakarrak ere ez zituen ezaugarri guzti horiek konpartitzen, *Atherix*, *Eleophila*, *Prionocera* eta *Wiedemannia* (S1. Taula) dira ezaugarri horietako batzuk dituzten taxaren adibideak, eta zaletasun horiek eragin zitzaizkienak. Honela, emaitza horiek muturluzeek harrapakin handi eta motelak positiboki hautatuko zituztela zioen gure lehenengo hipotesia gezurtatzera garamatzate hein batean. Bestalde, espero bezala, muturluzeek positiboki hautatu zituzten *gorputzaren malgutasun pixka bat* behintzat bazuten harrapakinak (zorrotuturik gabekoak) eta *agregazio-joera*

handia zuten harrapakinak eta, aldiz, *substratu finetan* bizi ziren harrapakinak negatiboki hautatu zituzten. Halaber, sakoneraren zaletasunari zegokionean *ur-zutabeen* zein *gune epibentikoan* bizi ziren harrapakinak, *ur-fluxu bizkorreko guneetan* bizi ziren harrapakinak, substratu gisa *blokeak* zituztenak, eta *igerilariak* zein *substratuari lotzeko mekanismoak* zituzten harrapakinak nahiago zituztela azpimarratu zuten gure emaitzek.

Positiboki hautatutako *trait*-kategoriei zegokienean, bi taldetan sailkatu litezke horiek. Alde batetatik, zuzenean urlasterrei loturik daudenak leudeke: urlasterretan bizi diren ornogabeetan esperotako kategoriak ur-fluxuarekiko *agerian* egotea, *substratuari lotuta*, substratu gisa *blokeak* izatea eta *abiadura bizkorreko* urak izatea dira. Badijadi kategorio horiek ez dutela muturluzeen hautuan zuzenean eragiten; aitzitik, muturluzeen habitat-hautespenaren ondorio direla dirudi. Muturluzeak lehentasunez urlasterretan bazkatzeen direnez, euren harrapakin ugarienek ere urlasterretan bizitzeko moldapenak erakustea espero zitekeen, muturluzeentzako izan dezaketen profitagarritasunaren edo harrapagarritasunaren efektua edozein izanik ere. Gainontzeko *trait*-kategoriek ulermen argiagoa eskain dezakete muturluzeen harrapakin-zaletasunei buruz: *ur-zutabeen* eta *gune epibentikoetan* bizitzeko zaletasuna duten harrapakinak (*Baetis*, *Epeorus* edo *Ecdyonurus* kasu), *2-8 mm*-ko tamaina potentziala dutenak (*Leuctra*, *Psychoda* edo Simuliidae kasu), *arintasun handiko* harrapakinak (*Alanites* edo *Echinogammarus* kasu), *gorputz-malgutasun pixka bat* (*ahula* edo *handia*) dutenak (*Ecdyonurus*, *Epeorus*, *Hydropsyche* edo *Psychoda* kasu), *taldekatuta* bizi direnak (*Hydropsyche*, *Leuctra*, *Odontocerum* edo Simuliidae kasu) eta *igerilariak* diren harrapakinak (*Alanites*, *Baetis* or *Echinogammarus* kasu). *Ur-zutabeko* eta *gune epibentikoetako* harrapakinak aldeko zaletasunak erraza dirudi ulertzen, harrapakin *hiporreikoen* kaltetan, ibilguaren hondoa zulatzen aritzeak garestia izan behar baitu energetikoki. Harrapakinak tamaina potentzialen hautespenak aldiz zailagoak dira ulertzen, energetikoki profitagarriagoak diruditen harrapakin handienekiko hautespen negatiboa erakutsi baitzuten muturluzeek. *2 mm baino txikiagoak* diren harrapakinekiko hautespen negatiboak aldiz bat egiten du Brose eta kideen (2006) irizpidearekin, kasu honetan kontsumitzaile-baliabide arteko \log_{10} gorputz-masa proportzioak ez baitzuen 6 balioa gainditzen. Aipagarria da ornogabeen laginketa 2mm-ko sare batez egin zela, *2 mm baino txikiagoak* ziren ornogabeen harrapaketa mugatuz, eta halaz ere hauek dietan zuten maiztasuna gure laginetan baino baxuagoa izan zela, hauekiko muturluzearen zaletasun negatibo sendoa erakutsiz. Harrapakinak arintasunak intuizioaren kontrako kriterioa dirudi muturluzeen zaletasunekiko, eta segur aski sakonerarekiko zaletasuna edo ur-fluxuarekiko *agerian* egotea bezalako bestelako *traitei* loturik egon daiteke. Gorputzaren malgutasunari zegokionez, muturluzeen hautespenak *gorputz-estalki gogorreko* (zorro, oskol, etabar.) harrapakinak saihesten zituztela islatzen zuen, manipulatzeko eta jateko zailagoak izan (Rychlik eta Jancewicz,

2002), eta hortaz, profitagarritasun txikiagoa izan zezaketeelako (Bertrand, 1992). Dena den, zenbait ikerketek erakutsi zuten Trikopteroeren hautespena ez zela zorroen presentziaren menpekoa, muturluzeek positiboki zein negatiboki hautatzen baitituzte zorrodun (Goeridae, Lepidostomatidae, Limnephilidae eta Odontoceridae kasu; *gorputz-malgutasunik eza*) zein zorrogabeko trikopteroak (Hydropsychidae, Polycentropodidae, Philopotamidae eta Rhyacophilidae kasu; *gorputz-malgutasun handia*) (4. Kapituluak; Biffi eta kideak, 2017b; Santamarina, 1992, 1993). Gauza bera behatu izan da habitata eta bazka-hautespena neurri batean muturluzeekin konpartitzen dituzten ur-zozoetan ere (Ormerod eta Tyler, 1991; Santamarina, 1990; Taylor eta O'Halloran, 1997, 2001). Litekeena da muturluzeen zorrodun trikopteroeren kontsumoa zorroak substratuari lotzeko duen indarrarekin lotuta egotea, izan ere, zorroa finkatuago duten trikopteroak (Glossosomatidae eta Goeridae kasu) finkotasun gutxiago dutenak (Limnephilidae, Odontoceridae eta Sericostomatidae kasu) baino gutxiago kontsumitzen baitituzte (4. Kapituluak; Vieira-Lanero, 2000).

Esan beharra dago harrapakin-hautespena ez dela *trait* bakarraren menpekoa. *Agregazio-joera handiko* harrapakin hautespen positiboa bat dator ikerketa-eremu berdinean egindako irati-telemetriaz deskribatutako bazka-jokabidearekin, non muturluzeek beste edonora mugitu aurretik denbora-tarte luzeak pasatzen zituzten urlaster jakinetan — gune beroak— (Esnaola eta kideak, 2018b). Muturluzeen harrapakin kasuan, agregazio-joerak antzeko baldintzak bilatzen dituzten banakoen ondorioa dirudi, harrizarren gainean iragazpenerako baldintza hidrauliko hobeezinak topatzen dituztenez, bertan dentsitate handitan pilatzen diren Simulidoak kasu. Kasu horietan, horrelako gune bat topatzen duten muturluzeek bertan modu intentsiboan bazkatuz kostu txikian energia asko irabaziko dute. Azkenik, muturluzeek *igerilariak* diren harrapakinetikiko duen hautespen positiboak Gammaridoen gisako taxaren aldeko zaletasuna islatzen duela dirudi, zeinak tamaina egokikoak diren, ibilguaren hondorekin azalean agertzen diren, eta erlatiboki motel mugitzen diren harrapakinak diren (Tachet eta kideak, 2002).

Bertrand-ek (1992), datu morfologiko taxonomikoetan oinarrituz, muturluzeek bentikoak eta erreofiloak diren eta gorputz tamaina ertaina-handia duten fauna jaten dutela ondorioztatu zuen, eta muturluzeek energia intrintsekoaren balio altua (zorrorik gabeko trikopteroak, Hydropsychidae, Rhyacophilidae eta Philopotamidae kasu) eta esklerifikazio baxua duten harrapakinak nahiago zituztela iradoki zuen. Bestalde, zorrodun trikopteroak oso gutxitan jaten zituztela esan zuen, baina hori gezurtatua izan zen beste ikerketa batzuetan (adb. Biffi eta kideak, 2017a,b; Santamarina eta Guitian, 1988; Santamarina, 1992, 1993). Nahiz eta ikerketa honetan ez den harrapakinaren energia-balioa aztertu, Esnaola eta kideek (4. Kapituluak) Amphipodaren eta zorrodun (*Odontocerum* eta Limnephilidae kasu) zein zorrogabeko trikoptero (*Hydropsyche* kasu) talde

batzuen hautespen positiboa baieztatu zuten. Bertrand-ek deskribatutako “esklerifikazio baxua” bat dator guk behatutako *gorputz-malgutasun ahula* edo *handiaren* hautespen positiboarekin. Era berean, Santamarinak (1992, 1993) ere gorputz malgua zuten taxa positiboki hautatzen zituztela esan zuen— *vs.* moluskuak, negatiboki hautatuak—. Hawlitschek eta kideek (2018) eta Biffi eta kideek (2017b) ere, euren ikerketa taxonomikoetako behaketetan oinarrituta, gorputz gogorreko eta zorro gogorreko ornogabeak negatiboki hautatzen zituztela iradoki zuten. Haatik, gorputz-malgutasunik gabeko harrapakinak, hots *Glossossoma*, *Protonemura* eta *Sericostoma*, oportunistikoki jan ziren gure lanean, nahiz eta balitekeen muga metodologikoez (gorotzetako FO *vs.* bentoseko ugartasunak, lagin tamaina, etabar) gure hautespen-proporzioetan eragina izan izana.

Hautatutako *traitak* eta urlasterrak

Aurretiaz Esnaola eta kideek (4. Kapituluua) egindako lanean ez zuten harrapakin eskuragarritasun osoan muturluzeen urlasterrekiko zaletasun sendoa azal zezakeen habitaten arteko alderik aurkitu eta, hortaz, zaletasun horrek bestelako arrazoiei —segur aski arrazoi mekanikoei— erantzungo ziela iradoki zuten. Hemen, ordea, harrapakin *traitak* aztertzerakoan, *trait* batzuen eskuragarritasunean agerikoak ziren habitaten arteko aldeak. Hala eta guztiz ere, urlasterretan bizitzeko ornogabeen moldaketak ziren hobetsitako *trait*-kategorietako asko, eta horrek segur aski ez zien muturluzeei bestelako abantailarik eskainiko. Hortaz, zaila da ezaugarri horien bidez espezie honen habitat-hautespena azaltzea, eta ziur aski habitataren beraren ondorioa besterik ez dira izango. Bestalde, harrapakin-eskuragarritasunak muturluzeen habitat-hautespena zein puntutaraino azal zezakeen aztertzeke, harrapakin profitagarritasunarekin lotutako *trait*-kategoriak urlasterretan ugariagoak ote ziren aztertu genuen. Gure emaitzen arabera, batzbestek, hobetsitako harrapakin kategoria guztiak izan ziren pixka bat ugariagoak urlasterretan, *gorputz-malgutasun handia* izan ezik. *Gorputz-malgutasun handia* Elaman soilik izan zen positiboki hautatua, bertan ugariagoa izanik urlasterretan, eta Leitzaranean aldiz *malgutasun ahula* izan zen positiboki hautatutako kategoria, bertan hori ere urlasterretan izanik ugariagoa.

Emaitza horiek gure bigarren hipotesia onartu eta muturluzeez urlasterrak bertako bazkarengatik hautatzen dituztela ondorioztatzen eraman gaitzakete. Kategorien arteko eskuragarritasun aldeak aldiz txikiak eta erreken artean aldakorak ziren. Horrez gainera, hobetsitako harrapakin motak urlasterretan ugariagoak izateak nekez frogatu dezake habitat hori bertako bazkarengatik hautatzen dela. Baliteke flotagarritasunari eta habitataren heterogeneotasun espazialari aurre egiteko muga ekomorfologiko edo funtzionalak, edo jarrerazko

mugak (Richard, 1986), edo bestelako arrazoi ezezagunak egotea zaletasun horren atzean. Almenar eta kideek (2013), espezie intsektiboro batean, bazkaguneen hautespenak sekuentzia hierarkiko bat jarraitzen zuela ikusi zuten, lehenik espeziearen espezializazio morfologikoaren eta gune zehatzetan ehizatze gaitasunaren, gero gune zehatzetan harrapakinak detektatzeko gaitasunaren, eta azkenik harrapakinaren ugaritasun erlatiboaren eraginpean zegoena. Balizko mailakako erabakitze-prozesu analogoak batetik, eta habitatetako eta lekuanlekuko *traiten* arteko elkarrekintzak bestetik, muturluzeen bazkatzearen kausa eta ondorioen arteko mugak desitxuratu egiten dira.

Erreken arteko aldeak

Muturluzeek funtsean harrapakin-*trait* berdinak hautatu zituzten bi erreketan, nahiz eta baziren zenbait alde, ziur aski erreken eta habitaten arteko harrapakin-eskuragarritasunaren aldeei zegozkienak. Eskuragarritasunean aldeak egon arren, FPCA-k muturluzeek bi erreketan ia *trait* berberak zituzten harrapakinak jaten dituztela erakutsi zuen, espeziearen espezializazio trofikoa azpimarratuz.

Gure azken hipotesia egiaztatuz, eskuragarritasuneko elipseak (FPCA grafikoa) handiagoak eta gainjarriagoak ziren Elaman, Leitzaranen txikiagoak eta gainjarri gabeak ziren bitartean. Elamako habitaten arteko antzekotasun handiagoak muturluzeen Elamako ur-bareen hautespen nulua (2. Kapitulua; Esnaola eta kideak, 2018b) ulertzen laguntzen du, hain zuzen ere, erreka horretan harrapakin eskuragarritasunari zegokionean urlasterrak ur-bareekiko antzekoagoak zirelako (3. Irudia).

Ondorioak

Euren bazka-jokabide selektiboaren ondorioz, kontserbazio-egoera eta harrapakin-eskuragarritasun desberdina zuten erreketan ia dieta berdina izan zuten muturluzeek *traitei* zegokienean. Habitaten artean harrapakin *traitetan* diferentziak egon arren, eta hobetsitako *traitak* oro har urlasterretan ugariagoak izan arren, ezin izan genuen muturluzeek urlasterrak bertako bazkarengatik edo habitat horretan jarduteko duten gaitasunarengatik hautatzen zituzten ondorioztatu. Oro har, espezie erreofilo honen bazka-habitat gisa urlasterrek duten garrantzia azpimarratzen du ikerketa honek eta emaitzek iradokitzen dute espezieak hobetsitako habitaten kontserbazioa eta errestituzioa gako izango dela kontserbazio eraginkor batetarako. Ur-erazketek urlasterren eskuragarritasunean eragina duenez, erreketako emarien kudeaketa funtsezko gaia da muturluzearen kontserbazioan.

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Informazio gehigarria

5. Kapitulua. Dietaren karakterizazioa harrapakinen *traitak* erabiliz

S1. Taula. Taxon bakoitzaren kategoria bakoitzarekiko afinitate balioak, *fuzzi coding* bidez adierazita (Chevenet eta kideak, 1994; Sánchez-Hernández, 2014). Daturik gabeko taxari maila taxonomiko baxuagoen balioen batzbestekoak esleitu zaizkie (grisez) edo analisietatik baztertuak izan dira (“-”).

Filuma	Klasea	Ordena	Familia	Especiea	Hiperoliko	Epibentiko	Ur-zutabea	Moleia	Eratina	Bizkerra	Blankeak	Trintarra-Uharris	Sedimentu finak	Nekrototoak	Orbela	Gordeta	Agenan	Igerilaria	Interstaria	Lotua	Ezer ez	Ahula	Handia	Handia	Handia	5.2 mm	2.8 mm	8.32 mm	> 32 mm	Ezer ez	Ahula	Handia				
					DEP1	DEP2	DEP3	CUV1	CUV2	CUV3	SUS1	SUS2	SUS3	SUS4	SUS5	FE1	FE2	MAS1	MAS2	MAS3	AG1	AG2	AG3	AG4	AG5	AG6	AG7	AG8	AG9	AG10	AG11	AG12	AG13	AG14	AG15	
Annelida	Clitellata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Arthropoda	Crustacea	Malacostraca	Oniscidae	<i>Oniscus asellus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Arthropoda	Insecta	Arachnida	Aturidae	<i>Aturidae sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Arachnida	Linyphiidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Coleoptera	Carabidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Coleoptera	Cucurionidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Coleoptera	Dytiscidae	<i>Platambus sp</i>	3	4	0	3	1	0	0	3	1	5	0	0	0	3	0	3	1	2	1	2	2	0	3	1	0	0	3	0	0	0		
Arthropoda	Insecta	Coleoptera	Elmidae	<i>Limnius sp</i>	1	3	0	3	3	2	2	5	4	5	0	3	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Arthropoda	Insecta	Coleoptera	Elmidae	<i>Oulimnius sp</i>	0.8	3.5	0	3	3	2	1	5	3	5	0	3	0	0	5	0	1	1	0	3	1	2	4	0	0	0	1	3	0	0		
Arthropoda	Insecta	Coleoptera	Gyniidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Coleoptera	Gyniidae	<i>Orectochilus villosus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Coleoptera	Hydrophilidae	<i>Berosus sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Coleoptera	Scirtidae	<i>Eldes marginata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Diptera	Athericidae	<i>Atherix ibix</i>	2	5	0	2	3	0	4	6	1	9	1	3	0	0	5	0	0	3	0	1	1	0	0	0	3	0	0	1	3	0	0	
Arthropoda	Insecta	Diptera	Chironomidae	-	2.7	4.2	2	3	2	1	3	5	5	7	1	3	0	2	5	1	0	1	1	3	1	0	3	2	0	0	0	0	0	0	0	
Arthropoda	Insecta	Diptera	Chironomidae	<i>Barbadocladius sp</i>	3	5	1	5	2	2	3	7	4	8	2	3	0	1	5	1	0	1	1	3	1	0	4	1	0	0	0	0	0	0	0	
Arthropoda	Insecta	Diptera	Chironomidae	<i>Brillia bifida</i>	3	5	1	5	2	2	3	7	4	8	2	3	0	1	5	1	0	1	1	3	1	0	4	1	0	0	0	0	0	0	0	
Arthropoda	Insecta	Diptera	Chironomidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Diptera	Empididae	-	2	2	0	0	1	2	3	4	3	7	1	2	0	3	0	0	3	0	1	1	0	3	0	0	0	0	1	3	0	0		
Arthropoda	Insecta	Diptera	Empididae	<i>Wiedemannia sp</i>	2	2	0	0	1	2	3	4	3	7	1	2	0	3	0	0	3	0	0	3	0	1	1	0	3	0	0	0	1	3	0	
Arthropoda	Insecta	Diptera	Limoniidae	<i>Eteobophila sp</i>	4	1	0	2	1	0	0	1	7	1	0	3	0	0	5	0	0	1	1	1	1	0	2	1	0	0	0	0	0	0	0	
Arthropoda	Insecta	Diptera	Limoniidae	-	4	3	0	3	1	0	1	3	9	1	3	3	0	0	5	0	0	3	0	1	1	0	3	1	0	0	0	0	0	0	0	
Arthropoda	Insecta	Diptera	Lonchopteridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Diptera	Mycetophilidae	<i>Gnoriste apicalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Diptera	Mycetophilidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Diptera	Pediciidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Diptera	Psychodidae	<i>Psychoda erminea</i>	2	2	0	2	0	0	1	0	1	0	2	3	0	0	5	0	0	3	0	0	3	0	3	0	0	0	0	1	3	0	0	
Arthropoda	Insecta	Diptera	Rhagionidae	<i>Chrysopilus sp</i>	0	5	0	2	2	1	0	0	3	6	2	1	1	0	5	0	0	3	0	0	3	0	3	0	4	0	0	3	0	0	0	
Arthropoda	Insecta	Diptera	Scatopsidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Arthropoda	Insecta	Diptera	Simuliidae	-	1	5	0	1	2	3	5	5	0	10	0	3	0	2	4	0	1	2	3	0	0	5	1	0	0	0	0	3	0	0	0	
Arthropoda	Insecta	Diptera	Syrphidae	<i>Herinia sp</i>	3	2	1	3	0	0	0	2	5	6	4	3	0	1	2	0	0	3	0	1	0	0	0	4	0	0	0	0	0	0	0	
Arthropoda	Insecta	Diptera	Tipulidae	-	4	1	0	5	1	0	0	4	4	6	4	3	0	0	5	0	0	3	0	1	1	0	3	2	0	0	1	3	0	0	0	
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Alainites muticus</i>	1	6	4	2	3	2	4	6	1	11	1	1	3	4	5	0	0	0	3	1	0	2	2	0	0	0	1	3	0	0	0	
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Baetis sp</i>	1	6	4	2	3	2	4	6	1	11	1	1	3	4	5	0	0	0	3	1	0	2	2	0	0	1	3	0	0	0	0	
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Centroptilum luteolum</i>	1	4.5	3	2	3	2	4	5	0	9	0	1	3	3	5	0	0	0	3	2	0	1	3	0	0	1	3	0	0	0	0	
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Procladius penulatum</i>	1	4.5	3	2	3	2	4	5	0	9	0	1	3	3	5	0	0	0	3	2	0	1	3	0	0	1	3	0	0	0	0	0
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	<i>Ephemerella ignita</i>	0	6	1	3	3	1	2	5	3	12	2	3	1	1	5	0	0	3	0	3	2	0	1	3	0	0	0	0	0	0	0	0
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	<i>Torleya major</i>	0	6	1	3	3	1	2	5	3	12	2	3	1	1	5	0	0	3	0	3	2	0	1	3	0	0	0	0	0	0	0	0
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	<i>Ephemera danica</i>	4	1	0	4	2	0	0	3	9	1	2	3	0	0	3	0	0	3	2	0	3	0	3	0	2	0	0	0	0	0	0	
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Ecdyonura sp</i>	0	6	1	3	3	2	2	5	2	1	0	1	3	1	5	0	0	3	1	1	1	0	0	3	0	0	0	0	0	0	0	0
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Electrogena lateralis</i>	0.4	5.6	1	2	3	3	2	6	1	1	1	1	3	1	5	0	0	3	1	1	1	0	4	0	0	0	0	0	0	0	0	
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Epeorus sp</i>	0	5	0	0	1	3	3	5	0	0	0	3	0	3	5	0	0	3	1	1	1	0	4	0	0	0	0	0	0	0	0	0
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	-	0.4	5.6	1	2	3	3	2	6	1	1	1	1	3	1	5	0	0	3	1	1	1	0	4	0	0	0	0	0	0	0	0	
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Rhithrogena sp</i>	1	5	0	1	0	2	3	7	1	0	1	0	3	1	6	0	0	3	0	3	2	0	1	3	0	0	0	0	0	0	0	0
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	<i>Habroplebia sp</i>	0	5	2	3	0	0	0	0	3	3	2	1	0	2	3	0																

S2. Taula. FPCA-ko *trait* bakoitzaren inertzia balioa.

<i>Trait</i>	Inertzia
Gorputzaren malgutasuna (zorroak/tutuak barne)	0,0651
Ur-fluxuarekiko esposizioa	0,0316
Arintasuna	0,0264
Tamaina potentziala	0,0196
Agregazio-joera	0,0145
Sakonera	0,0104
Substratuarekiko mugikortasuna/lotura	0,0090
Korrontearen abiadura	0,0083
Substratua	0,0030

S3. Taula. *Trait*-mailako RDA analisi globalaren ANOVA.

	a.g.	Bariantza	F	p-balioa
Dieta <i>vs.</i> Eskuragarritasuna	1	0,0006	1,01	0,348
Erreka	1	0,0215	34,16	0,001
Habitata	2	0,0081	6,48	0,001
Hondarra	56	0,0352		

6. Kapituluua



Eztabaida orokorra

Pirinioetako muturluzea: fosil bizia

Pirinioetako muturluzea ur bizkorreko mendiko erreketan bizitzera moldatutako (Charbonnel eta kideak, 2015) espezie errelikto bisa deskribatu izan da (Rümke, 1985). Bere banaketa mugatua eta monofilia (Arribas, 2004; Cabria eta kideak, 2006) kontutan hartuta, Pirinioetako muturluzea fosil bizitzat har genezake. Beste espezie erreofilo batzuekin batera (adb. ur-zozoa *Cinclus cinclus*, Hourlay eta kideak, 2008, Piriniotar uhandrea *Calotriton asper*, Valbuena-Urena eta kideak, 2013), babesleku glaziarretan bizi izan ziren azken glaziazioan, eta horren ostean berriro hedatu ziren Iberiar Penintsulan eta Pirinioetan (Gómez eta Lunt, 2007; Igea eta kideak, 2013). Muturluzeek gaur egun okupatzen dituzten habitatak iraganean segur aski zabalduago zeuden baldintzekin antza gehien dutenak dira, eta ziur aski antzina altitude baxuagoan bizi izandako eta garai hotzagoetan zabalduago zeuden espezieen adibide garbia dira (Ohlemüller eta kideak, 2008). Muturluzeak oso espezializatuta daude bazka zehatza duten ur hotzeko korrante bizkorreko biotopoetan bizitzera. Ordea, muturluzeak banaketa-eremu guztian atzerakada pairatzen ari dira, giza jardueraren (adb. ur-erauzketak) eta klima-aldaketaren eraginez (Araújo eta kideak, 2012; Charbonnel eta kideak, 2016; Ohlemüller eta kideak, 2008). Muturluzeek behar dituzten eremu erreofiloak murrizten ari dira, eta geroz eta erreka tarte gutxiago dituzte eskuragarri bizitzeko egokiak direnak. Testuinguru honetan, ugaztun honen espezializazio altuaren ondorioz, bere habitaten babesza gako da espeziearen kontserbaziorako.

Ekologia espaziala eta habitata

Muturluzearekin egindako lehenengo irrati-telemetria lanak laurogeigarren hamarkadan hasi ziren eta muturluzeek jarduera-erritmo bimodala zutela erakutsi zuten, egunean zeharreko aldi labur bat eta gauean zeharreko aldi nagusi batekin (Stone, 1985, 1987a). Lan berriagoek azaldu zuten, gauean zeharreko bazka-jarduerak bi edo hiru jarduera-aldi erakutsi ditzake (Melero eta kideak, 2014) eta muturluzeak normalean, beste banako batzuekin partekatzen dituzte babeslekuak, sexua, adina edo bizi-eremuan bizi ziren espezie bereko banakoen kopurua edozein delarik ere (Melero eta kideak, 2012). Behaketa hauek zalantzak sortu zituzten espeziearen ustezko jokabide lurraldekoia inguruan. Ikerketa horiek muturluzearen ekologiaren inguruko informazio berria eskaini zuten, baina ez zuten bere habitat zaletasuna aztertu. Melero eta kideen (2014) azken lanetik ez da irrati-telemetria lan gehiagorik egin.

Doktorego tesi honen garapenean zehar muturluzearen habitat-beharrizanak

modelizatu dituzte zenbait autorek. Lehenengo, Charbonnel eta kideek (2015), muturluzeen gorotzetan oinarrituz, espeziearen presentzia emariaren aldaketek, substratuen heterogeneotasunak eta babeslekuen eskuragarritasunak baldintzatua zegoela ikusi zuten, eta muturluzearen presentzian eragiten zuen habitat-faktore nagusia emaria zela ondorioztatu zuten. Bigarrenik, Biffi eta kideek (2016) muturluzearen habitata ibilguaren eta ibaiertzaren ezaugarrien arabera zela ondorioztatu zuten, urlasterrekiko, babeslekuen eta ibaiko substratuen heterogeneotasunarekiko, egitura irtenen ugaritasunarekiko, sedimentu finen eskasiarekiko, eta ibaiertzetan arroken proportzio altua eta luraren proportzio baxuarekiko zaletasuna erakutsiz. Hirugarrenik, Charbonnel eta kideek (2016) muturluzearen banaketa nagusiki aldagai hidrologikoek eta klima-aldagaiak baldintzatuta dagoela ikusi zuten, nahiz eta habitaten zatiketak, uraren eta substratuen kutsadurak, espezie inbaditzaileek, tokiko habitataren degradazioak, ibaiertzeko landarediaren aldaketek, lehiakideen zein harraparien presentziak eta ornogabeen komunitateen aldaketek bezalako beste faktore biotiko edota abiotikoek ere eragina izan dezaketen Pirinioetako muturluzearen helmenean uzkuetzean. Laugarrenik, Quaglietta eta kideek (2018) 1993-1996 eta 2014-2015 bitarteko iraungitze tasa %63,5 zela ondorioztatu zuten, eta banaketa-eremuaren murrizte gogorra ikusi zuten iturburuetarantz. Ez ziren behatutako aldaketak azaltzeko gai izan, baina mendiko errekek klima-aldaketa eta espezie inbaditzaileak bezalako giza-jatorriko estresatzaileen kontrako babesleku gisa duten garrantzia azpimarratu zuten. Azkenik, Biffi eta kideek (2019) giza-presioak, amonio kontzentrazioak eta *Neomys fodiens* ur-satitsuaren presentziak muturluzearen presentzian eragin negatiboa dutela, eta konduktibitate baxuak eta *Cinclus cinclus* ur-zozoaren presentziak presentziarekin korrelazio handia dutela erakutsi zuten. Gainera, beraiek neurtu ez zituzten zenbait aldagaik muturluzeen tokiko banaketan eragina izan dezaketela ondorioztatu zuten arren, ibilguan zuzenean eragiten duten jarduerak Pirinioetako muturluzearen populazioen kontrako eragina izan dezaketela iradoki zuten. Bestalde, muturluzeak aldagai biotiko eta abiotiko askorekiko aurkezten duen erantzun moderatuak espeziea ingurumen-baldintzekiko uste zena baino tolerantetagoa dela iradokitzen du.

Ikerketa horiek guztiek informazio baliotsua eskaini zuten Pirinioetako muturluzearen ekologia espaziala hobeto ulertzeko, muturluzeak agertzen ziren erreka-tartean habitat-faktore gakoak deskribatu zituztelako aurrenekoz. Horien oinarri ziren gorotz-laginen presentzia-datuek, ordea, emaitza alboratuak eman zitzaizketen (ikusi eztabaida 2. Kapituluak). Gure ikerketa oraindik ezezagunak ziren alderdietan sakontzen ahalegindu zen, besteak beste okupazioan eragiten zuten faktoreak eta mikrohabitat mailako habitat-erabileraren intentsitatea eta hautespena aztertuz. Informazio hori gako da muturluzearen tokian-tokiko banaketa zein faktoreren eraginpean dagoen ulertzeko eta espeziaren kontserbaziorako jarduerak gauzatzeko.

Aurreranzko pauso bat emanez, doktorego tesi honetan muturluzearen mikrohabitat mailako habitat-hobespena ikertu da, banakoak euren bizi-eremuen baitan irrati-telemetria bidez jarraituz eta bi erreka desberdinetan euren espazioaren erabilera aztertuz. Gure emaitzek aldez aurretik zenbait autorek aurreikusitako (adb. Biffi eta kideak, 2016) urlasterren garrantzia azpimarratu, eta muturluzeen habitat-hobespenaren inguruko informazio kontsistenteagoa eskaini dute. Ur-erauzketen eraginpean dagoen errekan urlasterren erabilera sendoagoa izateak espezie honen jokabide espazialean emariak duen garrantzia azpimarratzen du. Richard-ek (1986) esan zuen bezala, bizimodu frenetikoa du Pirinioetako muturluzeak. Horrez gain, Melero eta kideek (2012) iradoki zuten bezala, muturluzeek euren bizi-eremuak banako kopuru aldakorrekarekin partekatzen dituztela ikusi dugu, sexuaren edo adinaren arabera patroirik erakutsi gabe. Jarraitutako muturluzeen babeslekuak lehendik deskribaturikoen (Stone, 1987) berdinak izan dira: arroken arteko arrakala naturalak, harrizko hormak, zein ibaiertzeko zuhaitzen sustraien azpiko zuloak. Muturluzearentzako urlasterrak beharrezko habitata izatea eta euren bizi-eremuetan babesleku-eskuragarritasunak duen garrantzia oinarritzko aspektuak dira, etorkizuneko kontserbazio-jardueretan kontutan izan beharrekoak. Bestalde, Biffi eta kideekin (2016) bat eginez, gure datuek ere kudeaketa-jarduerak substratu heterogeneoak eta konektagarritasun-korridore egokiak dituzten habitat urtarrak faboratu beharko lituzketela sostengatzen dute.

Irrati-telemetriazko ikerketak Pirinioetako muturluzearen ekologia espazialaren inguruan ondorio sendoak ateratzea ahalbidetu arren, galdera ere ekarri zuen, habitat-erabilera hori habitaten arteko bazka-eskuragarritasunaren ezberdintasunei edo bestelako arrazoiren bati loturik (ikusi Richard, 1986) ote zegoen zalantza sortuz.

Ekologia trofikoa

Pirinioetako muturluzearen dieta gehienbat intsektiboroa dela aspaldi ezagutzen da (Cabrera, 1914; Puissegur, 1935). Dieta-azterketa berriek baieztatu egin zuten ezagutza hori (adb. Bertrand, 1992; Castián eta Gosálbez, 1995; Santamarina, 1992), eta muturluzeak Diptera, Ephemeroptera, Plecoptera eta Trichoptera larbez bazkatzen zirela eta euren dieta urtaroen eta erreken artean aldakorra izan zitekeela esan zuten. Ikerketa horiek, ordea, ez ziren bereizmen taxonomiko fina eskaintzeko gai izan (genero edo espezie mailakoa; Clare, 2014), eta hori oso interesgarria da arraroak, galzorian dauden edo kriptikoak diren espezieen ekologia trofikoa ikertzeko (adb. Bohmann eta kideak, 2014; Razgour eta kideak, 2011).

Honela, doktorego tesi honetan Pirinioetako muturluzearen dieta deskribatzea ere izan dut helburu, bere gorotzak “lehenengoz” DNA metabarcoding bidez era ez inbasorean aztertuz. Beste autore batzuek ere helburu bera izan dute, ordea, eta tesi hau aurrera joan ahala metabarcoding bidezko ikerketa asko argitaratu dira. Lehenengo, Gillet eta kideek (2015) hasle zehatz bat garatu zuten Pirinioetako muturluzearen eta bere harrapakinen bereizmen handiko identifikazioa ahalbidetzen zuena. Ondoren, Biffi eta kideek (2017a), DNA metabarcoding bidezko muturluzearen dietaren lehenengo erabateko deskribapena egin zuten, eta bertan harrapakin arraroen proportzio altua eta harrapakin lurtarren kontsumo handiko dieta zabala erakutsi zuten, espezieak uste zena baino dieta jeneralistagoa duela iradokiz. Geroago, Biffi eta kideek (2017b) Pirinioetako muturluzearen eta ur-satitsuaren (*Neomys fodiens*) dietak ez zirela askorik gainjartzen erakutsi zuten, muturluzeek nagusiki harrapakin urtarrak jaten zituztelako. Azkenik, Hawlitschek eta kideek (2018), Zamorako gorotz-lagin zahar eta freskoak alderatuz, lagin freskoek DNA-sekuentzia-irakurketa gehiago, itu ziren irakurketa-sekuentzien proportzio altuagoa eta potentzialki OTU gehiago ere ematen zituztela erakutsi zuten. Aipatutako metabarcoding bidezko dieta azterketa horiek guztiek, bakoitzak bere berezitasun metodologikoekin, xehetasunez deskribatu zuten muturluzearen dieta, eta espeziearen bazka-ekologiaren inguruko informazio baliotsua eskaini zuten. Haien eta gure emaitzak sakon eztabaidatu dira tesi honetan (ikus 3., 4. eta 5. Kapituluak), emaitzak konparatuz eta erabilitako hasleak bezalako xehetasun metodologikoen eragin ditzaketen alborapenak kontutan hartuz.

Errendimendu Altuko Sekuentziazio metodoen erabilera aurrerapen handia izan da arraroak, txikiak, urtarrak, gautarrak, bizkorak edota iheskorak diren, zein nagusiki gorputz biguneko organismoek bazkatzen diren espezieen bazka-ekologiaren inguruko ikerketetan (Berry eta kideak, 2017; Boyer eta kideak, 2013; Buglione eta kideak, 2018; Evans eta kideak, 2016; Piñol eta kideak, 2014; Shehzad eta kideak, 2012). Oraindik, ordea, ikerketa hauen diseinuan ez dago estandarizazio metodologikorik (Deagle eta kideak, 2019; Deiner eta kideak, 2017; Divoll eta kideak, 2018; Zizka eta kideak, 2019), eta horrek ikerketen erreprodukzio-gaitasuna, errepikagarritasuna eta konparagarritasuna zailtzen ditu (Alberdi eta kideak, 2018, 2019; Mata eta kideak, 2019), zenbait kasutan ondorio ekologiko egokietara heltzea zailduz (Andriollo eta kideak, 2019; Zinger eta kideak, 2019). Horrez gain, arazo dira halaber, besteak beste, erroreak detektatzea, erreferentziazko datu-baseen kalitate eskasa, espezie arraroen gainestimazioa eta bigarren mailako harraparitza saihestea (Clare, 2014; Pompanon eta kideak, 2012; Ruppert eta kideak, 2019); eta oraindik ez da garatu dieta kuantifikatzeko metodologiarik (Elbrecht eta kideak, 2017; Fonseca, 2018; Piñol eta kideak, 2015, 2019). Edonola ere, DNA metabarcoding-a ezinbesteko erraminta dela uste da espezieen ekologia ulertzeko (De Sousa

eta kideak, 2019), bai monitorizazio ekologikoetarako, baita mundu-mailako kontserbazio-ikerketetarako ere (Ruppert eta kideak, 2019).

Muturluzera itzuliz, bere dieta deskribatzeaz gain, maila taxonomiko orokorreko harrapakin-hautespena ere jorratu dute zenbait ikerketek (hots, Bertrand, 1992; Santamarina, 1992). Aurreranzko pauso bat emanez, Biffi eta kideek (2017b) muturluzeen dietaren osaketa molekularra harrapakin-eskuragarritasunarekin konparatu zuten eta muturluzeak maizen jandako harrapakinek erreketan ugarien ziren taxarekin bat egiten zutela ondorioztatu zuten. Horrez gain, muturluzeek harrapakinen zein ezaugarri estimatzen edo saihesten zituzten iradoki zuten, nahiz eta ez zuten euren bazka-hobespenen eta tokiko harrapakin-hautespenaren deskribapen zehatzik egin. Testuinguru honetan, muturluzearen mikrohabitat mailako bazka-hobespenak aztertu ditut tesi honetan bi erreka desberdinetan (4. eta 5. Kapitulua), irradi-telemetry bidez aztertutako habitataren erabilera, dieta eta harrapakin-eskuragarritasuna aldi berean ezaugarrituz hiru habitat desberdinetan (urlasterrak, ur-bareak eta putzuak). Laginketa-diseinu konplexu honek muturluzearen ekologia espazial eta trofikoaren arteko harremanak modu fidagarrian aztertzea ahalbidetu du, tokiko habitat-erabilera bazka-eskuragarritasunaren edo bestelako faktoreen arabera den aztertuz. Bestalde, harrapakinen ezaugarri bereizgarrien (*trait*) azterketak (Schmitz, 2017) muturluzeen bazka-zaletasunen inguruko informazio berria eskaini du, beste behin ere urlasterrekiko euren zaletasuna nabarmenduz. Hare gehiago, ikertutako bi erreketako harrapakinen ezaugarrien arteko antzekotasunek muturluzeak beharrezko dituen baliabideen deskribapena hobetzea ahalbidetu dute (ikusi 5. Kapitulua; Spitz eta kideak, 2014).

Laginen bilketa eta biltegiatze egokia lortzeko, babesleku artifizialak erabili dira tesi honetan (1. Eranskina; González-Esteban eta kideak, 2018), aztertutako laginak freskoak zirela segurtatzeko, eta beraz, kutsadura gutxiagokoak (McInnes eta kideak, 2017; Oehm eta kideak, 2011). Metodo hau etorkizuneko ikerketetan arruntki erabili beharko litzatekeela uste dut, muturluzea detektatzeko duen eraginkortasunarengatik eta gorotz freskoak biltzeko duen abantailarengatik.

Urlasterren garrantzia muturluzearen kontserbazioan

Pirinioetako muturluzearen biziraupenean ur-fluxu bizkorrek eta harrapakin mota zehatzen presentziak duten garrantzia azpimarratzen dut tesi honetan. Laburbilduz, bere espezializazioarengatik ur bizkorretan bazkatzea mugatuta

dagoen espezie estenotopiko gisa deskribatzen dut muturluzea eta, babestua izango bada, erreken hidrologian arreta jartzea ezinbestekoa izango dela nabarmentzen dut.

Erreken emariaren magnitudea, maiztasuna, iraupena, erritmoa (edo auresangarritasuna), aldaketa tasa (edo *flashiness*), eta abar bezalako ezaugarriak ibaietara lotuta bizi diren espezie askoren kontserbaziorako gakoak dira, ur-fluxuzko sistemen osotasun ekologikoa erregulatzen dutelako (Palmer eta Ruhi, 2019; Poff eta kideak, 1997). Eskualde mailako ibaien emariaren patroiak erreken tamainaren eta kliman, geologian, topografian eta landare-estalduran ematen diren aldakortasun geografikoen arabera dira (Poff eta kideak, 1997). Hala ere, giza jarduerak erregimen horiek aldatu egin ditzakete, besteak beste ureztaketarako, jarduera hidroelektrikorako, industriarako edo etxeen horniketarako ura erauzteko eraikitzen diren presengatik, zeinak erregimen hidrologiko naturala aldatzen duten (adb. Rosenberg eta kideak, 2000), aldaketa ekologikoak eraginez (Poff eta Zimmerman, 2010) eta uraren eta sedimentuen mugikortasunen arteko oreka dinamikoa etenez (Giulivo eta kideak, 2019). Etete horiek espezie urtar zein urbazterrekoen habitata osatzen duten eskala larriko zein fineko ezaugarri geomorfikoak itxuraldatzen dituzte (Chien, 1985), oronogabe urtarrengan eragin zuzenak izanik (Englund eta Malmqvist, 1996; James eta kideak, 2007; McIntosh eta kideak, 2002). Euskal erreketan eragin handia izan dute ur-erauzketa sistema horiek: Gipuzkoan soilik, 2000 km² baino txikiagoa den lurraldean, 900 presa baxu baino gehiago daude (Boletín Oficial del Estado, 2016; Ente Vasco de la Energía, 1995). Egitura horietako asko errota edo burdin-galdategiak bezalako jarduera zaharren ondareak dira, eta denen artean geldirik dagoen ur-eremu zabalak sortzen dituzte, segur aski, putzuak bezala, muturluzearentzako oso erabilgarriak izango ez direnak. Presa horiek emari ekologikoen erregimena errespetatzea ez da nahikoa izan erreken dinamika naturala mantentzeko, eta horiek ahal den heinean eraitsiak izan beharko lirakeela uste izan da, gaur egun erabiltzen direnak edo ondare-balioa dutenak soilik babestuz (2. Kapituluak). Oztopo horiek, klima-aldaketa globalarekin batera, aldaketa larriak ari dira eragiten iturburuetan, eta hortaz, zuzenean eragiten dute muturluzeek hobesten dituzten habitatetan. Izan ere, Pirinioetako glaziarrek atzerakada garrantzitsuak ari dira sufritzen (Rico eta kideak, 2017), erreken tenperaturak igotzen ari dira (van Vliet eta kideak, 2013) eta Iberiar Penintsulan geroz eta erreka gutxiagok mantentzen dute urte guztian zeharreko ur-emari esangarri bat (Lorenzo-Lacruz eta kideak, 2013). Gaur egun, faktore guzti horiek urlasterren eskuragarritasunean, eta beraz, muturluzea bizi daitekeen espazioaren eskuragarritasunean zuzenean eragiten dute.

Bada geroz eta handiagoa den gogo bat, nazio- eta eskualde-mailako politika eta legediek babesten dutena, ibaien eta horietara loturiko zingiren osasun ekologikoa eta funtzionamendua kontserbatzeko edo errestauratzeko, giza-

erabilerarako eta biodibertsitaterako. Asko dira norabide horretan lanean ari diren erakundeak. Gipuzkoako eta Nafarroako eskualdeetan, adibidez, LIFE IREKIBAI proiektuaren bidez, muturluzeak bizi diren erreka askotan presen eraisketak bezalako errestaurazio jarduera desberdinak egin dira (IREKIBAI, 2017). Proiektu horrek muturluzearekiko zuen helburu nagusia ibaien konektagarritasuna handitzea, populazioen zatiketa murriztea eta muturluzeen habitata hobetzea zen. Hala eta guztiz ere, ez proiektu honek, ez beste bakar batek ere, ez du emari ekologikoen gaia konpondu: hots, ez dago lan bakar bat ere Pirinioetako muturluzearen beharrian hidrologikoak kontutan hartuz emari ekologikoak ezartzen saiatu dena. Emari ekologikoa ibaien egoera ekologiko ona lortzen laguntzen duen eta modu jasangarri batean ekosistema urtarren zein hauei lotutako lurreko ekosistemen funtzionaltasuna eta egitura ahalbidetzen dituen emari txikiena gisa definitzen da (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2015). Sentikorrak diren espezieak —ez arrainak soilik— emari ekologikoen kudeaketaren politikan itu izan beharko lireratekeela azpimarratzen du definizio horrek. Ugaztun urtar desberdinak kontserbatzeko emari ekologikoen estrategiak zehaztearen desira azpimarratu izan da (adb. Grant eta Temple-Smith, 2003). Hala eta guztiz ere, ez da erraza erreketako emariak espezie horien beharrian ekologikoei erantzuteko erregulatzen dituzten lanak topatzea.

Etorkizunerako galderak (ezjakintasunak)

Lehenik eta behin tesi honetan ikertu diren gaietan arreta ipiniz, Pirinioetako muturluzearen ekologia espazialari eta habitatari dagokionean, gure ikerketak ez zuen espazioaren erabilera urtaroeekin edo banakoen sexu edo adinarekin nola aldatzen zen aztertu. Bestalde, muturluzearen habitataren karakterizazio zehatzagoa egitea oso interesgarria izango litzateke. Etorkizunean beharrezkoa izango da habitat-erabileraren patroiak eremu geografiko eta erreka mota desberdinetan errepikatzen ote diren ikustea. Muturluzearen ekologia trofikoari dagokionean, nahiz eta autore askok muturluzearen dietak denboran eta espazioan zeharreko aldaketak jasaten dituela esan (adb. Santamarina, 1993), ikerketa zehatzek eskatzen duten esfortzua eta kostuak ezagutuz, eta muturluzearen eredu trofikoak bere jokabide erreofiloak gidatzen dituela kontutan hartuz, horrelako ikerketetatik askoz irabazi gehiago ateratzerik ez dela izango uste dut.

Gure ikerketa eremuan muturluzeek beste lehiakide potentzial batzuekin, hala nola ur-satitsuarekin (*Neomys fodiens*) eta ur-zozoarekin (*Cinclus cinclus*), konpartitzen zuten habitata (Arzak eta kideak, 2014; Regla eta Arizaga, 2016; Ventura, 2007), eta oso interesgarria izango litzateke beraien arteko interakzio

ekologikoak aztertzea. Ur-satitsuak sakonera eta abiadura gutxiko uretan bazkatzen dira nagusiki (Churchfield, 1985; Mendes-Soares eta Rychlik, 2009; Rychlik, 1997), eta beraien dieta ez da muturluzearenarekin asko gainjartzen (Biffi eta kideak, 2017b). Hortaz, oso arraroa izango litzateke beraien artean bazkarengatik lehiatzea. Ur-zozoaren bazka-ekologia, ordea, muturluzearenaren antzekoagoa da, honek ere urlasterrak dituelako nahiago (Chen eta Wang, 2010; Santamarina, 1990), nagusiki ornogabe urtarrez bazkatzen delako (Taylor eta O'Halloran, 1997), eta emaria txikia denean bereziki selektiboak izaten direlako (Taylor eta O'Halloran, 2001). Ikerketa desberdinek ur-zozoek euren kumeak egunero bazkatzen dituztela erakutsi dute (Morrissey eta kideak, 2010). Horrez gain, jakina da euren dieta adinaren (Yoerg, 1994, 1998) eta sexuaren (Morrissey eta kideak, 2010) arabera, zein ugalketa-garaian egotearen (Morrissey eta kideak, 2010) edo beren burua edo beren kumeak bazkatzearen (Chiu eta kideak, 2009; Eguchi, 1990) arabera aldatzen dutela. Muturluzearen kasuan ere aldakuntza bat espero baliteke ere, orain arte ez da ikerketarik egin adinaren edo sexuaren araberako patroirik jarraitzen oten duten, ez eta euren kumeak bazkatzen ote dituzten erakusteko.

Pirinioetako muturluzea bere berezitasun morfologiko eta taxonomikoengatik aspalditik izan da ezaguna. Goiko lerroetan iradoki bezala, ordea, bere biologiarren aspektu ugari ezezagunak dira oraindik. Muturluzearen kontserbaziorako gako diren galdera asko geratzen dira erantzuteke. Alde batetik, emari aldaketek muturluzearen ekologian nola eragiten duten aztertzeke dago oraindik. Morueta-Holme eta kideek (2010) muturluzearen banaketa mugatzen zuten faktore klimatikoak beroketa globala, ur-fluxuaren aldakortasun handia eta prezipitazio baxua zirela esan zuten. Williams-Tripp eta kideek (2012) muturluzeen banaketaren modeloetan prezipitazioei loturiko faktoreek zuten garrantzia nabarmendu zuten. Auresandako aldaketek erreketako emaria murriztu eta ornogabe urtarrengan eraginak izango dituzte (Cowx eta kideak, 1984; Englund eta Malmqvist, 1996; Wood eta Petts, 1994). Berriki egin diren ikerketa guztiek (adb. Charbonnel eta kideak, 2015, 2016; Biffi eta kideak, 2016; eta baita doktorego tesi honek ere) muturluzearengatik babestutako eremuen erreketako emariak era egokian kudeatzeko emari-aldaketek muturluzearen ekologia espazialean nola eragiten duten aztertzearen garrantzia nabarmendu dute. Zehatzago esanda, beharrezkoa izango da isurpenen murrizketek urlasterren eskuragarritasunean nola eragiten duten aztertu eta, horretan oinarrituz, emari ekologikoak finkatzea. PHABSIM-aren (*Physical Habitat Simulation*; Bovee, 1982) antzeko hurbilpen baten bidez egin liteke hori, baina eredu sinpleago baten bitartez izan beharko litzakete, muturluzearentzako habitat-zaletasun kurbak zehaztea ia ezinezkoa litzatekeelako.

Erreketako emarien kudeaketa egokia eta emari ekologikoak betetzen direla ziurtatzea muturluzearen kontserbaziorako gakoak dira, baina oraindik ere

badira ebatzi beharreko beste zenbait gai ere. Adibidez, zein da muturluzearen habitat-erabileraren mugatzaile nagusia? Igeriketarako mugak? Zentzumen-gaitasunak? Oraindik ez dakigu muturluzeek gauez ur oso zurrumbilotsuetan bazkatzerakoan nola hautematen duten zer den jatekoa eta zer ez. Itsuak dira ia (Trutat, 1891) baina zentzumen-hartzaile oso onak dituzte euren tronpa itxurako sudurrean eta euren mingainean (Argaud, 1944; Richard eta Villard, 1969). Hala eta guztiz ere, oraindik ez dakigu beren harrapakinak Errusiako muturluzeak (*Desmana mochata*; Ivlev eta kideak, 2013), *Sorex palustris*ek (Catania eta kideak, 2008) eta *Condylura cristata*k (Catania, 2006) bezala, urpean aire burbuilak botaz eta arnastuz, usaimenez hautematen ote dituen. Ikerketa gehiago beharko dira muturluzeek euren harrapakinak urpean nola detektatzen dituzten, harrapakinaren ezaugarri bereizgarrietan oinarritutako hautespen-hobespenak zergatik dituzten, nola ehizatzen duten eta beren urpeko mugimendu-patroiak nolakoak diren azaltzeko. Muturluzearen zentzumenen-ekologiaren inguruko ezagutzan sakontzeak, halaber, muturluzeentzako tranpa hilgarriak izan daitezkeen jarduera hidroeletrikorako erauzketa-kanalen sarreretan muturluzeak uxatzeko nolabaiteko tresnak diseinatzea ahalbidetuko luke.

Espeziearen banaketa-patroien inguruan ere gutxi dakigu, nahiz eta arroen baitako mugimenduak ohikoenak direla eta ibaien arteko mugimenduak arraroak direla erakutsi duten ikerketa batzuek (Escoda eta kideak, 2017). Escoda eta kideek (2019) muturluzeek arro batetik besterako bidaia presa handi bat gurutzatzen baina errazago egin dezaketela iradoki zuten. Gillet eta kideek (2016) ere muturluzeek presak gaindi ditzaketela esan zuten. Oraindik informazio gehiago behar da gai horiek argitzeko, eta hori gorotzen banako mailako identifikazioa bezalako tekniken bidez lor liteke. Tesi honetan zehar muturluzeentzako espresuki diseinaturiko babeslekuak (ikusi 1. Eranskina; González-Esteban eta kideak, 2018) lagungarriak izan litezke, ziur aski, horrelako ikerketetarako, gorotz freskoen bilketa errazten daudelako. Gainera, ileen tranpak jar litezke horietan, beste ugaztun batzuetan egin izan den bezala (adb. Barja eta kideak, 2016; Roche, 2008), DNAREN bilketa hobetzeko. Populazioen dinamikari dagokionean, transponderren erabilerak eta muturluzeen babeslekuetan irakurle automatikoak jartzeak (zenbait saguzaharrekin egin den bezala, adb. van Harten eta kideak, 2019) informazio gehigarria eskaini lezake.

Azken ikerketek erakutsi dute dibertsitate genetiko baxuak, endogamia koefizienteen balio altuek eta populazioen tamaina-eraginkor txikiek iraungitze-arriskuan uzten dituztela muturluzeak etorkizunera begira (Gillet eta kideak, 2017). Bestalde, muturluzeek antzinako glaziazio garaiko babeslekuei loturik dauden eta genetikoki desberdinak diren populazioak dituzte (Querejeta eta kideak, 2016), eta egitura genetiko hori babestua izan beharko litzateke populazioa kontserbatzeko ahaleginak egin bitartean (Igea eta kideak, 2013;

Querejeta eta kideak, 2017). Muturluzeen banaketa-eremu osoan populazioen atzerakada azkarra eta odolkidetasun arazoak daudela kontutan hartuta, badirudi etorkizun hurbilean lekualdaketak (bersartzeak eta populazioen berrindartzeak) egokiagoak izango liratekeela gatibutasuneko hazkuntzak baino.

7. Kapitulua



Ondorioak

Ondorioak

Doktorego tesi honek Pirinioetako muturluzearen ekologia espazial eta trofikoa aztertu eta bere habitat zaletasunean eta bazka-erabakietan eragiten duten faktoreen ezagutza handitu ditu. Honakoak dira bere ondorio nagusiak:

- 1) Pirinioetako muturluzeak positiboki hautatzen ditu urlasterrak ur-bare eta putzuen aldean, hautespena altuagoa izanik jarduera hidroelektrikorako sistemen eraginpean dagoen errekan.
- 2) Pirinioetako muturluzearen dieta era ez inbasorean identifikatzeko eraginkorrena Gillet eta Zealeren hasleak konbinatzea izan zen; horrek harraparia bera ere gorotz laginetan identifikatzea ahalbidetu zuen.
- 3) Nahiz eta dibertsitate handiko dieta izan, muturluzeak uste zena baina espezialistagoak dira. Harrapakinen artean hainbat taxa positiboki hautatu zituzten, tartean Simuliidae eta Psychodidae (Diptera), Baetidae eta Heptageniidae (Ephemeroptera), Odontoceridae (Trichoptera) eta Gammaridae (Crustacea); eta negatiboki, aldiz, Planorbidae (Gastropoda), Goeridae (Trichoptera), Perlidae (Plecoptera) eta Athericidae (Diptera). Honek iradokitzen du muturluzeek substratuaren gainean agerian bizi diren ornogabeak eta materia organiko lodiari loturiko ornogabe zatitzaileak nahiago dituztela, eta aldiz, sedimentu finen barnean bizi diren gorputz biguneko taxa, oskol gogorreko ornogabeak eta harrapariak saihesten dituztela.
- 4) Harrapakinen hautespena desberdina izan zen bi erreka desberdinetan, muturluzeak oportunistagoak izanik okerren kontserbaturiko errekan.
- 5) Muturluzeek bazkatzeko urlasterrak erabiltzen dituzte, nahiz eta harrapakin eskuragarritasunean ez egon habitaten arteko alde esangarririk, ez kontsumitutako, ez eta positiboki hautatutako taxari dagokienean.
- 6) Harrapakinen ezaugarri —edo *trait*— biologikoen analisiak adierazitakoaren arabera, muturluzeek *ur-zutabe*an eta *gune epibentiko*an bizi diren harrapakinak, substratu gisa *bloke*ak dituzten *ur bizkorretan* bizi direnak, ur-fluxuarekiko *agerian* dauden harrapakinak, 2-8 mm-ko

tamaina potentziala duten harrapakinak, *gorputz-malgutasun pixka bat* duten *arintasun handiko* harrapakinak, *taldekatuta* bizi direnak, eta *igerilariak* zein *substratuari lotzeko mekanismoak* dituzten harrapakinak positiboki hautatzen zituzten. Negatiboki hautatzen zituzten, ordea, *ur-abiadura moteletan*, *substratu finen barruan*, ur-fluxuarekiko *gordeta* bizi diren harrapakinak, *2 mm baino* tamaina potentzial *txikiagoa* zuten harrapakinak, *agregazio-joerarik ez* zuten harrapakinak, eta harrapakin *herrestariak*. *Traiten* arteko lehen multzo batek ornogabeek urlasterretan bizitzeko beharrezko dituzten ezaugarriak islatu ditzakete, besterik gabe, baina bigarren multzo batek muturluzeen harrapakinaren profitagarritasunarekiko hautespen-irizpideak isla ditzake.

- 7) Hobetsitako harrapakin-kategoria guztiak, *gorputz-malgutasun handia* izan ezik, *pixka bat* ugariagoak izan ziren urlasterretan. Kategorien arteko eskuragarritasun-aldeak, ordea, txikiak ziren, eta erreken artean aldakorrak, eta honek iradokitzen zuen urlasterrekiko hobespena ez zela gertatzen harrapakin horiek bertan ugariagoak izateagatik. Litekeena da hobespen horren atzean muturluzearen flotagarritasunari eta habitat fisikoaren heterogeneotasunari aurre egiteko bestelako muga ekomorfologiko, funtzional edo jarrerazkoak egotea.
- 8) Muturluzearen urlasterrekiko hobespen sendoak ondorio garrantzitsuak ditu galzorian dagoen espezie honen kudeaketarako. Urlasterren eskuragarritasuna murrizten duten giza jarduerak, bereziki ur-erauzketak edo ur-desbideraketak, espezie honentzako kaltegarriak izan daitezkeela iradokitzen du honek.

1. Eranskina



Pirinioetako muturluzea
(*Galemys pyrenaicus*)
detektatzeko
laginketa-metodo berria

Argitalpena

Kapitulu honetan oinarrituriko artikulu bat *Hystrix, the Journal of Mammalogy* aldizkarian izan da argitaratua:

González-Esteban, J., Esnaola, A., eta Aihartza, J. (2018). A new sampling method to detect the Pyrenean desman (*Galemys pyrenaicus*). *Hystrix, the Italian Journal of Mammalogy*, 29(2), 190-194.

Laburpena

Pirinioetako muturluzea edo ur-satorra (*Galemys pyrenaicus*) erdi-urterra den ugaztun txikia da, eta bere populazioek atzerakada nabarmena jasan dute azken hamarkadetan. Populazioetan eta banaketan gertatzen ari diren aldaketak kuantifikatu ahal izateko, monitorizazio-programen premia du bere kontserbazioak. Xede horrekin gorotzen ikerketak egin izan dira, baina arrakasta baxua lortu izan dute, hau ibaien ibilguaren forma zein litologia bezalako tokiko ingurumen-baldintzen menpekoa delako. Muga metodologiko honen ondorioz, populazioaren parte bat oharkabean pasatzen da. Lan honetan espezie iheskor hau detektatzeko metodo berri bat probatu da. Ikerketa Euskal Herrian egin da (Iberiar Penintsularen iparraldean), berriki muturluzeak detektatu diren Elama eta Leitxaran erreketan. Modu erregularrean banatuta, babesleku artifizialak jarri dira bi erreketan, muturluzeei bazkatu bitartean atseden hartu eta kaka egiteko leku egokiak eskainiz. Muturluzeeak azkar eta maiz erabili zituzten babeslekuak, haien detekzio-tasa nabarmenki handituz. Landan gorotzen identifikazioa haien forma, kolorea, tamaina eta usainean oinarrituta egin ondoren, DNA metabarcoding azterketen bidez baieztatu zen. Metodo ez-inbasore berri honek adin ezaguneko gorotz freskoen laginak lortzea ahalbidetzen du, genomika, populazioen genetika, dieta-azterketa, ugalketa-analisi, eta bestelako ikerketetarako. Erabilitako materialen kostu baxuagatik, eta trebakuntza ostean muturluzeen gorotzak identifikatzeko ahalmenagatik, metodo hau optimoa izango da ikerketa sinkronikoak, eskualde-mailakoak edota boluntarioen bidezkoak egin behar direnean. Hortaz, babesleku artifizialen erabilera hobekuntza nabarmena da muturluzeen gorotzen bidezko ikerketa tradizionalen aurrean, eta galzorian dagoen espezie honen populazioen etorkizuneko monitorizazioetarako baliabideen indartze handia dakar.

Hitz-gakak

detekzioa, gorotzak, laginketa-metodoa, metabarcoding, ur-satorra

Sarrera

Galzorian dauden espezieen kontserbazioak, aldian-aldian, banaketa-eremuaz gainera populazioen parametro demografikoak zehaztu behar izatea dakar. Muga praktikoengatik, normalean, aplikatzeko errazak diren detekzio-metodo zehatz eta erlatiboki merkeak daudenean soilik izaten da posible aldizkako jarraipen hori. Ugaztun gehienen izaera iheskor eta ohitura gautarrak ikerketetarako euren arrasto eta seinaleekin fidatu behar izatera derrigortzen dituzte ikertzaileak (Wilson eta kideak, 1996). Espezie batzuen arrastoak nahastezinak dira, eta horrek aztarnen bilaketan oinarritutako ikerketa-protokoloak definitzea ahalbidetzen du (adb., Helle eta kideak, 2016). Ingurune batzuetan, ordea, zaila izaten da arrastoak detektatzea. Kasu horietan gorotzek alternatiba egokia eskain dezakete. Gorotzak erabilgarriak izan daitezke espezie bat kontrolatzeko, baldin eta zalantzarik gabe, espezie horri egokitzerik baldin bazaizkio, eta animaliek ikertzaileek aurreikusteko eta biltzeko moduko lekuetan eskuragarri uzten baldin badituzte. Gaur egun, genetika molekularreko teknikak gorotza egin duen espeziea zuzen identifikatzea ahalbidetzen dute (adb., Janecka eta kideak, 2008; Ruiz-González eta kideak, 2008; Gillet eta kideak, 2015; Walker eta kideak, 2016), baina metodo horiek prozedura garestiak dira eta teknikari espezializatuak eskatzen dituzte, eta beraz, gune geografiko zabaletan ikerketa intentsiboak egitea zaila izaten da. Behaketa bidezko identifikazio zuzena argiro hobesten da bideragarria denean. European aldian-aldian egiten diren igarabaren (*Lutra lutra*) ikerketak horren adibide garbia dira (Mason, 1986). Ikerketa horiek eskualde-mailan egiten dira normalean eta aldeztu beharrezko abileziak lortzeko trebatutako boluntario kopuru handiak barne-bildu ohi ditu. Igaraben gorotzen ezaugarri bereizgarriak (forma, usaina, kolorea, lurrean hartzen duen forma zehatza) posible egiten dute denbora-tarte laburrean nahikoa behaketa fidagarri biltzea, eskualde zabaletan (milaka km²-tan) igarabaren banaketaren zirriborroa egiteko.

Pirinioetako muturluzea (*Galemys pyrenaicus*) 60-70 g-ko ugaztun erdi-urtarra da. Nagusiki arroken arteko, harrizko hormetako edo ibai-ertzeko zuhaitzen sustraien azpialdeko zulo naturalak erabiltzen ditu gordeleku gisa; hauek normalean ez dute azaletik identifikatu ahal izateko ezaugarri nabarmenik izaten (Stone, 1987a). Batez ere ornogabe urtarrez bazkatzen da (Santamarina eta Guitián, 1988; Biffi eta kideak, 2017a; Hawlitschek eta kideak, 2018) eta, igarabak bezala, ibaia-ibilguko egitura irtenetan uzten ditu gorotzak (arroka, enbor edo sustraietan batez ere). Muturluzearen gorotzak, fresko daudenean, usain, kolore, forma eta tamaina kontutan hartuz (Nores, 1992), zalantzarik gabe esleitu dakizkioke espezieari; baina zahartu ahala, gorotzen usaina desagertu egiten da, kolorea aldatu, eta lehortzean erraz desegiten dira, *Neomys* sp. edo *Rattus* sp. bezalako beste ugaztun txiki batzuen gorotzekin nahasteko aukerak

handituz (ikusit Gillet eta kideak, 2015). Arazo horiek izan arren, gorotzen ikerketak erabili izan dira muturluzeen populazioak haien banaketa-eremuan zehar kontrolatzeko (Nores, 1992; Bertrand, 1992; Queiroz eta kideak, 1998). Ikerketa hauek arrakasta txikia lortu dute batzuetan (gorotz kopuru baxuak aurkitzeagatik ikertutako erreka-tarteetan) (González-Esteban eta kideak, 2011; González-Esteban, 2014; Charbonnel eta kideak, 2015), hau ibaien ibilguaren formaren eta litologiaren arabera izanik. Arroka irtenak edo enborrak bezalako substratu faboragarri gutxi dagoen erreka-tarteetan muturluzeek ibaiertzeko gordeleku edo zuloetan egiten dute kaka, ikertzailearen irismenetik kanpo (González-Esteban eta kideak, 2003a). Hori dela eta, errutinazko ikerketetan populazio batzuk ez dira detektatzen. Ausentzia faltsu hauek nola zuzendu eztabaida egon den arren, arazoak konpondu gabe dirau (Charbonnel eta kideak, 2014).

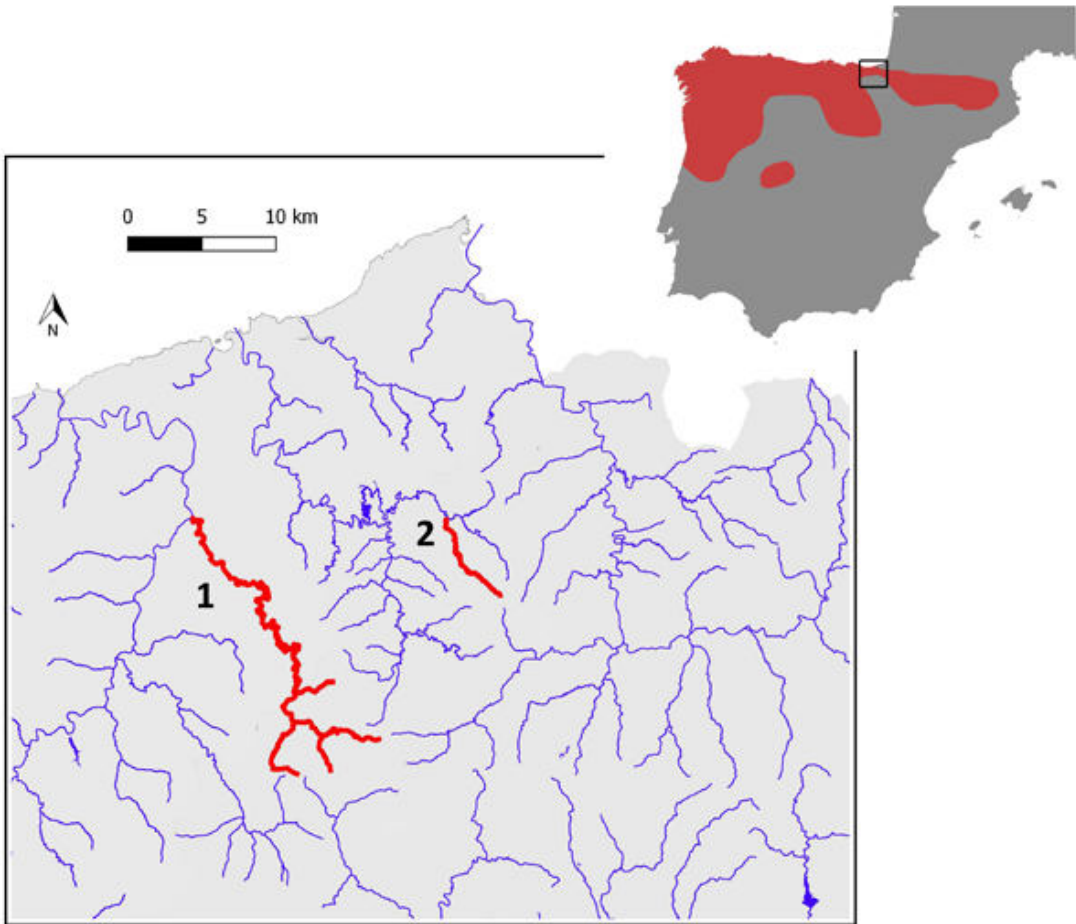
Pirinioetako muturluzea Bernako Hitzarmenaren (II. Eranskina) eta Europako Habitaten Zuzentarauaren bidez (II. eta IV. Eranskinak) babesturik dago (Council of the European Communities, 1992). Bere banaketa-eremua larriki murriztu da azken hamarkadetan (Charbonnel eta kideak, 2016; Quaglietta eta kideak, 2018), eta horrek Naturaren Kontserbaziorako Nazioarteko Batasunaren (IUCN) zerrenda gorrian Zaurgarri gisa sailkatzea ekarri du (Fernandes eta kideak, 2008). Presentzia-arrastoaren detekzioaren inguruan goian azpimarratutako arazoek —hots, arrakastaren aldakortasunak, kostu altuak eta negatibo faltsuen arriskuak— muturluzeari buruzko eskala eta zehaztasun handiko ikerketak eragozten dituzte. Ondorioz, bere gaur egungo banaketa partzialki soilik da ezaguna eta ez da ikerketa globalik egin oraindano. Hau arazo larria suerta liteke, espezie honentzako genetikoki, gutxienez, berriazko kudeaketa-programak beharko lituzketen bost kontserbazio-unitate diskretu identifikatu baitira (Querejeta eta kideak, 2016). Hemen, egungo ikerketetarako muga nagusia konpontzen lagun dezakeen detekzio-metodo berri bat aurkeztzen dugu, muturluzeen detekzio bizkorragoa eta merkeagoa egiteaz gain, ausentzia faltsuen arriskua ere murrizten duena.

Material eta metodoak

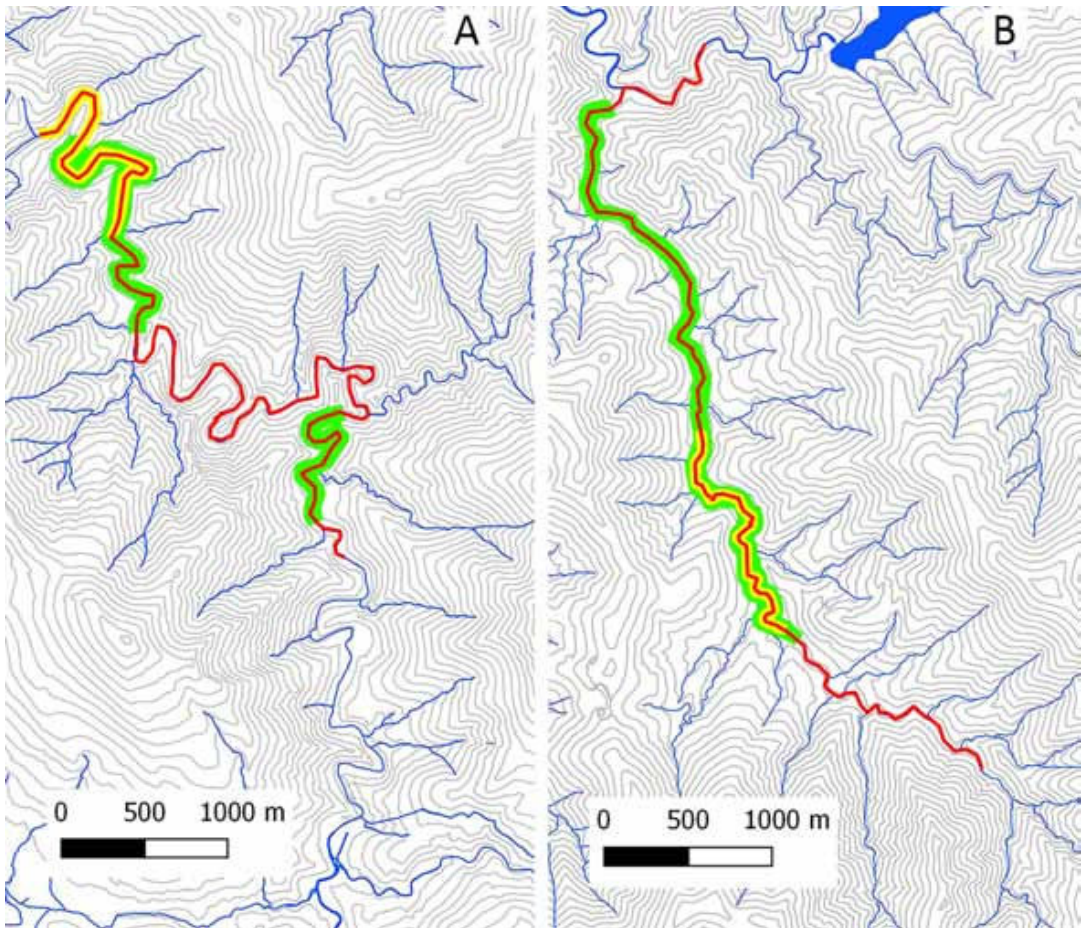
Ikerketa hau Pirinioetako muturluzearen ekologia espazial eta trofikoaren ikerketa zabalago baten parte da eta Iberiar Penintsulako iparraldeko mendiko bi erreketan egina izan da: Elama (batazbesteko altitudea 330 m; batazbesteko zabalera 7 m; batazbesteko emaria 0,7m³/s) eta Leitzaran (batazbesteko altitudea 290 m; batazbesteko zabalera 12 m; batazbesteko emaria 4,6m³/s) (1. irudia). Bi ikerketa-eremuak Europar Batasunaren Natura 2000 sarearen baitan babestuta daude, biek dute egoera ekologiko ona Uraren Europako

Zuzentarauaren arabera (Council of the European Communities, 2000), eta bietan Pirinioetako muturluzearen presentzia ezaguna izan da gutxienez azken 30 urteetan (2. Kapituluua; Esnaola eta kideak, 2018b; Nores, 1992).

Ingurumen-presio desberdinak pairatzen dituzte bi errekek. Elama (3. irudia) bigarren ordenako mendiko erreka da, biztanlerik gabeko granito eta eskistozko 1415 ha-ko arroa estaltzen duena, 1919-tik natura erreserba gisan zorrozki kudeatua izan dena, eta ondorioz pago eta haritzez osaturiko baso estaldura zabala duena (Castro, 2009). Gaur egun Elamaren arroan ez da baso-jarduerarik ematen. Bestalde, Leitzarari (4. irudia) laugarren ordenako ibaia da, kareharri, arbel eta hareharrizko 12 402 ha-ko arroa estaltzen duena. Elaman ez bezala, 3150 biztanle dituzten bi herri kokatzen dira Leitzararen arroaren goiko aldean, nahiz eta horren ostean errekek biztanlerik gabeko 25 km inguruko haran luzea zeharkatzen duen, non basogintza eta jarduera hidroelektrikoetarako ur-erazketak diren giza-eraginezko jarduera nagusiak (Izagirre eta kideak, 2013). Ibilguaren ezaugarriak desberdinak dira bi erreketan: Elaman urlasterrek eta ur-bareek antzeko eskuragarritasuna dute (%45), eta Leitzararen aldiz ur-bareak dira nagusi (%60). Putzuak dira eskuragarritasun baxueneko habitatak bi



1. Irudia. Ikerketa-eremua: (1) Leizarran eta (2) Elama errekek, Iberiar Penintsularen Iparraldean.



2. Irudia. Ikerketa-eremua, xehetasun handiagoarekin: (A) Leitzarar eta (B) Elama errekek. Muturluzeen gorotzen bilaketa aktiboko tarteen (lerro gorria), babesleku artifizialen lehenengo saiakerako tarteen (lerro horia) eta babesleku artifizialen bigarren saiakerako tarteen (lerro berdea) informazioa gehitu zen irudian.

erreketan (%10). Bloke eta enbor irtenak bi erreken ibilgu eta ibaiertzean agertzen dira. Elementu horiek ugariagoak dira Leitzararen, bertako muturluzeek jalkitze-gune (euren gorotzak uzteko gune) gehiago izanik. Landarediaren estaldura eskasa da bi erreketan, nagusiki belarkara eta zuhaixkaz osatua, behatzailea erraz sartzeko modukoa izanik.

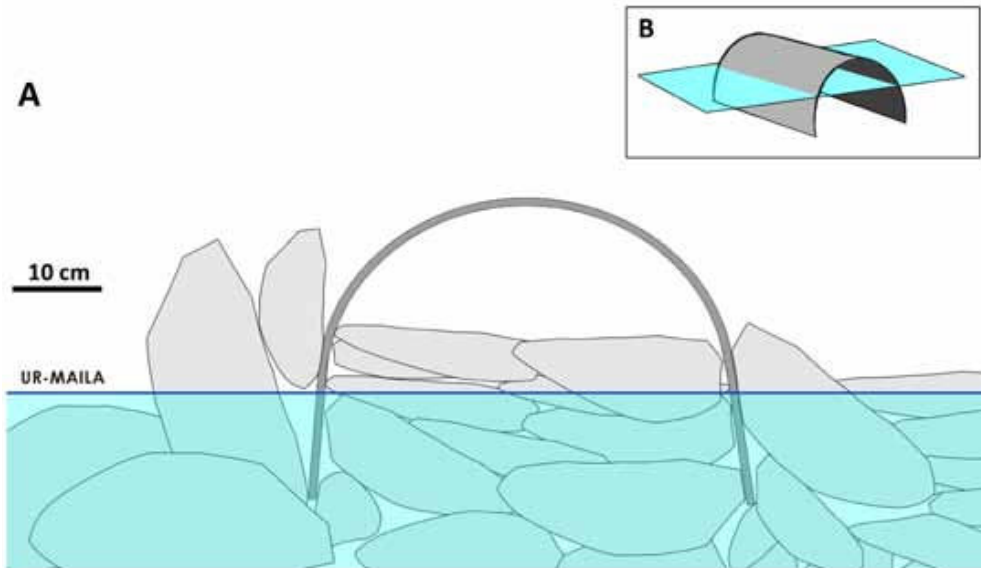
Aurretiazko lehenengo saiakera batean, 2016ko martxoan, muturluzearen gorotzak bilatu genituen Elama errekekako 6 km-tan eta Leitzarar ibaiko 8 km-tan, Queiroz eta kideek (1998) definitutako bilaketa aktibo arautuaren prozedurari jarraiki. Oinarrizko emariko egoeran, eta 10 egunez euririk egin ez zuelarik, esku-argi bat erabiliz muturluzeek gorotzak uzterik zituzten ibilguko eta ibaiertzeko egitura guztiak (arroka eta enborrak batez ere) arakatuz ikuskatu genituen errekek. Ibai-tarte bakoitza bere luzera guztian zehar miatu zen, elkarren segidako hiru egunetan, zatirik bereizi gabe, hiru pertsoez, batzabeste 200-300 m/h-ko abiadura estandarrean. Ikuskapenetan zehar erreketako uraren mailak ez zuen gorabeherarik izan.

Ondoren, etxean egindako babesleku edo kaka-leku artifizialek muturluzeak erakartzeko zuten eraginkortasuna aztertu genuen. Babesleku artifizial horiek goma-aparrezko dentsitate altuko etileno-binilo azetatozko (EVA) alfonbrak erabiliz eraiki ziren (erref. B517V1, mottez.com, 6 € bakoitza, 630x630x12 mm). Alfonbra bakoitza sakonera txikiko (10-30 cm-ko sakonera) urlasterren erdian, harritzar pilaketa baten gainean tolestu zen, 0,25 m² inguruko azalera lehorra estaltzen zuen 'kanpin-denda' bat eratuz. Alfonbra arrokak erabiliz lotu zen, zeinak muturluzeak erraz heltzeko eta gorotzen iraunkortasuna bermatzeko moduko azalera lau bat eskaintzeko moduan zeuden pilatuta (3. eta 4. irudiak) eta, horrez gain, soka bidez hurbileko adarretara ere lotu zen, uholdean galtzea ekiditeko. Arroken eta 'kanpin-dendaren' sabaiaren arteko tartea ez zen 15 cm baino handiagoa. Amaierako egiturak, hortaz, babesleku edo kaka-leku txiki bat simulatzen zuen, muturluzeek urpean harrapatutako bazka jan bitartean atsedenerako erabiltzen dituztenen antzekoa (Niethammer, 1970). Egitura hori urlasterretan kokatzen zen, muturluzeentzako bazka harrapatzeko toki hoberenak direlako (2. Kapituluak; Esnaola eta kideak, 2018b; Richard, 1986).

Euren eraginkortasuna aztertzeko, babesleku artifizialen bidezko lehenengo laginketa-saiakera bat egin zen, ibai-tarte berean, hasierako bilaketa aktiboan muturluzeen gorotzik topatu ez zen bi azpi-tarte txikiagotan (1,8 km Leitzaranean eta 1,4 km Elaman). Leitzarango azpi-tartea 2016ko apirila-ekaina artean lagindu zen, martxoan egindako lehenengo bilaketa aktibo arautuaren probaren segidan. Elaman laginketa 2016ko ekainera arte atzeratu zen. 12 babesleku artifizial ezarri ziren Leitzaranean eta 18 Elaman (2. irudia); kopuru eta kokapenak urlasterren eskuragarritasunaren arabekoak izan ziren, eta haien arteko batazbesteko distantzia 160,9 m-koa izan zen Leitzaranean (DE: 60,2 m, tartea: 55–210 m) eta 82,9 m-koa Elaman (DE: 22,9 m, tartea: 55–135 m). Leitzaranean 92 egunez mantendu ziren babeslekuak eta 10-15 egunero ikuskatu ziren; Elaman 13 egunez mantendu, eta 6-7 egunero ikuskatu ziren. Esan beharra dago Elamako laginketa bertan-behera geratu zela 13 egunen ostean, babeslekuak gorotzez gainezka eginda zeudelako. Laginketa-garaian oinarritzko emaria eta euri gutxi edo bat ere ez ziren erregistratu bi erreketan. Bisita bakoitzean topatutako gorotzak bildu eta etanol absolutuan gorde genituen, ontzi indibidualetan, eta babesleku guztiak garbitu egiten genituen, hurrengo behaketan gorotz berriak zirela bermatzeko.

Ondoren, 2016ko iraila-urrian egindako harrapaketa eta irrati-telemetria lan batek (2. Kapituluak; Esnaola eta kideak, 2018b) bi erreketako ikerketa-eremuetan muturluzeek okupatutako ibai-tarteen informazio zehatza eman zuen, eta lan horri etekina atereaz saiakera berri bat egin genuen, babesleku artifizialen bidez lagintzearen eraginkortasuna hobeto ebaluatzeko. Honela, 2016ko urrian, orduan ere oinarritzko emariarekin eta plubiositate txikiarekin, 16 babesleku ezarri ziren erreka bakoitzean, harrapaketa eta irrati-telemetria bidez

(Esnaola eta kideak, 2018) muturluzearen presentzia berretsi zen guneeetan. Aldi honetan 3,5 km-ko luzerako tartea lagindu genuen Leitzaranean (1,0 km eta 2,5 km-ko bi azpi-tartetan) eta 4 km-ko luzerako tartea Elaman, eta babeslekuak sakonera txikiko urlasterretan zehar banatu ziren berriro (2. irudia). Babeslekuen arteko batazbesteko distantzia 233,3 m-koa zen Leitzaranean (DE: 47,3 m;



3. Irudia. Scheme of a shelter. Babesleku baten eskema.



4. Irudia. Babesleku baten argazkia. Argazkia: Amaiur Esnaola.

tartea: 190–360 m) eta 270,3 m-koa Elaman (DE: 67,3 m; tartea: 170–410 m). Babesleku oro 24 orduro izan zen ikuskatua, elkarren segidako 3 egunez, eta gorotzak goian deskribatu bezala gorde ziren. Ur-mailaren gorabeheren eraginez gorotzak galtzeko arriskua gutxitzeko, goizeko lehenengo orduan ikuskatzen ziren babeslekuak, muturluzeen gauean zeharreko jarduera-aldia bukatu bezain pronto. Bisita bakoitzaren ostean garbitu egiten ziren babesleku guztiak, hurrengo bisitan babeslekuan topatutako gorotzak berriak izango zirela bermatzeko.

Landan gortzen hautaketa egiten zen eta muturluzeen ezaugarriak zituzten (musketa usaina, esfera txikien fusioz eratutako garun luzearen forma, 15-30 mm-ko luzera) gorotzak soilik gordetzen ziren etanol absolutuan, ontzi indibidualetan.

DNA metabarcoding bidezko espeziearen identifikazioa

Gorotzaren egilea identifikatzeko DNA metabarcoding-a erabili zen, lehenengo saiakerako zorizko 15 gorotz eta bigarren saiakeran bildutako ia gorotz guztiak erabiliz (N = 150), azken hau muturluzearen dieta aztertzeko egindako ikerketa molekular baten parte gisa (4. Kapituluak). DNA *Quiagen Powerfecal DNA kit* (Qiagen Iberia, S.L. Madril) erabiliz erauzi zen, fabrikatzailearen jarraibideak jarraituz. Ondoren, erauzkinetako DNAtik Mitokondrioko c zitokromo oxidasaren I azpiunitatearen genea (COI) PCR bidez amplifikatu zen Euskal Herriko Unibertsitateko UPV/EHU Ikerkuntzarako Zerbitzu Orokorretan (SGIker), Gillet eta kideek (2015) deskribatutako hasleak erabiliz. Laginak purifikatu eta bigarren erreakzio bat egin zen *Illumina Nextera v2 Kit*-a erabiliz, amplifikatutako produktu bakoitza indexatu eta Illuminako lotailuak eransteko. Amplifikazioak *Quiagen Multiplex PCR Kit* protokoloarekin egin ziren, 12,5 µL Quiagen 2X (1X amaieran), 1,25 µL aurreranzko hasle (10 µM; 0,5 µM amaieran), 1,25 µL atzeranzko hasle (10 µM; 0,5 µM amaieran), 8 µL H₂O eta 2 µL DNA erabiliz, amaierako 25 µL-ko bolumenean, 95 °C-tan 15 minutuko aktibazio pausoa 40 zikloz jarraituz (94 °C-tan 30 segundoz desnaturalizatu, 45 °C-tan 45 segundoz suberatu, 72 °C-tan 30 segundoz hedatu) eta amaieran 72 °C-tan 10 minutuko hedapen fasea izanaz.

Anplifikatu ostean, PCR-aren ekoizpenak *MiSeq® Reagent Kit v3 (600 ziklo)* erabiliz *Illumina MiSeq NGS* plataforman (parekatutako muturreko 2x300 bp-ko irakurketen sekuentziak) sekuentziatu ziren, fabrikatzailearen jarraibideak jarraituz. Sekuentziak Euskal Herriko Unibertsitateko UPV/EHU Ikerkuntzarako Zerbitzu Orokorretan (SGIker) egin zen.

USEARCH erabiliz (Edgar, 2010, 2013; Edgar eta Flyvbjerg, 2015) parekatutako muturreko sekuentzia-irakurketak bateratu ziren, hasleen bidez demultiplexeatu, eta adaptadoreen eta hasleen sekuentziak ezabatu; eta CUTADAPT erabiliz (Martin, 2011) sekuentzia-irakurketak kalitatearen eta luzeraren arabera filtratu ziren. Ondoren, sekuentzia-irakurketa bakanak (edo *singletonak*) ezabatu eta gainontzeko sekuentziak USEARCH erabiliz miatu ziren, kimerak topatzeko. Sekuentziak euren artean %97-ko antzekotasun-atalasearen arabera elkartu (ikus Alberdi eta kideak, 2017) eta OTU-ak (*Operational Taxonomic Units*) sortu ziren, UPARSE algoritmoa (Edgar, 2013) erabiliz. Azkenik, Genbank nt datu-basea erabili zen BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) erabiliz OTU-ei taxonomia esleitzeko. Clare eta kideei (2013) jarraiki, espezie mailako esleipenak sekuentziek %100-eko antzekotasuna eta gainezartze-balioak eskuratutakoan soilik onartu ziren.

Emaitzak

Bilaketa aktibo arautuaren protokoloan (Queiroz eta kideak, 1998) oinarrituriko aurretiatzko laginketaren bidez ez zen gorotzik topatu Elama errekan, eta bi gorotz soilik topatu ziren Leitzaran ibaian (0,25 gorotz/km). Muturluzeentzako diseinaturiko babeslekuek, aldiz, gorotz kopuru askoz handiagoa topatzea ahalbidetu zuten. Babesleku artifizialekin eginiko lehenengo saiakeran (apirila-ekaina), lehenik muturluzearen presentzia egiaztatu ez zen ibai-tarteetan, babeslekuen %50ak izan zituzten gorotzak, eta hauen agerpena mantendu egin zen denboran zehar (1.A. taula). Gorozkien ratioa handiagoa izan zen Elaman Leitzaranen baino (km eta ikerketako 24,5 *versus* 11,1 gorotz, hurrenez hurren). Bada, lehenengo saiakeran, muturluzeen erabilera intentsiboa zela-eta, babeslekuak ikuskatzeko Elamara egindako bisitak bertan behera utzi behar izan ziren 13 egunen ostean. Babeslekuak astero bisitatzean, gorotzak pilatu egiten ziren eta horrek oztopatu egiten zuen laginen indibidualizazioa. Egoera hori ez zen behatu Leitzaranen.

Babesleku artifizialekin eginiko bigarren saiakeran (urrian), lehenik muturluzearen presentzia egiaztatu zen ibai-tarteetan, hirugarren gaurako babeslekuen %80 izan zen erabilia (1.B. taula). Lehenengo saiakeran bezala, gorozkien ratioa handiagoa izan zen Elaman Leitzaranen baino (km eta ikerketako 10,6 *versus* 4,5 gorotz, hurrenez hurren).

Udaberriko laginetan 12.667.575 DNA sekuentzia gordin lortu ziren gorotzetatik, eta kopuru hau 11.788.479ra murriztu zen, kalitatearen arabera filtratu ostean. Udazkenean, ostera, 10.297.382 DNA sekuentzia gordin lortu ziren, eta filtratu ostean 8.724.646 inguru geratu ziren. Analisi bioinformatikoek eta

1. Taula. Babesleku artifizialen bidez ikertutako erreketan bildutako gorotz kopurua. Ikuskapen bakoitzerako babeslekuak eraiki zirenetik pasatako denbora (T, egunak), muturluzeek bisitatutako babesleku kopurua (P) eta bildutako gorotz kopurua (N) adierazi dira.

A) Lehenengo saiakera. Leitzarari ibaiko babeslekuak 2016ko apirilaren 13an eraiki ziren eta Elama errekekokoak aldiz 2016ko ekainaren 11n.

ikuskapena	Leitzarari (12 babesleku)			Elama (18 babesleku)		
	T	P	N	T	P	N
1	16	7	32	6	13	27
2	28	5	17	11	12	34
3	41	3	7	13	12	42
4	56	9	20			
5	68	4	15			
6	81	10	32			
7	92	8	17			
	Guztira	10	140	13	103	

B) Bigarren saiakera. 24 orduko ikuskatutako laginketak. Elama errekekoko babeslekuak 2016ko urriaren 1ean eraiki ziren eta Leitzarari ibaikoak aldiz 2016ko urriaren 30ean.

	Leitzarari (16 babesleku)			Elama (16 babesleku)		
	T	P	N	T	P	N
1	5	15	1	11	38	
2	6	15	2	14	42	
3	11	24	3	12	32	
	Guztira	13	54	15	112	

BLASTen erabilerak gorotz guztietan Pirinioetako muturluzeari zegozkion DNA-sekuentzia asko zeudela erakutsi zuten, erreferentziazko sekuentziekin %100-eko antzekotasuna zutenak (batazbeste, filtratutako 100.353 sekuentzia-irakurketa lagineko udaberrian, eta 10.153 udazkenean). Lagin bakar bat ere ez zen baztertua izan sekuentzia-irakurketa kopuru baxuagatik edo sekuentzien kalitate txarragatik. Ez zen beste gorotz-egile potentzialik identifikatu.

Eztabaida

Pirinioetako muturluzearen populazioen dentsitatea 4-8 banako/km ingurukoa izan ohi da (Nores eta kideak, 1998), eta muturluze banakoek euren bizi-eremua egunero esploratzen dute (Stone, 1987a). Nahiz eta kaka egiteko ratioa ezezaguna izan, eguneko eta km-ko hamarnaka gorotz egiten dituztela estimatzea ez litzateke gehiegizkoa izango, zeinak, galera ratio handi bat izan

arren, km-ko ehundaka gorotz eskuragarri egotea eragingo lukeen, oinarrizko emariko garai lehorretan behintzat. Hala eta guztiz ere, zenbaki hori ez dator bat ikuskapenetan jaso ohi diren kopuru baxuagoekin (1,7–1,8 eta 5,8 gorotz/km artekoa; Charbonnel eta kideak, 2015). Muturluzeak presente dauden zenbait ibaietan euren gorotzak topatzeko zailtasunaren eraginez ausentzia faltsuak ematen dira gorotzen bilaketa aktiboetan zehar (adb. González-Esteban eta kideak, 2003), eta hori kezka-iturri da 1980tik eskualde zein nazio mailan inbentarioak egiten dituzten administrazioentzako. Azken ikerketek arazo hori zuzentzeko egin dute lan (Charbonnel eta kideak, 2014).

Muturluzearen gorotzak topatzeko probabilitatea ibaien ibilguen osaketa eta egiturekin lotua dagoela dirudi (González-Esteban eta kideak, 2003a), ikertzailearentzako eskura dauden bloke irten ugari dituen ibilguetan handiagoa izanik. Ikerketa honek honako hipotesi hau sostengatzen du: muturluzeek modu azkar eta jarraian erabiliz erantzuten diote egitura irtenen igoera artifizialari , eta horrek espeziea detektatzea errazten du. Hala ere, ikerketa gehiago egin beharko litzateke egitura irten edo babesleku ugari ibaietan muturluzeek kaka-leku artifizialei nola erantzuten dioten aztertzeko.

Bestalde, zaila da ikertutako erreketan babesleku artifizialek erakutsitako eraginkortasunaren diferentziak azaltzea, eta gure laginketa-diseinua ez zen horretarako pentsatu. Lortutako emaitzek Elaman muturluzeen populazio-dentsitate altuago bat (2. Kapituluak; Esnaola eta kideak, 2018b) edo, besterik gabe, muturluzeen erantzun sendoagoa islatu lezake, egitura artifizialen eraikuntzak sortutako azalera unitateko egitura irtenen azalera erlatibo handiagoak eragindakoa. Tokiko gorotz-egite-erratioa faktore askok baldintza dezakeenez, ikertzaileek ez litzukete gorotz kopuruak erabili behar erabaki kuantitatiboak ondorioztatzeko.

Edozein kasutan, hemen aurkezten den laginketa-metodo berriak muturluzeen detektagarritasuna handitzen du, espeziearen presentzia/ausentzia ausentzia faltsuen arrisku txikiagoaz aztertzeko tresna aurreratua eskainiz. Gainera, gorotzen adina finkatzea errazten du, material fresko ugari behar duten metodoen eraginkortasuna hobetuz, hala nola genomika, populazioen genetika, paisaia-genetika, dieta-azterketak, edo ugalketa-analisietan. Bereziki, autore askok azpimarratu izan dute muturluzeen gorotz freskoen bilketa ahalbidetuko lukeen metodo baten beharra (Gillet eta kideak, 2016; Hawlitschek eta kideak, 2018).

Areago, metodo hau erabilterrazak diren material merkeetan dago oinarritua; babeslekuak eraikitzea saio bakarrean erraz ikasi daitekeen zerbait da, eta bi pertsonen artean 4-6 babesleku eraiki daitezke ordubeteetan. Horrez gain, nahiz eta laginketa prozedura honek ikerketa-eremura beti bi bisita egin behar izatea

eskatuko duen —lehenengo babeslekuak finkatzeko eta gero ikuskatzeko—, esan beharra dago gorotzak puntu finkoetan biltzea askoz errazagoa dela —eta denbora gutxiago eskatzen duela— ibai-tarte batean zehar balizko kaka-leku guztiak miatzen ibiltzea baino. Gainera, gorotz fresko ugari eskura izateak *in situ* identifikazioa errazten du oinarrizko trebakuntzaren ostean. Hortaz, honek metodo optimoa dirudi eskualde-mailako ikerketa sinkronikoetarako, bereziki boluntarioak barne-biltzen baditu, beste ugaztun espezie batzuekin erabili izan den bezala, adibidez, Erresuma Batuan *Arvicola amphibius* ur-arratoiarekin (Ur-Arratoiaren Monitorizazio Nazionalerako Programa; (<https://ptes.org/get-involved/surveys/countryside-2/national-water-vole-monitoring-programme/>)).

Babesleku artifizialek, hortaz, muturluzeen gorotz-bilaketa tradizionaletik hobekuntza nabarmena eskaintzen dute, eta izugarri areagotzen dute etorkizunean metodo ez-inbasoreetan oinarrituz galzorian dagoen espezie honen monitorizazioak egiteko aukera. Ikerketa horietan erabili beharreko protokoloa etorkizuneko lanek definitu beharko lukete (laginketa-unitateen kopurua, esfortzua, urtarokotasuna, eta abar).

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**The spatial and trophic ecology
of the Pyrenean desman
Galemys pyrenaicus:
key factors for conservation**



PhD Thesis by
Amaiur Esnaola Illarreta

Leioa, 2020
University of the Basque Country

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Universidad
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Unibertsitatea

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Let us try to teach generosity and altruism,
because we are born selfish
(Richard Dawkins, The selfish gene)

The spatial and trophic ecology of the Pyrenean desman *Galemys pyrenaicus*: key factors for conservation

A thesis submitted by **Amaiur Esnaola Illarreta**
to the University of the Basque Country
for the degree of Doctor of Philosophy,
under the supervision of
Dr. Joxerra Aihartza and Dr. Arturo Eloseggi.

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Publications

Article 1

Esnaola, A., González-Esteban, J., Elosegi, A., Arrizabalaga-Escudero, A., & Aihartza, J. (2018). Need for speed: Preference for fast-flowing water by the endangered semi-aquatic Pyrenean desman (*Galemys pyrenaicus*) in two contrasting streams. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28(3), 600-609.

Article 2

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Summary

The Pyrenean desman *Galemys pyrenaicus* (É. Geoffroy Saint-Hilaire, 1811) is an endangered semi-aquatic insectivore mammal that lives in headwater streams and has mostly nocturnal habits. It is endemic to the northern Iberian Peninsula and the Pyrenees. Although the current situation of desman populations is poorly known, it is estimated that its total distribution area has shrunk more than 50% in the last decades, and it is currently listed as a vulnerable species in the Red List categories by the International Union for Conservation of Nature (IUCN). The reasons behind this decline are not well known, since the population decline occurs even in regions such as the Basque Country, where pollution has been abated as a consequence of improved sanitation and water-treatment schemes. This suggests that there are other potential factors causing the decline, among which the degradation of physical habitat stands out. However, many aspects about the desman ecology are still poorly known, which complicates the design of measures for conservation. Thus, research and management are urgent issues for desman conservation.

In this thesis I studied the spatial ecology of the Pyrenean desman in two contrasting streams, one in excellent conservation condition (Elama), and the other affected by diversion hydropower schemes (Leitzaran). The aim was to determine the foraging habitat preferences of the desman within its home range to identify factors affecting its habitat suitability. Radiotracking showed that the Pyrenean desman positively selects riffles over runs and pools, especially in the stream affected by water diversion. However, radiotracking could not tell whether the desman selects riffles for the food in them or for other reasons. Therefore, I simultaneously studied the trophic ecology of the Pyrenean desman. To that end, I first analysed the efficiency of several primer sets for DNA metabarcoding in diet studies, analysing the taxonomic coverage provided by each primer set, as well as by their combination. Gillet and Zeale primer sets resulted the most cost-effective combination to identify the widest taxonomic range of prey, as well as the desman itself. Therefore, I studied the diet and the prey selection of the desman in the two contrasting streams analysing desman faecal samples collected in latrines. I DNA metabarcoded 94 faeces per stream to identify prey consumed and I also characterized available prey from 10 Surber samples taken at each habitat type per stream. Prey availability and desman diet varied between streams, desmans being more selective in the stream with better ecological status. Overall, desmans positively selected Simuliidae and Psychodidae (Diptera), Baetidae and Heptageniidae (Ephemeroptera), Odontoceridae (Trichoptera) and Gammaridae (Crustacea), while Planorbidae (Gastropoda), Goeridae (Trichoptera), Perlidae (Plecoptera) and Athericidae (Diptera) were selected against. Food availability did not differ

among habitats, and therefore did not explain the preference of desmans for riffles. Therefore, I re-analysed the data focusing on prey biological traits to identify what drives diet selection by the Pyrenean desman. I analysed whether trait preference can explain desman habitat selection, and assessed whether preferred traits differed between the two streams. Trait selection was inferred from 9 traits potentially affecting desman prey selection, among which 4 (depth preference, current velocity preference, substrate preference and preference of exposure to the flow) were likely influenced by the type of habitat (riffle, pool or run), and the other 5 (type of mobility/attachment to substrate, agility, aggregation tendency, maximal potential body size and body flexibility) were considered to be independent. On the one hand, desmans positively selected prey living in the water column and epibenthic zone, in fast facies with blocks as substrate, prey exposed to the flow, prey with the potential size of 2-8 mm, highly agile prey with some flexibility, clustered prey, and prey either swimmers or with mechanisms to attach to the substrate. On the other hand, they negatively selected prey living in water with slower velocities, in fine substrates, protected from the flow, prey with the potential size below 2 mm, prey without a tendency to aggregate, and crawlers. All preferred prey categories (except the high body flexibility) were slightly more abundant in riffles, but differences were small and varied between streams, so there are probably other reasons, likely the natural buoyancy of desmans, that explain the preference of this animal for riffles.

This thesis, overall, highlights the high specialization in terms of habitat use and diet of this species. It also highlights that clean water is not enough for desmans, riffles and stream connectivity being key factors for desman conservation.

Chapter 1



General introduction

The global “biodiversity crisis” and freshwater ecosystems

The current massive loss of biodiversity on our planet Earth caused by human activity has been named as a global “biodiversity crisis” (see Abell, 2002), and some authors argue that we are facing a sixth mass extinction (Barnosky *et al.*, 2011; Leakey & Lewin, 1997; Myers, 1990). Land-use change, management practices, pollution and human demography shifts are all drivers of this decrease (Root *et al.*, 2003; Rosenzweig *et al.*, 2008). Human-induced climate change makes this trend even worse (Walther *et al.*, 2002).

Freshwater ecosystems harbour a disproportionate amount of biodiversity for their area (Dudgeon *et al.*, 2006). These ecosystems, and in particular headwater streams, are experiencing declines in biodiversity far greater than those in the most affected terrestrial ecosystems (Richardson, 2019). Reid *et al.* (2019) included among the major threats to inland waters expanding hydropower, changing climates, infectious diseases and cumulative stressors, some moderate threats such as e-commerce and invasions, harmful algal blooms, light and noise, freshwater salinization and declining calcium, and others such as contaminants.

In free-flowing rivers, biodiversity can persist because organism dispersal can be effective in both upstream and downstream directions, while in fragmented and regulated rivers such dispersal can be strongly limited. River connectivity can be compromised by physical infrastructures in the river channel, by alterations of river flow and by changes in water quality that produce barrier effects (Grill *et al.*, 2019). Nilsson *et al.* (2005) showed that over half of the world’s large river systems they analysed were affected by dams. Europe is the continent with the smallest proportion of unaffected large river systems. Vörösmarty *et al.* (2010), jointly considering human and biodiversity perspectives, showed that multiple stressors have serious impacts on freshwater resources (Figure 1). They found that in 2000 nearly 80% of the world’s population was living in areas where either incident human water security or biodiversity threat exceeded the 75th percentile, and that 65% of global river discharge and the aquatic habitat supported by this water were under moderate to high threat. Only a strikingly small fraction of the world’s rivers were unaffected. The authors highlighted that massive investment in water technology enables rich nations to offset high stressor levels without remedying their underlying causes, while less wealthy nations remain vulnerable.

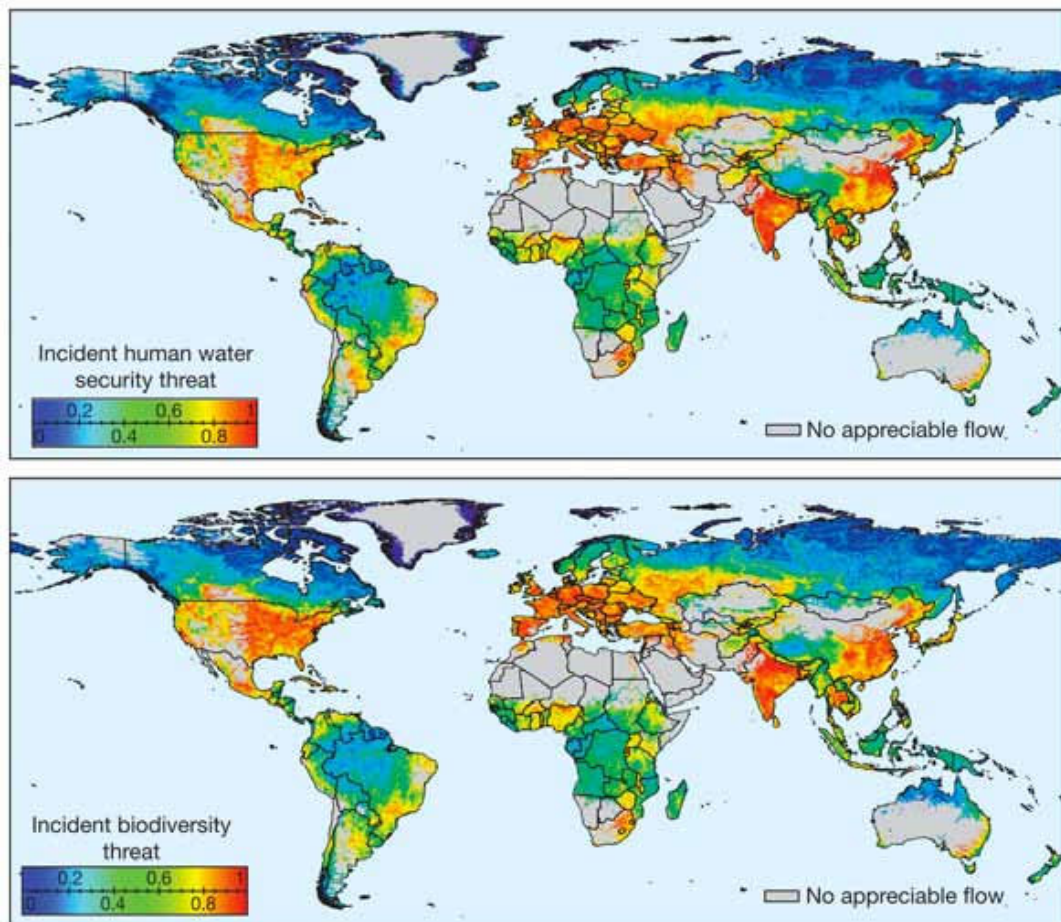


Figure 1. Global geography of incident threat to human water security and biodiversity. The maps demonstrate pandemic impacts on both human water security and biodiversity and are highly coherent, although not identical. Figure modified from Vörösmarty *et al.* (2010).

The forecasted biodiversity loss caused by the global environmental change will alter the functioning of ecosystems and their ability to provide society with goods and services (Cardinale *et al.*, 2012), directly impacting humans. Freshwater biodiversity provides a broad variety of valuable goods (e.g. food in the form of fishes) and services (e.g. clean water and waste assimilation) to human societies, some of which are irreplaceable (Covich *et al.*, 2004). Inland waters constitute a valuable natural resource in economic, cultural, aesthetic, scientific and educational terms, and their conservation and management are critical to the interests of all nations and governments (Dudgeon *et al.*, 2006). For instance, in the Mekong River Basin local people rely on fishing and rice farming, both dependent on the quality and quantity of the water in the river. However, intense exploitation for commercial aquaculture and the construction of hydropower dams, reduce fish stocks, prevent migration, and affect farming due to the artificial droughts and floods (Coates *et al.*, 2005; Hurtle, 2009). Such alterations threaten the livelihoods of the local population,

who rely heavily on natural resources to make a living. Freshwater pearls have also declined. Historically, freshwater mussels were very abundant through the world, but currently they are among the most endangered groups of organisms (Moorkens *et al.*, 2017). Human action has caused the decline in distribution of many species, including fish (e.g. the European eel *Anguilla anguilla*; Jacoby & Gollock, 2014) and amphibians (e.g. the Pyrenean frog *Rana pyrenaica*; Bosch *et al.*, 2009), and other freshwater megafauna such as turtle, crocodile and dolphin species (He *et al.*, 2019). A worrying issue is that as soon as one species is lost, the local society tends to forget it and thus, is not aware of how much rivers have departed from their natural status (Soga & Gaston, 2018). This trend has been called the “shifting baseline” (McHarg, 1969). For instance, the Atlantic sturgeon (*Acipenser sturio*) and the Eurasian beaver (*Castor fiber*) are good examples of forgotten species. Part of this neglect occurs because trade-offs among services occur at very different spatial and temporal scales: the gains from simplifying ecosystems tend to be local and short term, whereas the costs are transmitted to people in other locations, or to future generations (Cardinale *et al.*, 2012).

Being among the most sensitive freshwater ecosystems, headwater streams are unique environments, highly connected to their surroundings. They tend to have seasonally moderated temperatures, enemy-free space (none or few large-bodied predators) and relatively stable physical environments that provide refuge for many species (Richardson, 2019). The dendritic structure of river ecosystems has interesting implications for the biota, since it affects the mobility of strictly aquatic organism, but less so for amphibian species. The dispersal of freshwater animals is limited within stream networks (Hughes, 2007) and headwaters may be isolated by distances to tributaries and then back up to other headwaters (Fagan, 2002). Isolation of populations, coupled with small population sizes and limited gene flow, make genetic differentiation between populations and endemism high (Sterling *et al.*, 2012). Groups of highly diverse organisms include North American crayfish (Taylor *et al.*, 2007) and freshwater mussels (Inoue *et al.*, 2014). Currently, many freshwater species are threatened, and indicators suggest rapid population declines and a large extinction risk (Reid *et al.*, 2019).

Dudgeon *et al.* (2006) argued that if trends in human demands for water remain unaltered and species loss continues at current rates, the opportunity to conserve much of the remaining biodiversity in fresh water will vanish. Thus, a major change in attitude toward freshwater biodiversity and ecosystem management is needed. Reid *et al.* (2019) presented some tools and techniques for freshwater conservation. However, most of the species in freshwater communities are rare (Sheldon, 1988) and, thus, their natural histories (ecology, biology, etc.) and the causes for their decline tend to be poorly known. The first step to conserve

freshwater species is to stop causes of degradation, such as pollution, habitat modification and overharvesting. Although there would remain many species whose threats are poorly known, claiming that more research is the first step to nature conservation leads the path to extinction since there is simply no time to acquire all relevant information before we lose most endangered species (see Croxall *et al.*, 2012).

The Pyrenean desman

Morphological characteristics

The Pyrenean desman *Galemys pyrenaicus* (É. Geoffroy Saint-Hilaire, 1811) (Figure 2) is a semi-aquatic insectivore belonging to the family of the moles (Talpidae). Its body-length is 11.5-13.5 cm, tail-length 10-16 cm, and adult's mass 44-76 g (Nores, 2012). Unlike its relatives, the moles — *Talpa sp.* —, the desman is adapted to swimming, with wider hindfeet than forefeet (Palmerim & Hoffmann, 1983). They have strong claws that allow a strong attachment to the bottom, and their digits are webbed at the base to facilitate swimming. Forefeet are small and not adapted to digging. Hindfeet are larger, covered by scales and with a strip of long, strong and compact hairs in the outside. The tail, almost naked, is covered by scales and scarce hard hairs, has a cylindrical section except in the flattened tip, and can be used as a rudder when swimming. Under the tail they have a gland that secretes a musky liquid, likely used to mark their territories (Palmerim & Hoffmann, 1983; Richard & Viillard, 1969). The snout is the most distinctive feature of the Pyrenean desman: it consists of a hairless trunk, at the end of which, on the dorsal side, the nostrils open. Their elongate rostrum supports conspicuous anterior upper incisors (Figure 4), and their dental formula is 3.1.4.3/3.1.4.3 (Palmerim & Hoffmann, 1983). Like the mole, the Pyrenean desman has very small eyes and outwardly invisible ears, which are reduced to a cylindrical cavity.

Desmans vibrissae, much longer and thicker than in moles, are located on both sides of the snout, decreasing in size distally (Richard, 1981). Woolly hairs and guard hairs are distributed along the body. Desmans have two types of guard hairs, the straight guard hairs (*leithaar*) and the sharply bent *grannen* (Poduschka & Richard, 1985). The latter are easily identifiable because they lack a medulla, are folded inwards in their distal half and present an apical flattening in the form of a spearhead. These characteristics make desman hair of special significance for excrement identification, as they are accidentally ingested as a result of grooming (Stone, 1987b).



Figure 2. The Pyrenean desman. Photo by Amaïur Esnaola.

Although the desman body is brownish, underwater it reflects bronze and metallic glows, due to its particular hair types: the spatulate part of the *grannen* produces the reflections and iridescence observed on the fur of the animal, either alive or dead (Puissegur, 1935). Trunk and limbs have blackish skin, with silvery hairs. The ventral region is paler than the dorsal. The tail is pale flesh-coloured and has whitish or yellowish hairs. Sexual dimorphism with regard to colour, size and mass is inconspicuous, if present at all. Moreover, it is also hardly perceptible in the genital system, both sexes having a penniform organ. Desmans have a very thin pelvic arch that can help in sex identification (Peyre, 1957): in adult females a soft gap of about 5 mm can be identified, while in adult males the arch is always closed. During the mating season, females can be distinguished by the open and depigmented vaginal opening (Richard, 1986). González-Esteban *et al.* (2003b) presented a new method to identify sexes independent of season: applying slight pressure on the external genitals to evaginate them, males show a cone-shaped glans while females have a long and narrow urinary papilla (Figure 3).

Desmans age cannot be inferred from their weight and nobody has ever seen a new born desman. Richard (1976) and González-Esteban *et al.* (2002) established some criteria for age determination. On the one hand, they proposed a quantitative criterion using an index (D) based on the relative value of the height of the upper canine crown (HCC) in relation to the condylobasal length (LCB), where $D = (CCH/LCB) \times 100$. However, the most used criterion

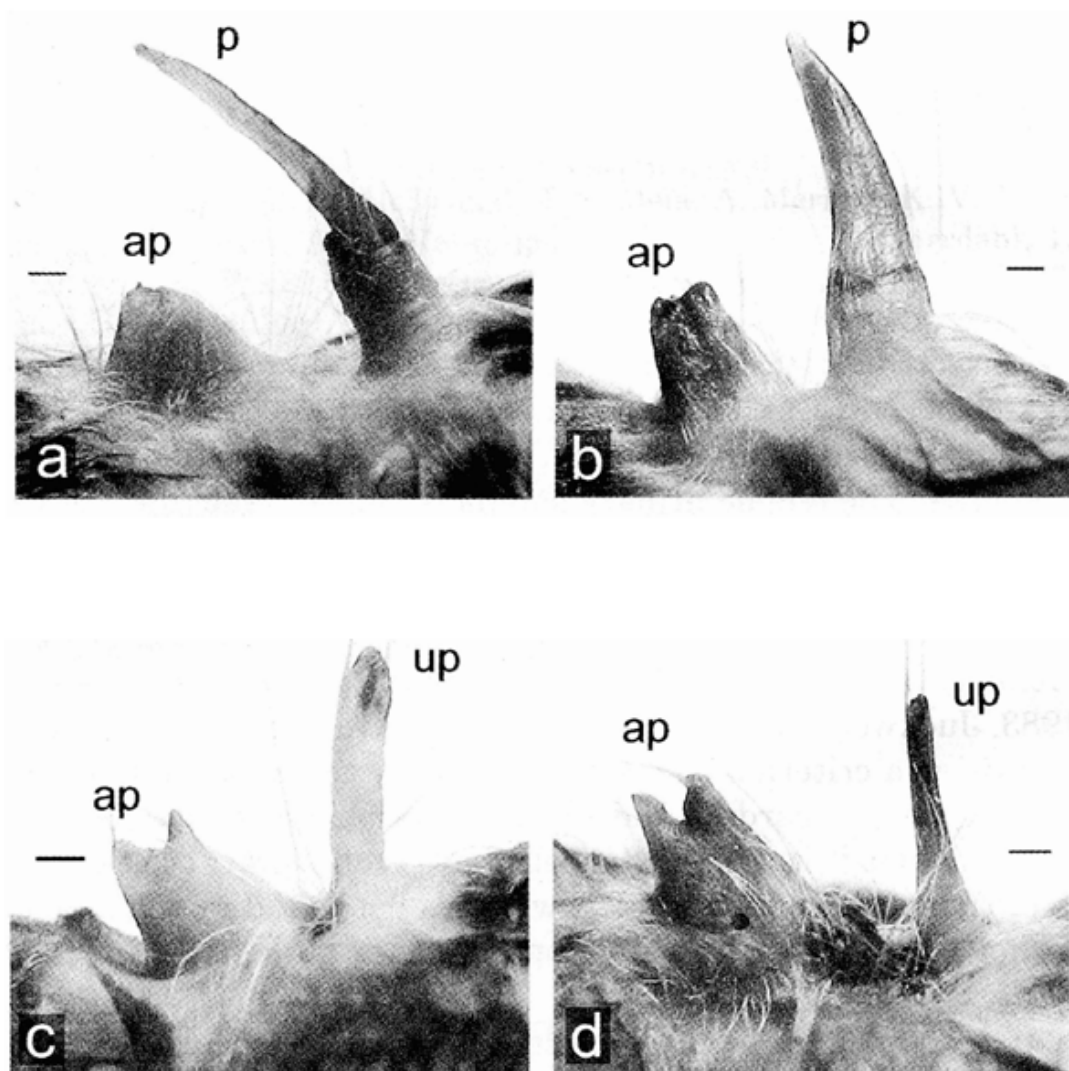


Figure 3. Lateral view of the external genitals of the Pyrenean desman: (a) a young male (under 1 year), (b) an adult male (over 2 years), (c) a young female (under 1 year), and (d) an adult female (over 2 years). Anal papilla (ap), penis (p) and urinary papilla (up) have been represented in the figure. The prepuce was removed in males to expose the glans. Scale bar: 1 mm. Figure recovered from González-Esteban *et al.* (2003).

is the semiquantitative one, where they set the following classes based on the wear of the upper canine (Figure 4): 0) 1st year of age, with an intact or slightly blunted canine; 1) 2nd year of age, canine with a blunt cusp, with wear affecting about a fourth of the crown; 2) 2nd-3rd year of age, wear affecting half of the crown; 3) 3rd-5th year of age, wear reaches the maximum width of the crown affecting approximately three-fourths of the tooth; 4) 3rd-6th total wear of the crown, the two roots of the tooth can be seen separately and are similar in size and shape to incisors 2 and 3.

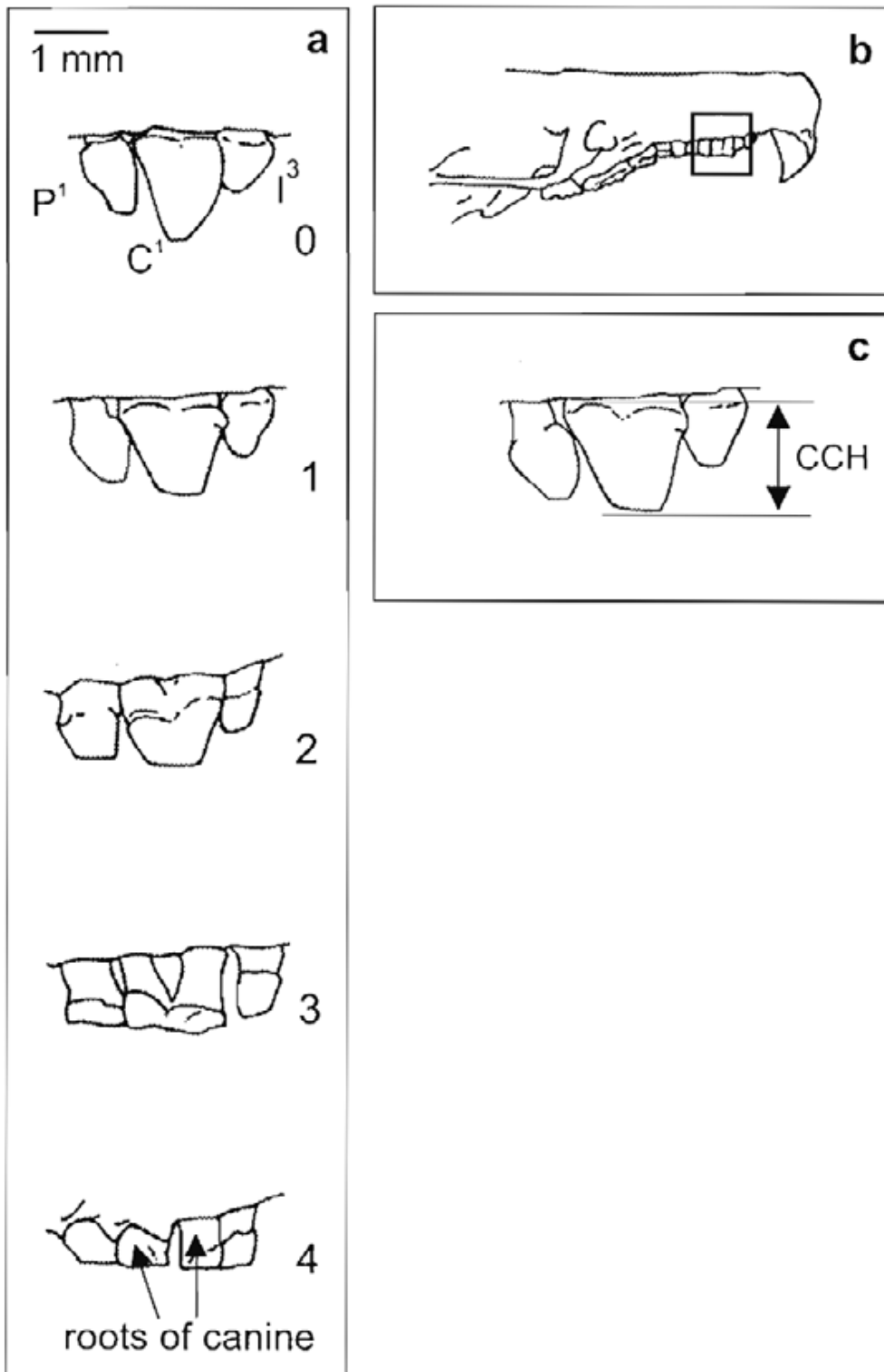


Figure 4. Dental wear in Pyrenean desmans. (a) Shape of the upper canine (labial side) for the five wear classes established; 1st upper premolar (P¹), upper canine (C¹) and 3rd upper incisor (I³) have been represented. (b) Facial region of a desman skull (lateral standard); the square shows the upper maxillary fragment represented in (a). (c) Height of the crown of the upper canine (CCH) used in an index. Figure recovered from González-Esteban *et al.* (2002).

The sensory world

Desmans are almost blind, they are likely able to perceive changes in light intensity, although they seem unable to locate food by sight (Richard, 1973). Desmans can emit some peculiar noises (described as a piercing scream) when manipulated or when encountering members of the same species, although they usually remain silent (Richard, 1973). They have also been reported to perform a strange “drumming”, hitting the surface of the water with the front legs (Richard, 1973). Although Niethammer (1970) interpreted this behaviour as “cleaning the hands after meals”, Richard (1981) proposed that it was performed to detect obstacles in the surface of the water by means of vibrations. Desmans seem to have a very developed mechanoreceptors, with large and complex vibrissae and Eimer’s organs (Bauchot *et al.*, 1973), which make the desman snout highly sensitive. As in most aquatic animals, the vibrissae are numerous, long and strong, immobile at their roots, and with rich innervation (Richard, 1985b). Over three quarters of the snout section is filled with efferent nerves of the vibrissae, which perceive both the contact of nearby objects and the movement of passing water. Eimer’s organs, exclusive of Talpidae, numbering about 100 000 over a few mm² at the end of the snout, have piled-up cell structures around the nerve tissue which rise up to the epidermis (Richard, 1981, 1985b). They are sensitive to contact and to variations in water pressure caused by the movement of the animal, allowing the detection of obstacles in water. Both the vibrissae and Eimer’s organs have been compared to the lateral line of the fishes (Richard, 1973; Sarko *et al.*, 2007). The trunk serves to avoid collision with underwater as well as to detect food (Richard, 1973, 1985b).

As for the chemoreception, the scarce taste organs in the desman tongue (Richard, 1986) suggest that desmans detect their immersed prey by smell (Richard & Micheau, 1975; Richard, 1986). Jacobson’s organ has also been suggested as important for desman chemoreception of liquid material (Richard, 1985b). It consists in a simple internally lined dimple with an olfactory mucosa that passively receives the odorous molecules dissolved in the water around the mouth (Richard, 1986). The oral openings of this double organ are difficult to find, since they are hidden by two valves. These are located just behind the upper incisors, on the soft palate, avoiding the return of the contents from the mouth to the nasal cavity, and letting in the water that is taken to analyse by olfaction. It seems that the lower incisors favour the opening of these valves, pressing them in the soft palate (Richard, 1986). That unique olfactory organ is usually absent from adult mammals, but it persists in insectivores and is particularly developed in Talpidae. The desman emits bubbles under water from the nostrils (Richard, 1986), so, it seems that desmans also smell their prey underwater, exhalating and inhaling air-bubbles, as described in the Russian

desman (*Desmana mochata*, Ivlev *et al.*, 2013) and other mammals with similar ecology, such as the American water shrew *Sorex palustris* (Catania *et al.*, 2008), the water shrew *Sorex palustris* and the star-nosed mole *Condylura cristata* (Catania, 2006).

Reproductive biology

After histologic analysis of over 500 individuals from the French Pyrenees, Peyre (1962) reported that the heat period and the period of births of desmans concentrate during the first half of the year, and that females have postpartum oestrus, with a maximum of three litters per year. Litters range from 1 to 5 offspring, with a mode of 4 (Peyre, 1956). Unfortunately, neither desman offspring nor nest with young has ever been reported in the wild. A recent study suggested that desmans do not reproduce until they reach the age one; that they are not monogamous, females mating with different males in different breeding events; and that only the female provides parental care (Escoda *et al.*, 2019).

Home range and movement

Richard and Viallard (1969), based on mark-and-recapture techniques, first attempted to define desman home range, an essentially linear stretch along the stream, whose length they described as ranging from a few tens of meters to a maximum of 200 meters. They reported that males and females can share the home range. They studied animals held in captivity, and stressed that although they are mainly nocturnal, have a peak of activity at noon to eat. Palmerim and Hoffmann (1983) reported that desmans seem to have a “memorized” knowledge of their home range, as the displacement of any object appears to confuse the animal, and that desmans defend their territories, even among sexes.

Although Richard (1985a) reported captive desmans being more active in autumn and winter than in spring and summer (probably related to the time of reproduction), it was not until the use of radiotracking that it was possible to study desman activity in the field. In the first such study, Stone and Gorman (1985) reported that whereas some individuals were solitary, adults share largely exclusive home ranges with individuals of the opposite sex. Females occupied home ranges (301 ± 6.8 m; $n = 7$) within the longer ranges of their male partner (429 ± 10.0 m; $n = 7$) and although the ranges of neighbouring males were largely exclusive, they showed some degree of overlap. No overlap was seen among neighbouring females neither between neighbouring males

and females. On the other hand, juveniles occupied relatively small ranges 249-300 m long. The activity of neighbours appeared to be synchronized, as the members of each pair moved upstream from their nest in one active period and downstream in the next, covering their whole range in 24-h. Thus, confrontation among neighbours was avoided by temporal isolation.

Stone (1985) reaffirmed the strong fidelity of desmans to their home ranges, and described their population as comprising sedentary groups, consisting of a single adult male and female cohabiting the same section, and transient individuals, which may be juveniles or nomadic adults. That description of transient (solitary) desmans could be confusing, since there might be other individuals not captured in their home ranges. Stone added that females and juveniles spent a greater proportion of their time close to nest sites than at the ends of their ranges, unlike adult males. He suggested that the regularity of male movements was an important method of territorial demarcation – with the continual renewal of scent marks, both faecal and from sebaceous sub-caudal gland –, whilst, at the same time, it worked as deterrent against the influx of neighbouring and transient animals. According to Stone, all desmans (with the exception of one male) used only one main nest, exclusive to each individual. Stone (1987b) reported that the juveniles used both exclusive and totally overlapping ranges with neighbouring resident desmans and with other juveniles. He suggested that the spatial organization of desmans is a form of territoriality, since they are extremely aggressive towards conspecifics and they attain a high familiarity with each part of their range by following a routine pattern of movements each day. A study using automatic nest recorders (Stone, 1987a) reported that desmans maintained two shelters, one temporary used irregularly for brief (10-15 min) periods of rest and the other, a main nest site for longer periods. As described previously, individuals displayed two distinct periods of activity during each diel period, with a short diurnal period and a longer nocturnal one. He suggested that such pattern could be related to being active at a time when prey activity is at its highest.

Chora and Quaresma (2001) published the first radiotracking study in Portugal, which assessed the effects of small hydropower schemes on desman movements. They reported that desmans can overcome low weirs. Melero *et al.* (2012) reported that in Catalonia desman home ranges average 523 ± 50.85 m, without significant differences between sexes, and that the species is not strictly territorial. Furthermore, they observed no aggression between conspecifics, with frequent social interactions and reported individuals sharing resting sites regardless of sex or age. The entrance of resting sites usually were submerged or partially submerged. Melero *et al.* (2014) showed that the desman was mainly nocturnal and its activity pattern changed from bimodal in spring, to trimodal-tetramodal in autumn. Diurnal movements were always

straightforward, without forward and backward movements. In contrast to previous observations, authors observed encounters between individuals without evidence of aggressive behaviour. Thus, Melero *et al.* suggested that desmans do not alter their activity or their spatial behaviour to avoid encounters with conspecifics.

Barriers and isolation

Escoda *et al.* (2017), with DNA samples from La Rioja, reported little genetic relationship between neighbouring populations, indicating that desmans do not frequently move between rivers. Further, results suggested overland movement across watershed divides. Escoda *et al.* (2019) applied a pedigree-based approach to DNA samples from Zamora to assess the influence of different obstacles (i.e. watershed divides and dams). They found a dispersal distance per generation of 4 km and greater philopatry among females. They inferred that the most important barrier in the area was the watershed divide between rivers, followed by large dams, smaller dams having a minor influence on dispersal. The authors concluded that fragmentation and reduced connectivity may lead to inbreeding depression and, eventually, local extinction.

Habitat

The first known description of desman habitat (Trutat, 1891) stated that they prefer small rivers, mill channels and swampy meadows to large mountain streams. Cabrera (1914) reported that desmans live near streams and large rivers, and shelter in cracks and cavities of the banks or in galleries opened by water rats. Peyre (1956), in the first complete description of the habitat of this species, explained that the Pyrenean desman lives in any type of water courses, from small mountain streams to large rivers, even mill channels, provided they meet two conditions: first, permanent, cold and oxygenated water, with very rich fauna, basically composed of insect larvae and crustaceans, and second, the presence of natural or artificial shelters. Peyre also described desman behaviour, stressing high activity and nocturnal habits, although it can make fast outings during the day, especially in the breeding season, to look for food. He explained that desmans rarely swim on the surface and that they make long dives instead, where they seem to walk along the bottom, lifting the small stones with the trunk or digging in the sand.

In recent decades several models have been made to clarify the factors that explain the presence of the desman. Barbosa *et al.* (2009) presented the first model for desman distribution in Spain, Portugal and the Iberian Peninsula. Next,

Morueta-Holme *et al.* (2010) modelled the distribution of the desman in Spain to assess the affect of the climate change. Morueta-Holme *et al.* reported that the current climate, in particular water balance and mean summer temperature were the main determinant of the species distribution, even though dispersal probably also strongly limits the distribution at a broader scale. In contrast, after a new modelling research in France, Williams-Tripp *et al.* (2012) emphasized the importance of factors linked to precipitation and not temperature. Three years later, analysing data from the French Pyrenees, Chabonnel *et al.* (2015) suggested that the flow variation, substrate heterogeneity and the presence of shelters affect detectability of desman faeces, while the flow is the main factor driving habitat occupation by the desman. They argued that high flow is related to the abundance of invertebrates, which are the main prey of the Pyrenean desman, and that the importance of the flow suggests a preference for riffles, fast-flowing facies.

All the model studies published before my research had coarse spatial resolutions and relied only on data from stream sections where desman faecal detection was possible. The results of these studies can be influenced by the substrate heterogeneity in each stream section and do not allow characterizing the spatial preferences of the desman at the microhabitat scale, nor the intensity of use of the space. As for the previous radiotracking studies, they focused more on social aspects than on the use of the habitat, so they neither allowed the description of desman requirements at local level. Since this thesis started new research have been published: first, Biffi *et al.* (2016) provided information about habitat factors measured at the local scale in river stretches occupied by desmans in the three hydrological regions from the French Pyrenees, while Charbonnel *et al.* (2016) compared historic and current desman distribution in French Pyrenees; subsequently Quaglietta *et al.* (2018) measured the extinction rate across two periods in Portugal; and more recently Biffi *et al.* (2019) characterized the habitat at the local scale in the French Pyrenees. However, none of these studies has analysed the intensity of the use of different microhabitats within desman home range. These studies are discussed in Chapters 2 and 6.

Foraging ecology

Early studies based on direct observation (Cabrera, 1914) reported desmans to feed mainly on insects, although they also chase fish and crustaceans. The first analyses of stomach contents (Puissegur, 1935) reported insect larvae (probably Plecoptera and Trichoptera), small insects (especially terrestrial) and crustaceans, but no fish remains. Peyre (1956) reported similar results and concluded that Gammarids are the basis of the diet, since they are

available throughout the year. He reported that captive desmans eat spleen and mealworms (*Tenebrio molitor*), as well as trout, which they eat completely without leaving remains. Richard and Viallard (1969) reported that in captivity desmans preferred small aquatic fauna, including gastropods, but accepted terrestrial prey, from worms to arthropods. They described how desmans explored the entire bottom of the aquarium, preferably capturing large to small prey (15 vs. 5 cm) and mobile to non-mobile prey, and eating them out of the water. The amount of food eaten by desmans was large, as would be predicted from a small insectivore.

Santamarina and Guitian (1988) analysed eight gut contents from Galicia and found the most frequently consumed prey to be Ephemeroptera (35.8% of items), Trichoptera (32%), Diptera (24%) and Plecoptera (4.3%). When measuring consumed volumes, though, Trichoptera (67.4%) and Ephemeroptera (20.7%) were the most important groups. Baetidae, Leptophlebiidae, Simuliidae and Limnephilidae were the most common families, the latter having the highest percentage values in volume. Comparing what was consumed with what they had available, Santamarina and Guitian reported that desmans selected against molluscs and aquatic beetles (Dryopidae and Elmidae). Bertrand (1992) published the results of 521 gut samples collected for a year in the French Pyrenees. The groups eaten were Amphipoda, Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Diptera, and their frequency varied among seasons. Desmans positively selected benthic prey, prey with high intrinsic energy value (Trichopterans without cover, vs Limnephilidae) and prey with little sclerification. Santamarina (1992) analysed 2 guts in summer, 2 in autumn and 2 in winter in Galicia. Trichoptera was dominant in the diet, followed by Ephemeroptera, Amphipoda, Annelidae, Diptera and Odonata. Santamarina (1993) highlighted that desman mainly consumed Sericostomatidae and Ephemera in summer, shifting in autumn to Gammaridae, Lumbricidae, Philopotamidae and Tipulidae, and in winter to Sericostomatidae, Limnephilidae, Lepidostomatidae and Hydropsychidae. He added that desmans exhibited selection against Hydrobiidae, Elmidae and Chironomidae in all seasons examined, and that they also avoided Ephemerellidae in autumn and Leptoceridae in winter. Castián and Gosálbez (1995) analyzed 46 gut samples from Navarre and found Ephemeroptera (Heptageniidae), Trichoptera (most important in biomass), Diptera (Simuliidae, Chironomidae, Limoniidae and Athericidae) and Plecoptera to be the most important prey. Ephemeroptera and Trichoptera changed little among seasons, while Plecoptera were more common in autumn and spring, and Diptera more common in winter. Terrestrial species were scarce in desman diet (< 4%). Castián and Gosálbez (1995) characterized the desman as a stenophagous species that feeds mainly on large, not very mobile invertebrates (Trichoptera), plus other small but abundant taxa (Ephemeroptera larvae). No fish was identified in their samples.

From an anecdotic point of view, Quaglietta and Beja (2019) reported that semi-captive desmans captured and ate trout and frogs in Portugal.

Metabarcoding diet studies

Recently, molecular methods such as High Throughput DNA Sequencing have been revolutionary for diet studies, as faecal DNA allows identification of consumed items at species level, even from remains that cannot be distinguished visually (Clare *et al.*, 2009; Valentini *et al.*, 2009; Bohmann *et al.*, 2014; Pompanon & Samadi, 2015). Gillet *et al.* (2015) were the first to apply DNA metabarcoding to desman diet. The analysis of 16 faecal samples from the French Pyrenees yielded 19 prey species from four orders (Diptera, Ephemeroptera, Plecoptera and Trichoptera) and 11 families. An advantage of DNA metabarcoding was that it also allowed clear confirmation the Pyrenean desman produce the faeces.

These molecular methods have however some constraints. On the one hand, DNA is degraded by its transition across the gut, as well as by exposure of scats to environmental conditions (Deagle *et al.*, 2010; McInnes *et al.*, 2017; Oehm *et al.*, 2011; Symondson, 2002). Besides, the high sensitivity of molecular methods may produce abundant false positives as a consequence of secondary predation, scavenging, or contamination from any source (Sheppard *et al.*, 2005). Moreover, results from DNA metabarcoding depend on methodological details such as the primers used, PCR strategy, sequencing workflows, bioinformatic decisions or the information available in databases (Alberdi *et al.*, 2018; Brandon-Mong *et al.*, 2015; Divoll *et al.*, 2018; Piñol *et al.*, 2019). False positives and false negatives in dietary studies can have strong implications in the interpretation of predator ecology, as well as in ecosystem management (Hines & Gessner, 2012). Thus, successful identification of relevant prey taxa is key to sound conclusions about ecological role and trophic specialization (Schwarz *et al.*, 2018; Waraniak *et al.*, 2018).

When my research began, these new methods had not been applied to desman diet yet, and so, there was little knowledge about its trophic ecology at fine scale, which could be a key aspect for the conservation of the species. Afterwards, though, several studies have been published: first, Biffi *et al.* (2017a) described desman diet in the French Pyrenees from 383 faeces using the primers tested by Gillet *et al.* (2015); subsequently, Biffi *et al.* (2017b) analysed 184 faecal samples from the French Pyrenees to compare the diets of the desman and the Eurasian water shrew (*Neomys fodiens*); and more recently, Hawlitschek *et al.* (2018) described desman diet from 41 faecal samples in Zamora (Spain) with a different set of primers. These studies are discussed in Chapters 4, 5 and 6.

Interactions with other species

Although desmans share their habitat with Eurasian water shrew (*Neomys fodiens*), white-throated dipper (*Cinclus cinclus*) and brown trout (*Salmo trutta*), their diets are not expected to overlap much (Santamarina, 1993), as it has been recently verified for the water shrew (Biffi *et al.*, 2017b). On the other hand, desmans predators include the Eurasian otter (*Lutra lutra*, Adrian *et al.*, 1988; Callejo & Delibes, 1987), the grey heron (*Ardea cinerea*; González & Román, 1988), the white stork (*Ciconia ciconia*; Hernández, 1988), the common buzzard (*Buteo buteo*; Bustamante, 1985), the brown owl (*Strix aluco*; Nores, 2012) and the barn owl (*Tyto alba*; González & Román, 1988; Purroy *et al.*, 1988). It is believed that the invasive American mink (*Neovison vison*) has decimated desman populations (Nores *et al.*, 2007), although there is not direct evidence for this. Finally, desman parasites include mites (*Eadidea desmanae*; Luckoschus, 1969), flukes (*Omphalometra flexuosa* var. *peyrei*, Vaucher, 1975; *Maritrema pyrenaica*, Jourdane, 1979; *Matovius galemydis*, Mas-Coma *et al.*, 1985) and nematodes (*Aonchotheca galemydis*, Álvarez-Mascato, 1991; *Paraucaria hispanica*, Álvarez *et al.*, 1994).

Phylogeny and taxonomy

Geoffroy Saint-Hilaire (1811) named the Pyrenean desman as *Mygale pyrenaica*. Afterwards, Kaup (1829) reclassified it within the genus *Galemys*. Finally, Miller (1912) established the current nomenclature. Currently, desmans are grouped within the order Eulipothyphla, family Talpidae and subfamily Desmaninae.

Molecular data show that desman and Eurasian mole lineages separated 37 Ma ago (Douady & Douzery, 2003), in the equator of the Cenozoic period, in a small ice age, precluding the greater cooling of the end of the Eocene. Such divergence indicates the withdrawal of the excavator line of desmans due to a growing aquatic specialization. The fossil record shows many species of desmans in Europe and Asia since the Miocene, 9.0 to 5.3 Ma ago (Rümke, 1985).

Nowadays, only two desman species survive: the Pyrenean desman and the Russian desman (*Desmana mochata*, Linnaeus (1758)). These two species constitute the subfamily Desmaninae (Cabria *et al.*, 2006) and diverged 10.05 Ma ago (Colangelo *et al.*, 2010). Their geographic ranges are disjunct, and the species are easily distinguishable (Palmerim & Hoffmann, 1983). The Russian desman is much larger, its tail is laterally compressed throughout its length, and has a proportionally shorter snout.

Distribution

Fossil *Galemys* are known from the end of the Pliocene era, 5.33 to 2.58 Ma ago, when they were distributed along most Europe, from Polonia to Portugal and from Hungary to England (Harrison *et al.*, 1988; Rümke, 1985). However, the distribution of this genus was reduced to the southwest from the early Pleistocene, 2.58 to 0.78 Ma ago (Arribas, 2004), and currently *Galemys pyrenaicus* is restricted to the Ibero-Pyrenean area. Such contraction could be explained by a growing specialization of the smaller desmans to mountain streams (Nores, 2012), since they probably found refuge in the Iberian mountains that did not suffer as intense glaciation processes as in other European mountains.

During recent decades the desman distribution has shrunk by more than 50% in the Iberian Peninsula and in the north of the Pyrenees (MAGRAMA, 2013; Charbonnel *et al.*, 2015). Currently, its distribution is limited to the mountain ranges of the north of the Iberian Peninsula, from the Central System and the Serra da Estrela to the Iberian system and Pyrenees (Nores *et al.*, 2007). In the Basque Country, northern Iberian Peninsula, where my research was performed, rivers with extant desman populations include the Leitzaran, Urumea, Bidasoa, Larraun, Basaburua, Ultzama, Arga, Irati, Salazar and Ezka (Castián & Gosálbez, 1992). Their status is poor, as these populations have decreased even in streams where the conservation situation has improved as a consequence of pollution abatement (Ihobe, 2012; Rubio, 2016; URA, 2017).

Threat factors

Fragmentation of the populations, habitat degradation and food shortage have been identified as the main threat for desman conservation (Nores, 2012), but these are in turn consequences of multiple human activities (Figure 5). One of the likely causes of fragmentation are dams and reservoirs. The reservoirs are of little or no use to desmans, and their effects on flow regimes can affect long reaches downstream. In many of the mountain areas occupied by the desman, an additional activity is water diversion, especially linked to small hydropower plants, which results in extremely low flow in long bypassed river sections (MAGRAMA, 2013; Queiroz *et al.*, 1996). Additionally, diversion canals could be an important cause of death for desmans (Chora & Quaresma, 2001; Elosegi, 2010). Also, river channelling and civil works, such as rip-rap and other flood defences, affect riverbeds and riverbanks, causing habitat deterioration and loss of stream heterogeneity, which in extreme cases could make the stretch totally unsuitable. Destruction of riparian vegetation has also been mentioned

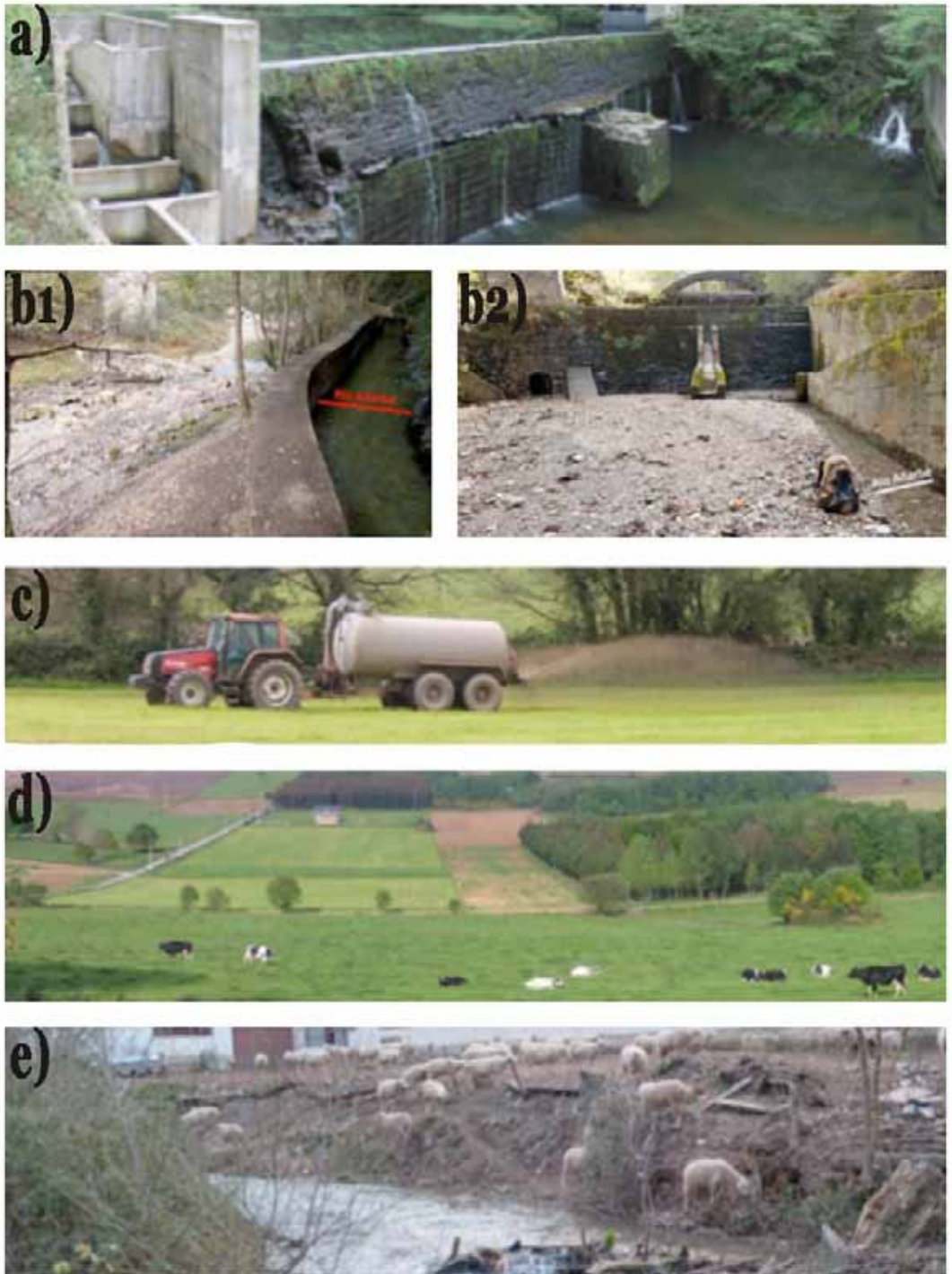


Figure 5. Examples of some of the threat factors. (a) A dam in Leitzaran stream; (b) a water diversion almost drying out a section of Añarbe stream; (c) the intensive agriculture Ulla; (d) modification of the land for human use in Miño; (e) destruction of river banks in Tobia. Photos by Jorge González-Esteban and Amaïur Esnaola.

as a damaging activity, as it reduces nesting sites, increases water temperature and can affect invertebrate productivity (Queiroz *et al.*, 1996). Of course, water pollution can be a threat factor, especially as a consequence of intensive agriculture and the use of phytosanitary products (diffuse contamination), as well as of point-source inputs of pollutants, associated to towns, farms or industry. Although in many parts of the original desman range water pollution has decreased during the last decades, it has also become more complex, with a large number of emerging contaminants found in many rivers and streams (Petrovic *et al.*, 2016). In some sites, aquatic sports can be an additional detrimental factor for the desman, either by affecting food production, or by unwillingly disturbing the animals. Gravel extraction can be also important, especially in braided sections of piedmont rivers, as it seriously affects channel form and river bottom stability (Brown *et al.*, 1998). Illegal fishing is also known to cause accidental death of desmans in trammel nets (Queiroz *et al.*, 1996), or through poisoning rivers with hypochlorite. Besides, some anglers captured desmans as they believed they were a threat to trout stocks (Fernandes *et al.*, 2008). Furthermore, over-eager collectors were another threat of direct persecution (Richard, 1976). Invasive species, especially the American mink (*Neovison vison*), could also negatively impact desman populations (Fernandes *et al.*, 2008). And finally, climate change is a threat over all the biodiversity, including freshwater species.

Conservation status

Regression trend

Although it is hard to obtain precise estimates of population size and decline rate for this species (González-Esteban *et al.*, 2003a), climate and hydrology models suggest that desmans potential distribution will continue decreasing (Araújo *et al.*, 2011). Moreover, Charbonnel *et al.* (2016) reported the range contraction of the Pyrenean desman in the last three decades to be much faster than what could be explained by changes in climate, hydrology and land use. This clearly shows that other factors, such as probably those listed above, have a strong influence on the species.

Population genetics

Of the four species of *Galemys* described from fossil remains (Rümke, 1985), only *G. pyrenaicus* remains alive. Although some authors (Cabrera, 1914; Ellerman & Morrison-Scott, 1951) distinguish two subspecies (*G. p. pyrenaicus* in the Pyrenees and the Cantabrian Mountain Range and *G. p. rufulus* in the Central System), recent studies refute this idea (González-Esteban *et al.*, 1999). Rather, desmans are separated in four genetic clades with clear geographical

delimitation and a prominent parapatry, grouped in two main groups (Figure 6; Igea *et al.*, 2013). Clade A1 appears in the northeast of the Iberian Peninsula, from Portugal and Galicia to the centre of the Cantabrian Mountain Range; clade A2 is found in the Central System and south of the Iberian System; clade B1 from the centre of the Cantabrian Mountain Range to north of the Iberian System; and clade B2 from the eastern foothills of the Cantabrian Mountain Range to the Pyrenees. The genetic diversity values found in these populations correspond to the expected patterns of postglacial recolonization in the Holocene of different areas from certain glacial refugia. Exchanges between these clades have not been observed in the contact areas, although no apparent barriers to desmans dispersal seem to exist (see also Querejeta *et al.*, 2017).

However, there is discrepancy among the resulting clades. Querejeta *et al.* (2016) presented a possible population structure based on five genomic clusters, which coincided with the main mountain ranges: Pyrenees, Occident, Cantabrian Mountains, Iberian Range and Central System (Figure 7). The clusters built with nuclear data correspond fairly well to the mitochondrial lineages detected by Igea *et al.* (2013) at the initial subdivisions (A and B), but had some remarkable differences at higher subdivisions. Additionally, focusing on the French Pyrenees and using nuclear data, Gillet *et al.* (2017) showed that three genetically and geographically distinct clusters could be found (the

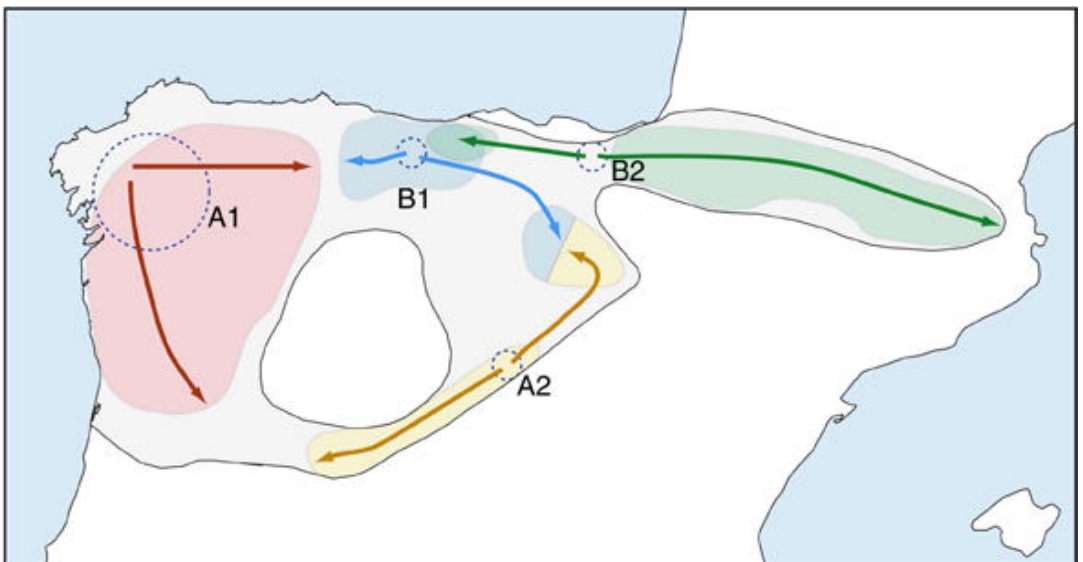


Figure 6. Schematic representation of the evolutionary history of *Galemys pyrenaicus* and the four clades defined by Igea *et al.* (2013). The greyed area represents the historical species distribution while the current areas of the four mitochondrial lineages are shown in different colours. Hypothetical glacial refugia are depicted by dotted circles. Circle sizes represent the relative importance of the refugia and arrows indicate colonization routes from them. Figure recovered from Igea *et al.* (2013).

eastern, central, and western) within the B2 clade described by Igea *et al.* (2013). The low level of genetic diversity observed in the French clusters by Gillet *et al.* (2017), as well as the heterozygote deficiency highlighted by the high inbreeding coefficient values and the relatively low effective population sizes within clusters, suggest an increasing extinction risk for this species.

Legal protection

The Pyrenean desman is protected under the Bern Convention (Appendix II) (OJEC, 1982) and the European Habitats Directive (Annexes II and IV) (OJEC, 1992). The decline of the Pyrenean desman populations warranted its inclusion in several red lists. Currently, it is listed as Vulnerable (A2ac+3c+4ac) in the Red List categories by the International Union for Conservation of Nature (IUCN) (Fernandes *et al.*, 2008), Endangered in the Basque Autonomous Community (EHAA, 2011) and as Special Interest in Navarre (Boletín Oficial de Navarra, 1995). In the French territory it is protected under the ministerial order of the 23rd of April of 2007 (JORF, 2007).

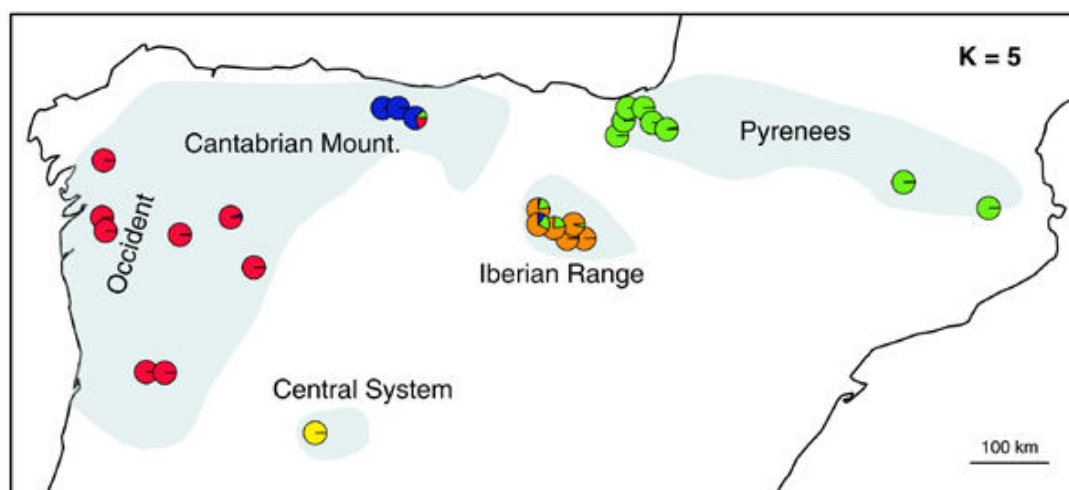


Figure 7. The five clusters proposed by Querejeta *et al.* (2016), largely coincident with the main mountain ranges where the species occurs. Individuals were represented by pie charts and admixture proportions of each of them were plotted with different colours. The shadowed area represents the current species distribution, according to different sources. Figure recovered from Querejeta *et al.* (2016).

The context of the thesis

Taking into account the critical situation of the Pyrenean desman, it was considered that there still was an important lack of knowledge, particularly about essential aspects for its conservation, such as the spatial and trophic ecology of the species. As for the spatial ecology, the last research on desman habitat provided information about local habitat factors correlated with the presence of desman faeces in river stretches, but they did not identify the factors controlling their occupancy at the microhabitat scale, which is key for understanding the species' needs. On the one hand, faecal samples only provide presence data of the species —false negatives included—, but do not allow for assessment of the intensity of use of different habitat types. On the other hand, previous studies explored the relation of desman distribution with different environmental factors —some of them maybe correlated to each other—, but did not determine the ultimate factors defining the species' distribution at microhabitat level. Moreover, as selection patterns change spatially according to differences in availability (Ayllón *et al.*, 2010; Boyce *et al.*, 2016), it is necessary to study the behavioural response of desmans in contrasting environments to understand the key factors shaping their habitat preferences. Regarding the trophic ecology, the observational works prior to this thesis showed the main groups of prey the desmans eat. However, they did neither provide information with a fine taxonomic resolution, nor they studied desmans prey in terms of their morphofunctional —morphoecological— characteristics. Moreover, I considered critical to clarify whether local-scale habitat use depended on food availability or on other factors, maybe concerning desmans deeper adaptations/constraints.

Aims of the Thesis

Based on the aforementioned reasoning, this Doctoral Thesis aims to give insight into the spatial and trophic ecology of the Pyrenean desman, to gain a more detailed understanding for its conservation. The specific objectives of the thesis are:

- 1) To determine the foraging habitat preferences of the desman within its home range to identify the factors affecting its habitat suitability.
 - Test if habitat selection occurs and which habitat (riffles, runs and pools) are positively and negatively selected.
 - Check if habitat selection differs between two contrasting streams.
- 2) To test different primers for the metabarcoding-based non-invasive determination of diet and the identity of the desman itself.
 - Assess how different primers affect the characterization of a phylogenetically diverse diet and the predator identity from faecal samples.
 - Set the best primer combination to define desman diet.
- 3) To test whether desman preference for any specific habitat responds to food availability or to other reasons, comparing diet and prey availability in three habitats (riffles, runs and pools).
 - Describe desman diet at consumed species level.
 - Assess desman prey selection.
 - Analyse whether there are differences in the consumed and/or preferred prey among habitats and see if those differences explain habitat selection.
 - Check whether prey selection differs between two contrasting streams.
- 4) To test which prey traits guide foraging preferences of the desmans and to test whether that analysis could shed some light into the desman preference for riffles. To do so, diet and prey availability in three habitats (riffles, runs and pools) were compared characterizing prey using biological traits.
 - Describe desman diet using prey traits.
 - Describe desman prey selection using prey traits.

- Analyse whether there are differences in the consumed and/or preferred prey among the habitats using prey traits and see if those differences explain habitat selection.
- Check whether prey selection using prey traits differs between two contrasting streams.

Chapter 2



Need for speed:
Preference for fast-flowing water
by the endangered semi-aquatic
Pyrenean desman (*Galemys pyrenaicus*)
in two contrasting streams

Publication

An article based on this chapter was published in the journal *Aquatic Conservation: Marine and Freshwater Ecosystems*:

Esnaola, A., González-Esteban, J., Elosegi, A., Arrizabalaga-Escudero, A., & Aihartza, J. (2018). Need for speed: Preference for fast-flowing water by the endangered semi-aquatic Pyrenean desman (*Galemys pyrenaicus*) in two contrasting streams. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28(3), 600-609.

Abstract

1. The Pyrenean desman (*Galemys pyrenaicus*) is an endangered, semi-aquatic, insectivore mammal, endemic to the northern Iberian Peninsula and the Pyrenees. Owing to its small populations, evasive behaviour, and nocturnal activity, knowledge of its ecological requirements is still limited. Continuing population decline over most of its distribution range – even in regions where water quality has clearly improved – points to other factors as the main conservation threat. Nevertheless, at present there is a lack of information on its habitat preferences within its area of occupancy (e.g. stream microhabitat characteristics), a key point for assessing or improving its habitat.

2. This study used radio-telemetry data to determine the use of space at microhabitat level by the desman, and how this changed depending upon environmental conditions.

3. Desmans were studied in two contrasting rivers in the Basque Country (northern Iberian Peninsula): Elama, a nearly pristine stream, and the Leitzaran, a clean-water stream affected by hydropower diversions. Fifteen desmans were captured and radio-tracked in Elama and 16 were captured and radio-tracked in the Leitzaran, and nocturnal activity points were assigned to one of three habitat types: riffles, runs, or pools. Habitat use was compared against availability to measure habitat selection in each stream and between streams.

4. Desmans selected riffles positively and pools negatively, with this selection being stronger in the Leitzaran.

5. The results highlight the ecological relevance of riffles as foraging habitats of desmans, and therefore as key features for their conservation. It suggests that channel modifications that reduce the areal cover of riffles impair habitat quality for this species. In addition, water diversion for hydropower is likely to be detrimental for desmans, as it reduces discharge and flow velocity in the bypassed river sections.

Keywords

behaviour, endangered species, habitat management, hydropower, mammals, river, stream

Introduction

The Pyrenean desman *Galemys pyrenaicus* (Geoffroy, 1811; Figure 1) is an endangered semi-aquatic mammal endemic to the northern Iberian Peninsula and the Pyrenees (Palmerin & Hoffman, 1983), specialized for life in cold streams, with paddle-like hind feet and a long and laterally compressed tail, adapted to swimming and diving (Richard, 1986). The Pyrenean desman is protected under the Bern Convention (Appendix II) and the European Habitats Directive (Annexes II and IV) (Council of the European Communities, 1992). Its distribution area has been severely reduced during recent decades, and it is currently listed as a vulnerable species in the Red List categories by the International Union for Conservation of Nature (IUCN) (Fernandes *et al.*, 2008). The reasons behind this decline are not well known, but seem to be mainly linked to anthropogenic factors such as water pollution, habitat degradation (canalization, river-bank degradation, flow modification), and stream fragmentation (Charbonnel *et al.*, 2016; Queiroz *et al.*, 1996; Williams-Tripp *et al.*, 2012). It must be noted that the population decline occurs even in regions such as the Basque Country (Ihobe, 2011; Rubio, 2016; Ura, 2017), where pollution has abated as a consequence of improved sanitation and water-treatment schemes. This suggests that there are other potential factors causing the decline, among which the degradation of physical habitat stands out.

Desmans typically use home ranges of several hundred metres in length, and tend to have a bimodal activity rhythm, with one short bout of diurnal



Figure 1. The Pyrenean desman (*Galemys pyrenaicus*) in the Elama Stream. Photo by Jorge González-Esteban.

activity and one principal bout of nocturnal activity (Stone, 1985, 1987a). The night foraging pattern in turn shows two or three activity peaks (Melero *et al.*, 2014). It is unclear whether the desman demonstrates territorial behaviour and whether individuals share shelters independently of sex, age, or number of conspecifics sharing the home range (Melero *et al.*, 2012). Desmans usually shelter in natural crevices between rocks, in stone walls, or below the roots of riparian trees; usually there are no outwardly visible signs of the shelters from the surface (Stone, 1987a). They feed mainly on freshwater invertebrates (Biffi *et al.*, 2016; Santamarina & Guitian, 1988). Many factors can affect freshwater invertebrate abundance and composition, from pollution to drought, or changes in physical habitat (Cowx *et al.*, 1984; McIntosh *et al.*, 2002; Wood *et al.*, 2000). Invertebrates typically have a patchy distribution in rivers, with their composition and abundance differing between habitat types such as riffles, runs, and pools (Dewson *et al.*, 2007), as well as depending on sediment grain size (Elosegi *et al.*, 2011). Therefore, freshwater invertebrate abundance and diversity can decrease as a consequence of stressors such as water diversion (McIntosh *et al.*, 2002).

Charbonnel *et al.* (2015) suggested a positive influence of stream flow and substrate heterogeneity on desman occupancy, based on occupancy models applied in one French Pyrenean catchment. Desmans seem to be rheophilic, i.e. have a strong preference for fast-flowing waters, which we here call a 'need for speed'. Charbonnel *et al.* (2015) hypothesized that this preference is caused by a higher abundance and richness of invertebrates in fast-flowing reaches, whereas Richard (1986) explained it mechanically, arguing that high water velocity may help the animal to counteract its natural buoyancy. Morueta-Holme *et al.* (2010) highlighted the importance of discharge, as more water offers better habitat conditions and greater food availability. Biffi *et al.* (2016) provided information about habitat factors measured at the local scale in river stretches occupied by desmans, but did not provide information about factors controlling occupancy at the microhabitat scale: key information for understanding the needs of desman. Moreover, as habitat selection patterns change spatially according to differences in habitat availability (Ayllón *et al.*, 2010; Boyce *et al.*, 2016), it is necessary to study the behavioural response of desmans in contrasting environments to understand better the key factors shaping their habitat preferences. Most of the studies have coarse spatial resolutions, because environmental data have not been calculated in the field. In addition, almost all habitat studies for the Pyrenean desman have been based on stool detections as signs of presence (Barbosa *et al.*, 2009; Charbonnel *et al.*, 2015, 2016; Morueta-Holme *et al.*, 2010; Williams-Tripp *et al.*, 2012). None of these studies have been made using telemetry data, which could give new relevant information for individual identification and the tracking of displacements.

The aim of this study was to determine the foraging habitat preferences of the Pyrenean desman within its home range to identify factors affecting its habitat suitability. It was hypothesized that: (i) desmans prefer riffles, which are more productive in terms of prey availability (Dewson *et al.*, 2007) and are easier for foraging by a buoyant predator; and (ii) habitat preference patterns, or intensity of habitat selection, change with stream characteristics. Therefore, this study was carried out in two contrasting streams: the Elama, a nearly pristine stream, and the Leizaran, a stream affected by water diversions for hydropower.

Methods

Study area

The study was conducted in two mountain streams in the northern Iberian Peninsula (in the Basque Country; Figure 2). Both study sites are protected within the European Union Natura 2000 network (Council of the European Communities, 1992), and both are in good ecological condition according to the European Water Framework Directive (Council of the European Communities, 2000), but contrast in the environmental pressures that they experience. The Elama (Figure 3) is a second-order headwater stream draining an uninhabited basin of 1415 ha over granite and schist that has been managed strictly as a nature reserve since 1919, resulting in extensive cover of beech and oak forests (Castro, 2009). At present there is no extractive activity in the Elama basin. On the other hand, the Leizaran (Figure 4) is a fourth-order stream draining a basin of 12 402 ha over limestone, slate, and sandstones. Contrasting with Elama, in the headwaters of Leizaran there are two towns totalling 3150 inhabitants, but then the stream enters a long, uninhabited valley approximately 25 km in length where forestry and hydropower diversion schemes are the main human activities (Izagirre *et al.*, 2013). The hydropower diversion schemes consist of low weirs (2-4 m in height) that divert water from the stream channel to diversion canals several km long, and lead to hydraulic turbines before returning the water to the stream. The storage capacity of the reservoirs above the weirs is very small, but the proportion of water diverted is high. Most hydropower concessions must leave an environmental flow, which is usually set at 10% of the average flow calculated for each month (BOE, 2016). Nevertheless, some old concessions still have no environmental flow requirements, and thus practically dry out entire stream sections. Hydropower plants in the area tend to operate throughout most of the year, stopping only in summer or early autumn for maintenance operations, or when the water authorities compel them to ensure environmental flows (Iñaki Bañares, Province Government of

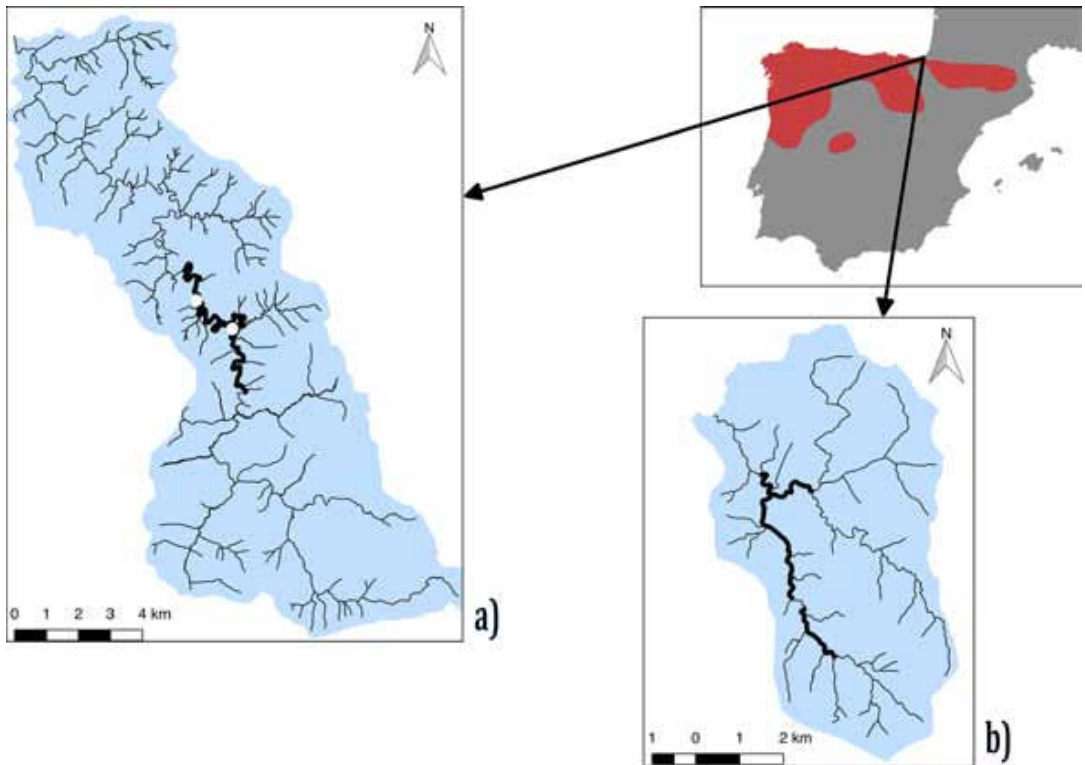


Figure 2. Study area: (a) Leitzaran and (b) Elama streams in their basins. The study section of each stream has been depicted with bolder black lines. The white dots mark the dams present in the study sections. The distribution of the Pyrenean desman in the Iberian Peninsula has been represented in red (Fernandes *et al.*, 2008).

Gipuzkoa, pers. comm., September 2017). Hydropower schemes are typically set in close succession, with diversion weirs being located almost immediately below the outflow from hydropower turbines. Therefore, in streams affected by hydropower, the bypassed sections are much more abundant than sections with natural discharge. In the mid- and low-Leitzaran stream, over 70% of the main-stem channel length is bypassed by diversion canals, and an additional 4% is converted into slow-flowing areas above weirs (Izagirre *et al.*, 2013).

The research was carried out on a 4-km section of the Elama Stream (from 43°12'40"N, 1°48'36"W to 43°11'14"N, 1°48'4"W; mean altitude, 330 m; mean width 7.08 m), and on a 10-km section of the Leitzaran Stream (from 43°8'57"N, 1°57'26"W to 43°6'55"N, 1°56'9"W; mean altitude, 290 m; mean width, 12.42 m) (Figure 2). The section lengths were different because of the greater difficulty in trapping desmans in the Leitzaran Stream.



Figure 3. The Elama Stream, Basque Country. Photo by Amaïur Esnaola.

Habitat availability and characteristics

Both streams were surveyed in September and October 2016 by walking the entire sections with the aid of a GPS and topographic maps. Three habitat types were identified, following Overton *et al.* (1997): riffles with fast, turbulent water, uneven surface level, and white water; runs with close to laminar flow and even depth; and pools with slow flow on riverbed depressions.

Each stream section was divided into subsections according to the dominant habitat type: riffle, run, or pool. Boundaries of habitat types were recognized

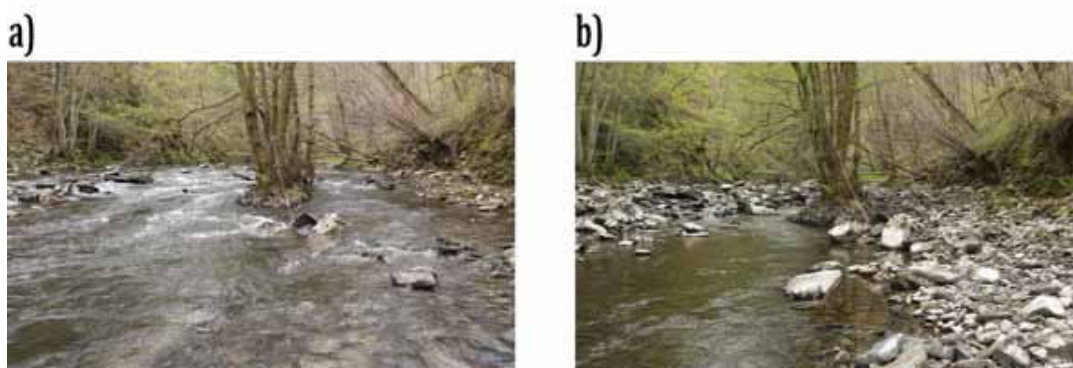


Figure 4. The Leitzarain Stream, Basque Country: (a) a stream stretch occupied by desmans with its natural flow (21 April 2016); (b) the same stream stretch 5 days later (25 April 2016), when a hydroelectric plant diverted most of the discharge. The contrast of both images shows the effect that water diversion has on the distribution of hydromorphological habitats, especially the reduction of both total wetted area and riffle area. Photos by Jorge González-Esteban.

by identifying the breaks in stream channel slope along the thalweg of the channel bottom. Each habitat type was characterized by 10 transversal transects conducted for each section. More precisely, in each transect the wetted width was measured, and at regular intervals (0.5 m in the Elama; 1 m in the Leitzarán) the depth and water velocity at 0.6 of maximum depth (current meter Martin Marten Z30, Barcelona, Spain) were measured. Substrate was also characterized by visually assigning the bottom to size categories: sand, gravel, pebble, cobble, boulder, and others (rock, bedrock, or organic) (Díez *et al.*, 2000). In these surveys, the study sections were marked with plastic tape every 25 m to improve the GPS location.

Desman capture and tracking

This research was performed outside of the desman mating and breeding season (from January to August; Palmerin & Hoffman, 1983) to avoid disturbing pregnant females or females with cubs. Desmans were captured in September and October 2016 using wire-mesh traps placed partially submerged in the streams during the night, and checked every 3 h to minimize stress (González-Esteban *et al.*, 2003a). Two trapping sessions were carried out in each stream, with 12 traps used per night, and with each trapping session lasting two or three nights. Captures were less frequent in the Leitzarán, where a greater trapping effort was required to capture a similar number of individuals (four capture nights in the Elama versus six capture nights in the Leitzarán).

The age and sex of captured desmans were determined visually (González-Esteban *et al.*, 2003b; González-Esteban *et al.*, 2002), the hair on their back (approximately 1 cm²) was trimmed with scissors, radio-transmitters (0.75 g in weight, model A2435, Advanced Telemetry Systems, <https://atstrack.com>) were glued with Dermabond™® and animals were set free where they were captured. The process typically lasted 15-20 min per animal.

Tagged desmans were tracked for five nights, as a compromise to obtain enough information about the activity of each individual in the shortest possible time. This also prevented the radio-transmitters from falling off and avoided the occurrence of major environmental changes (such as strong weather disturbances) during the tracking period, which could distort the results. Radio-tracking was carried out on foot using two types of radio receivers and antennae (TRX1000S, Wildlife Materials Inc., Carbondale, IL, USA, with hand-held three-element Yagi antennae; AOR AR8200 Mark III B, Universal Radio Inc., OH, USA, with H-type antenna). Desmans were tracked as close as possible to identify their activity overnight. Periods of activity and rest were distinguished from the variations in radio signal. To minimize spatial autocorrelation, successive

position recordings, i.e. fixes, were taken at least 5 min apart, as in that time each individual could reach any habitat type (Stone, 1987a). Resting locations were later excluded from the analysis.

Desman capture and handling protocols met the guidelines for treatment of animals in research and teaching (Animal Behaviour Society, 2012). The study met local legal requirements and was approved by the Ethics Committee for Animal Welfare of the University of the Basque Country (ref. CEBA/M20/2016/022).

Data analysis

Habitat information and radio-tracking fixes were transferred to a GIS system (ArcView 3.2; ESRI, Redlands, CA, USA). Individual habitat availability and home range (length in m, area in m²) were determined, calculating preferential foraging areas with the Animal Movement 2.0.β extension for ArcView 3.2 (Hooge & Eichenlaub, 1999), considering only active locations. Preferential foraging areas were determined by kernel home ranges (Kernel Density Estimation (KDE) 95%), which are based on the probability of use derived from the number and spatial arrangement of locations, and the relative length of time an animal spends in a given area (Worton, 1989). Kernels were calculated by the fixed kernel method and the smoothing factor was estimated by means of least-squares cross-validation. Differences in home ranges (KDE95%; in length and in area) and in available riffle, run, and pool areas (from KDE95%) between study sites were tested statistically with the nonparametric Mann-Whitney *U*-test.

Independence among habitat availability and use was tested with χ^2 goodness-of-fit (log-likelihood ratio G). Habitat types were ranked in order of relative preference according to their average selection ratio w_i (Manly *et al.*, 2002): the average of the ratios of the observed proportion of each habitat type used by each animal to the known proportion of each habitat type available to the population, for the whole population of animals in each stream. To assess the selection or rejection of a given class of habitat, Bonferroni's confidence intervals were constructed, following Manly *et al.* (2002), to estimate habitat availability and use individually. Comparisons between streams were made using the average selection ratios, taking into account Bonferroni's confidence intervals. For all tests, α was set to 0.05 corrected by the number of simultaneous comparisons. The confidence intervals were computed at the 95% level, also corrected by the number of simultaneous comparisons. Individual selection was represented by one habitat selection diagram for each stream (SOILTEXTURE package in R).

Results

Habitat availability and characteristics

In the Elama, riffles and runs were similarly available (proportions of 0.48 and 0.49, respectively; Table 1), whereas runs dominated the Leitzaran (0.62; Table 1). Pools were the least abundant habitat in both streams (0.03 in the Elama, 0.11 in the Leitzaran; Table 1). In general, riffles were slightly narrower, considerably shallower, and much faster than runs and pools. Water depth was in general greater in the Leitzaran, whereas overall differences in water velocity among streams were small. In the Elama, boulders prevailed in riffles, cobbles in runs, and other types of substrates prevailed in pools. In the Leitzaran, boulders dominated in riffles and runs, and other types of substrates dominated in pools.

Captures

Thirty-one desmans were captured and successfully tracked: seven female and eight male desmans in the Elama, and 10 females and six males in the Leitzaran (Table 2).

Home range size and habitat selection

Overall, more than 200 activity points were recorded for each individual, and animals were followed for more than four nights (Figure 5; Table 2). Length of home ranges (KDE95%) did not differ significantly between streams (length of individual foraging area, LIFA; $w = 135$, $P = 0.5665$), but their area was significantly larger in the Leitzaran than in the Elama (area of individual foraging area, AIFA; $w = 26$, $P < 0.0001$; Table 2). Although riffles covered a smaller proportion of the stream channel in the Leitzaran, the total area of riffles within home ranges was higher there (KDE95%; $w = 29$, $P = 0.0003$; Table 2), as well as the total area of runs (KDE95%; $w = 63$, $P = 0.0255$; Table 2), because this stream was significantly larger. There were no significant differences between the total area of pools (KDE95%; $w = 109$, $P = 0.6663$; Table 2).

In the Elama, 63.7% of the active locations were recorded in riffles, 33.1% in runs, and only 3.2% in pools (Table 3). Riffles were used disproportionately to their availability (i.e. there was positive habitat selection; $X^2 = 361.2$, d.f. = 30, $P < 0.0001$) and runs were selected against, whereas the data were not

Table 1. Habitat characteristics in the Elama and Leitizaran streams.

Stream	Habitat	Availability	Channel width (m) mean ± SD	Depth (cm) mean ± SD	Water velocity (ms ⁻¹) mean ± SD	Sand (%) ± SD	Gravel (%) ± SD	Pebble (%) ± SD	Cobble (%) ± SD	Boulder (%) ± SD	Other (%) ± SD
Elama	Riffle	0.48	6.7 ± 2.4	9.13 ± 2.78	0.31 ± 0.13	0.8 ± 2.6	5.7 ± 8.1	10.1 ± 10.1	28.5 ± 20.3	32.6 ± 20.6	22.2 ± 29.8
	Run	0.49	7.4 ± 1.3	16.79 ± 4.10	0.09 ± 0.03	6.7 ± 9.7	18.6 ± 13.2	19.0 ± 15.8	29.6 ± 13.2	21.8 ± 16.7	4.3 ± 6.6
	Pool	0.03	7.1 ± 2.8	57.74 ± 16.59	0.03 ± 0.02	18.6 ± 15.8	9.4 ± 8.6	12.4 ± 10.9	14.3 ± 16.1	14.3 ± 11.7	31.0 ± 19.4
Leitizaran	Riffle	0.27	11.5 ± 2.8	11.28 ± 2.81	0.36 ± 0.11	0	2.5 ± 7.9	0	25.1 ± 9.1	65.3 ± 14.5	7.1 ± 12.8
	Run	0.62	12.1 ± 2.0	28.86 ± 9.95	0.07 ± 0.04	5.6 ± 9.1	12.0 ± 10.2	5.7 ± 8.9	20.3 ± 21.6	34.7 ± 28.6	21.6 ± 23.7
	Pool	0.11	13.6 ± 4.7	73.56 ± 27.01	0.02 ± 0.02	2.5 ± 4.3	12.9 ± 10.7	7.7 ± 9.6	19.1 ± 21.1	14.1 ± 13.9	43.6 ± 27.5

'Availability' shows the proportion of each habitat type in the entire study section of each stream.

Table 2. Details of animals followed in the Elama and Leitizaran streams.

Stream	Desman capture nights	Tracked desmans	Active location points:		Tracking nights: mean ± SD	LIFA (m) mean ± SD	AIFA (m ²): mean ± SD	Riffle area (m ²): mean ± SD	Run area (m ²): mean ± SD	Pool area (m ²): mean ± SD	
			Females	Males							
Elama	4	15	7	8	268.9 ± 44.9	4.73 ± 0.57	328 ± 72	2215.6 ± 345.3	1103.3 ± 394.7	1038.7 ± 494.0	73.6 ± 78.6
Leitizaran	6	16	10	6	200.4 ± 32.9	4.19 ± 0.83	368 ± 231	6176.9 ± 5201.9	2379.3 ± 1298.1	3295.2 ± 3497.8	502.5 ± 761.9

LIFA (KDE95%), length (m) of individual foraging area (kernel 95%); AIFA (KDE95%), area (m²) of individual foraging area (kernel 95%); Available riffle, run, and pool mean areas (kernel 95%) are also included.

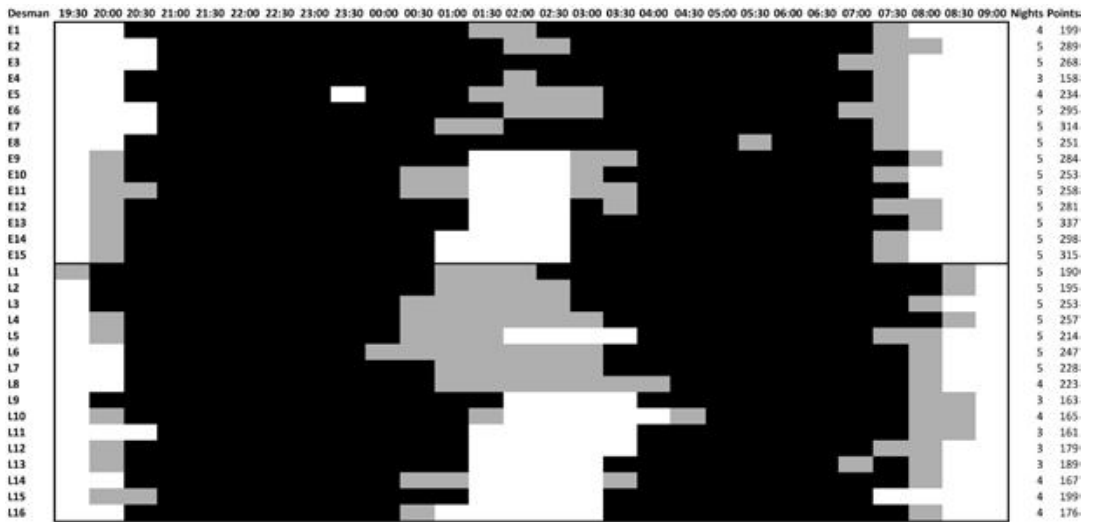


Figure 5. Periods of time each animal was radio-tracked during the night: black areas represent activity periods, grey areas represent inactivity periods, and white areas show radio-tracking lapses. The codes in the ‘Desman’ column refer to individual animals in the two streams (E, Elama; L, Leitzarar). The number of tracking nights that each animal has been followed (‘Nights’) and the number of active location points recorded for each individual (‘Points’) are also indicated in columns on the right.

Table 3. Habitat use, Bonferroni’s confidence intervals of habitat use, habitat availability, and average selection ratio (Manly et al., 2002) in the Elama and Leitzarar streams.

Stream	Habitat	Habitat use u_i	Bonferroni’s confidence intervals for the proportions of habitat use (α_i)		Habitat availability (KDE95%) Π	Selection	Bonferroni’s confidence intervals for the average selection ratio (\hat{w}_i)	
			Lower	Upper			Lower	Upper
Elama	Riffle	2176	0.615	0.659	0.502	Positive	1.265	1.347
	Run	1130	0.310	0.352	0.466	Negative	0.642	0.729
	Pool	110	0.024	0.040	0.033	Null	0.864	1.308
Leitzarar	Riffle	2289	0.818	0.856	0.490	Positive	1.626	1.719
	Run	406	0.130	0.166	0.441	Negative	0.293	0.396
	Pool	40	0.009	0.021	0.068	Negative	0.046	0.384

‘Habitat use’ shows the successive active position recordings – location points or fixes – taken with time lapses of 5 min or greater. ‘Habitat availability’ shows the proportion of each habitat type in each stream (kernel 95%).

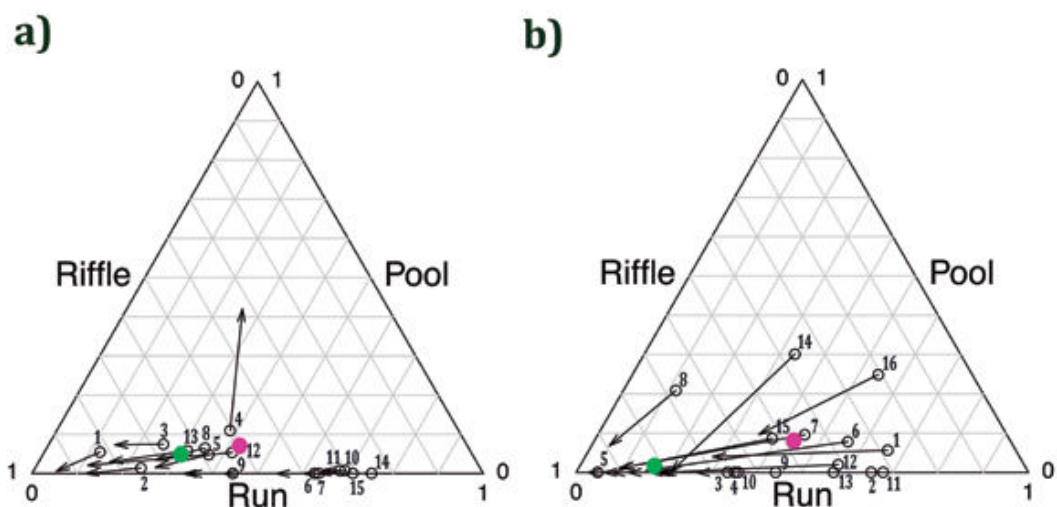


Figure 6. Habitat selection diagrams in the Elama (a) and Leitzarán (b) streams. Circles mark the combination of habitats available for each animal (taking into account the proportion of available habitat between 0 and 1 for each microhabitat), whereas the arrows point to the habitat combination used (taking into account the proportions of habitat used between 0 and 1 for each microhabitat). Pink dots mark the mean habitat availability and green dots mark the mean habitat use in each stream section.

statistically significant for pools (Table 3). In the Elama, one desman did not follow this general pattern and positively selected pools instead (Figure 6).

In the Leitzarán, 83.7% of the active locations were recorded in riffles, 14.8% in runs, and 1.5% in pools. Riffles were selected positively ($\chi^2 = 819.9$, d.f. = 32, $P < 0.0001$), and runs and pools were selected negatively (Figure 6; Table 3). Overall, habitat selection was stronger in the Leitzarán than in the Elama, as shown by a higher average selection ratio (1.70 *versus* 1.26) and a greater distance between the pink and green dots in Figure 6.

While out of their burrows, desman movements showed uneven patterns. Commonly, individuals ranged every night along a stretch of a few hundred metres in length, concentrating their foraging activity for several minutes in specific areas (generally riffles), before moving on to the next foraging spot. However, some animals moved swiftly some hundreds of metres from the nest to a riffle in a few minutes, and then remained there for 1-3 h. In those ‘hot spots’, changes in the intensity of radio signals suggested constant movement but restricted to a few metres. The long stays happened especially in riffles where the stream channel branched and where there were large boulders or fallen logs, which trapped branches and leaf litter.

Discussion

The present research shows the Pyrenean desman to select riffles positively and pools negatively. It also shows that the intensity of habitat selection changes among streams, with selectivity being higher in the stream that was affected by hydropower schemes. These results add valuable information about the factors likely to affect desman habitat quality, and on potential management actions for improving the conservation status of this endangered mammal.

Human pressures such as habitat destruction and water pollution are blamed for the population decline of the Pyrenean desman (Queiroz *et al.*, 1996; International Union for Conservation of Nature (IUCN), 1995). In particular, local extinctions have been attributed to artificial barriers to dispersal, such as dams or highly altered reaches in a dendritic network where biogeographic barriers already abound (Morueta-Holme *et al.*, 2010). Comparing the historical with the present distributions of the species in France, Charbonnel *et al.* (2016) concluded that the range contraction observed is larger than expected from the decrease in habitat suitability caused by climate, and hydrological and land-use changes. They speculated that the decline may be caused by additional factors such as habitat fragmentation, pollution, changes in prey availability, or invasive species (e.g. the American mink *Neovison vison*) preying on the desman, but gave no suggestions on the relative importance of this list of potential factors. Therefore, this list is still too generic a foundation for conservation, which is reflected in the paucity of conservation actions tailored to the desman. As an example, both the French Conservation Action Plan for the Pyrenean Desman (Némoz & Bertrand, 2008) and the Spanish Desman Conservation Strategy (Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA), 2013) include a wealth of actions on research and science, but almost no specific actions devoted to habitat improvement.

Stream flow velocity has been reported as an important environmental factor for the Pyrenean desman (Charbonnel *et al.*, 2015). Biffi *et al.* (2016) performed an Ecological Niche Factor Analysis (ENFA) based on the detection of faeces. They concluded that the habitat of the Pyrenean desman is characterized by in-stream variables (substrate heterogeneity, fast-flowing water, absence of fine sediment), as well as by bank characteristics (rocky banks), with desmans preferring sites with abundant emergent structures and a high heterogeneity of shelters. Their results might be strongly biased, however, because these reaches are precisely the type where desman faeces are more detectable. In reaches without emerging structures desmans tend to deposit their faeces in bank refugia, where they are hardly detectable, resulting in false absences (Charbonnel *et al.*, 2015; González-Esteban *et al.*, 2003a; Nores *et al.*,

1999). These research studies can only indicate the type of streams where desman faeces are more easily detectable, and do not show how desmans use the space in the streams that they inhabit. Our data partly fills this gap, showing a consistent need for speed, i.e. a strong preference for riffles and a clear avoidance of pool habitat, thus confirming the first hypothesis. Only one individual showed a preference for pools. After close inspection of the home range of this individual, we could find no clear explanation for its behaviour. It might have resulted from some patches of high prey availability, but, given the precision of the locations, this hypothesis could not be tested without engaging in highly destructive benthos sampling.

Apart from this one individual, the rest showed preference for swift microhabitats, probably related to food, shelter, or hydraulics. Regarding food, riffles have been reported to harbour higher invertebrate diversity and biomass than stream habitats with slower water velocities (Dewson *et al.*, 2007; Hussain & Pandit, 2012). They also seem to harbour higher secondary production (Buffagni & Comin, 2000; Polis *et al.*, 1997), although the trend is far from universal (Wohl *et al.*, 1995). Alternatively, riffles may offer better shelter than either runs or pools because typically they have more emerging boulders, among which the desman can hide when eating the prey captured under water (Niethammer, 1970), or perhaps the boulders provide protection for desmans against rapid increases in flow (either natural or artificial). In addition, the fast water current in riffles may make it easier for desmans to overcome their natural buoyancy, as they often crawl on the bottom, using their strong claws to take hold (Richard, 1986).

The second hypothesis was also confirmed, as desmans used the habitat differently depending on the environmental conditions of the stream. Although animals can be expected to be less selective in lower quality areas, where they are forced to use suboptimal habitat in order to survive (Goiti *et al.*, 2003; but see Garshelis, 2000, or Railsback *et al.*, 2003), the opposite was observed. The pattern found for the desman may result from the least preferred habitats being worse in the Leitzarán, the stream affected by water diversions for hydropower, or reflect a decrease in selectivity in the Elama, as a consequence of a higher population density. In fact, the difficulty in capturing the same number of animals in the Leitzarán suggests a lower density of the population there. It is worth noting that foraging ranges were longer in the Leitzarán than in the Elama, which emphasizes either poorer habitat suitability or larger population pressure in the Leitzarán. Moreover, the smaller foraging ranges observed in the Elama suggest that the use of pools in this river was not a consequence of a higher population pressure and intraspecific competition. Thus, the overall results indicate that the less preferred habitats in the Elama were not as unsuitable

as in the Leitzaran, either in prey availability or in physical characteristics (e.g. shallower depth, slower flow).

Whatever the reason, the results show that the Pyrenean desman positively selects riffles rather than runs or pools, and more so in rivers affected by water diversion. Water diversion reduces the wetted channel (Stanley *et al.*, 1997), alters hydraulics (McIntosh *et al.*, 2002), reduces the populations of benthic invertebrates (James *et al.*, 2008), and exerts impacts on stream ecosystem functioning (Arroita *et al.*, 2017). The results suggest that water diversion can also have detrimental effects on the habitat of the endangered Pyrenean desman by reducing the availability of its preferred microhabitat. Aymerich (2004) proposed that the artificial reduction of flow regime (e.g. as a result of hydropower production or irrigation) is likely to have an adverse impact on the Pyrenean desman, especially in mountain rivers that are more sensitive to flow reduction. In addition, desmans are observed at times in the diversion canals of hydropower schemes (MAGRAMA, 2013), probably when animals travelling downstream in search of new territories follow the canal instead of the water-scarce stream channel. It is unclear what happens to these animals, but dying in the turbines is not an unlikely outcome (Elosegi, 2010). Reduced flows in general, and water diversion in particular, are also known to have detrimental effects on other stream species, including salmonids (Bradford & Heinonen, 2008; Heggenes *et al.*, 1996) and the dipper (Chen & Wang, 2010), a genus of birds that also favours riffles (Logie *et al.*, 1996) and apparently exploits a trophic niche similar to that of desmans (Santamarina, 1993).

As for the management implications of the present findings, we suggest that environmental flow allocations should be revisited in streams with desman, especially in those such as the Leitzaran Stream that are designated as Special Areas of Conservation (SACs) within the Natura 2000 network. Instead of relying on the simple hydrological formulae used at present, specific hydraulic studies should be undertaken to determine how discharge affects the abundance and distribution of riffles in each bypassed section, and to discern whether there is any tipping point that should not be crossed, i.e. a threshold of discharge diverted beyond which habitat availability falls rapidly. Arroita *et al.* (2017), using experimental flow manipulation in a stream similar to the Elama, demonstrated that environmental flows, as currently defined in the Basque Country, still severely reduce flow velocity and riffle abundance, thus strongly affecting stream ecosystem functioning. Therefore, it is likely that the very abundant diversion schemes in headwater streams in the Basque Country (Ente Vasco de la Energía (EVE), 1995), and elsewhere, are severely affecting desman habitat quality.

In addition to environmental flows, management actions that reduce riffle availability should be avoided. In the province of Gipuzkoa alone, where the Leitzaran Stream is, there are more than 900 low dams and weirs in an area of less than 2000 km² (BOE, 2016; EVE, 1995). Most of these structures are legacies of past activities such as mills or iron foundries, and collectively create large areas of stagnant water, probably of little use for desmans as pools. These weirs should be taken down where possible, preserving only those currently in use or with heritage value. Channelization has also been described as detrimental to desman habitats (MAGRAMA, 2013), although with little empirical evidence. The present results suggest that channelization can be especially detrimental when it reduces flow velocity and heterogeneity, which is common in engineered channels with trapezoidal cross sections designed to increase flood conveyance capacity. Among restoration activities that might improve desman habitat is the introduction of large wood in the river, especially forming deflectors to create riffle-like areas, as has often been used to improve salmonid habitat (Nagayama & Nakamura, 2010).

The present research points to the availability of fast-flowing riffle areas as a key factor in habitat quality for the Pyrenean desman, thus highlighting specific management activities that could improve the conservation status of this speed-needing species. Given the highly fragmented characteristics of the desman population, these actions should, most likely, be combined with others, such as genetic studies and individual relocation, if the endangered status of this species is to be improved.

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Chapter 3



Determining diet from faeces:
Selection of metabarcoding primers
for the insectivore Pyrenean desman
(*Galemys pyrenaicus*)

Publication

An article based on this chapter was published in the journal *PLoS ONE*:

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Abstract

Molecular techniques allow non-invasive dietary studies from faeces, providing an invaluable tool to unveil ecological requirements of endangered or elusive species. They contribute to progress on important issues such as genomics, population genetics, dietary studies or reproductive analyses, essential knowledge for conservation biology. Nevertheless, these techniques require general methods to be tailored to the specific research objectives, as well as to substrate- and species-specific constraints. In this pilot study we test a range of available primers to optimise diet analysis from metabarcoding of faeces of a generalist aquatic insectivore, the endangered Pyrenean desman (*Galemys pyrenaicus*, É. Geoffroy Saint-Hilaire, 1811, Talpidae), as a step to improve the knowledge of the conservation biology of this species. Twentyfour faeces were collected in the field, DNA was extracted from them, and fragments of the standard barcode region (COI) were PCR amplified by using five primer sets (Brandon-Mong, Gillet, Leray, Meusnier and Zeale). PCR outputs were sequenced on the Illumina MiSeq platform, sequences were processed, clustered into OTUs (Operational Taxonomic Units) using UPARSE algorithm and BLASTed against the NCBI database. Although all primer sets successfully amplified their target fragments, they differed considerably in the amounts of sequence reads, rough OTUs, and taxonomically assigned OTUs. Primer sets consistently identified a few abundant prey taxa, probably representing the staple food of the Pyrenean desman. However, they differed in the less common prey groups. Overall, the combination of Gillet and Zeale primer sets were most cost-effective to identify the widest taxonomic range of prey as well as the desman itself, which could be further improved stepwise by adding sequentially the outputs of Leray, Brandon-Mong and Meusnier primers. These results are relevant for the conservation biology of this endangered species as they allow a better characterization of its food and habitat requirements.

Keywords

COI gene, diet, endangered species, molecular ecology, next generation sequencing, predator-prey interactions

Introduction

The diet of many consumers is difficult to determine: direct observation can be methodologically challenging and visual identification of prey remains in faeces difficult (Clare *et al.*, 2009), especially in the case of generalist feeders, which prey upon a large variety of species. Consumers rarely forage at random and prey choice can be affected by prey defence and escape strategies, by nutritional quality, or by spatial and temporal distributions of predators and prey, among other factors (Symondson, 2002). New molecular tools such as DNA metabarcoding allow non-invasive studies of diet, as well as assignation of the consumer species that produced a scat, thus preventing identification errors. These new techniques allow analysing the environmental DNA (eDNA) extracted from faeces (Bohmann *et al.*, 2014; Sheppard & Harwood, 2005; Taberlet *et al.*, 1999) and the identification of both soft- and hard-bodied prey to species level, what was impossible by means of traditional morphological techniques (Razgour *et al.*, 2011). Furthermore, DNA metabarcoding facilitates high-resolution dietary analyses further disclosing trophic and habitat requirements of consumers and providing an invaluable tool to unveil food web structures (Pompanon *et al.*, 2012), particularly useful for elusive and endangered species (Casper *et al.*, 2007; Greenstone *et al.*, 2005; Piggot & Taylor, 2003; Valentini *et al.*, 2009). These innovative techniques make diet studies useful for species conservation. For instance, a cost-effective screening of multiple DNA metabarcodes in faeces detected a broad diversity of plants (99 taxa) in the diet of the vulnerable Italian hare (*Lepus corsicanus*), including items that leave no solid remains or that lack diagnostic taxonomic features (Buglione *et al.*, 2018). Similarly, Schwarz *et al.* (2018) documented dietary differences consistent across site and year in the diet of male and female harbor seals (*Phoca vitulina*), likely affecting commercial prey such as salmon. Moreover, the foraging ecology of the Alpine long-eared bat *Plecotus macrobullaris* was inferred from the molecular analysis of faeces and from the ecological requirements of prey, which would be impossible by traditional radio-tracking methods (Alberdi *et al.*, 2012). Furthermore, the analysis of the prey consumed showed that the Mediterranean horseshoe bat *Rhinolophus euryale* does not only rely on the habitats where it directly hunts, but also on other habitats of vital importance for its prey's larval stages, where bats do not forage (Arrizabalaga-Escudero *et al.*, 2015). Results like these help setting up guidelines for species management.

High throughput sequencing methods are effective when applied to the dietary analyses of predators (Clare *et al.*, 2013) since they enable the examination of very degraded, fragmented and different DNA pieces without previous knowledge of prey identity (Clare *et al.*, 2009; Valentini *et al.*, 2009; Pompanon

& Samadi, 2015). However, some methodological constraints must be taken into account. On the one hand, DNA quality is affected by its transition time across the gut, as well as the exposure of scats to environmental conditions (e.g. temperature oscillations, sun, rain, humidity and fungal attacks) (Deagle *et al.*, 2010; Oehm *et al.*, 2011; Symondson, 2002). On the other hand, the high sensitivity of molecular methods may produce abundant false positives as a consequence of secondary predation, scavenging, or contamination from any source (Sheppard *et al.*, 2005). Finally, the completeness of diet characterization depends on methodological details such as the taxonomic coverage of primers, the spectrum of consumed prey species, PCR strategy, sequencing workflows, bioinformatics decisions or the information available in databases (e.g. Alberdi *et al.*, 2018; Brandon-Mong *et al.*, 2015; Divoll *et al.*, 2018).

Studies that target terrestrial animals usually rely on markers within the mitochondrial cytochrome c oxidase subunit I (COI) region, since it has a high copy number and variation suitable for allowing species-level identification (Elbrecht & Leese, 2017; Hebert *et al.*, 2003). Indeed, the COI has one of the most complete reference databases nowadays, particularly well represented for many invertebrate taxa in GenBank (<http://www.ncbi.nlm.nih.gov>) and BOLD System (<http://www.boldsystems.org/>). Nevertheless, even though the COI is short enough to be identified in fairly fragmented DNA sequences, for very fragmented sequences such as those usually found in faeces, shorter markers have been used successfully (e.g. Deagle *et al.*, 2006). The choice of the markers is usually guided by DNA reference databases, but also depends on the research question (Creer *et al.*, 2016). In this sense, Alberdi *et al.* (2018) showed that the results of dietary analyses depend on the selection of marker regions, amplicon sizes, primers, as well as the taxonomic level required. While various primers do detect some prey species, detection of others is primer-dependant. If too long regions are amplified, some species will not be identified, resulting in false negatives. This effect will inflate the difference between samples or individuals, thus yielding skewed information about intraspecific variability. Therefore, the selection of the marker region and primers is a critical decision in any DNA metabarcoding study, as factors such as primer length or specificity have a great effect on the results (Elbrecht & Leese, 2017; Gibson *et al.*, 2014).

False positives and false negatives in dietary studies can have strong implications in the interpretation of predator ecology, as well as in ecosystem management (Hines & Gessner, 2012). Successful identification of relevant prey taxa is key to obtain sound conclusions about the ecological role, trophic specialization and conservation of any predator—or their consumed prey (Schwarz *et al.*, 2018; Waraniak *et al.*, 2018)—. Besides, the need for simultaneous identification of diet and predator identity from faeces makes methodological decisions more demanding, as a broader phylogenetic spectrum must be targeted.

Here we conducted a DNA metabarcoding study to compare different primers for noninvasive determination of the diet and the identity of an aquatic predator. In particular, we aimed at assessing how different primer pairs—or their combinations—affect both the characterization of a phylogenetically diverse diet and the predator identity.

We chose as a model species the Pyrenean desman (*Galemys pyrenaicus*, É. Geoffroy Saint- Hilaire, 1811, Insectivora, Talpidae) a semi-aquatic insectivorous generalist mammal that lives in cool and clean mountain streams (Fig 1). It is endemic to the northern Iberian Peninsula and the Pyrenees, but its distribution area has been severely reduced during the last decades, being currently listed as Vulnerable in the Red List Categories by the IUCN (Fernandes *et al.*, 2008). Recent research has shown the desman to feed mainly on freshwater invertebrates (Biffi *et al.*, 2017a; Hawlitschek *et al.*, 2018) and to prefer riffles to runs or pools (Chapter 2; Esnaola *et al.*, 2018b). Even so, there is still a lack of information to adequately manage this endangered species (MAGRAMA, 2013; Némoz & Bertrand, 2008). Namely, it is still unknown to which extent the diet of desmans depend on prey availability, and which types of prey they select for; moreover, it is still unclear whether desmans' reported habitat selection within



Figure 1. A Pyrenean desman in the Elama Stream. Photo by Joxerra Aihartza.

rivers (Chapter 2; Esnaola *et al.*, 2018b) reflects differences in prey availability or other factors. These and other questions ask for detailed diet studies.

Our study provides valuable information about differences in primer efficiency when using DNA metabarcoding in diet studies, offering an overview of the taxonomic coverage provided by each primer set, as well as by their combinations. Moreover, regarding desman ecology, our results will be useful to better understand and compare the output of published studies (e.g. Biffi *et al.*, 2017a; Hawlitschek *et al.*, 2018), as well as to unveil the habitat requirements of this endangered species. This information is necessary to design and implement specific management actions for its conservation.

Table 1. Location of analysed faecal samples.

Basin	River	Number of faeces	Collected in artificial shelters ^a	Coordinates ^b
Urumea	Asura	3	No	43°08'03"N 1°48'10"W
				43°07'46"N 1°47'07"W
				43°08'21"N 1°49'39"W
Ollin		3	No	43°07'44"N 1°51'01"W
				43°07'44"N 1°51'01"W
				43°07'37"N 1°51'00"W
Añarbe		3	No	43°13'09"N 1°51'17"W
				43°13'18"N 1°51'05"W
				43°13'18"N 1°51'05"W
Elama		9	Yes	43°12'37"N 1°48'38"W
				43°12'37"N 1°48'38"W
				43°12'37"N 1°48'38"W
				43°10'59"N 1°47'59"W
				43°10'59"N 1°47'59"W
				43°10'58"N 1°47'56"W
				43°10'58"N 1°47'56"W
				43°10'57"N 1°47'56"W
				43°10'57"N 1°47'56"W
Oria	Leitzaran	6	Yes	43°08'59"N 1°57'19"W
				43°08'59"N 1°57'19"W
				43°08'59"N 1°57'19"W
				43°08'59"N 1°57'18"W
				43°09'00"N 1°57'19"W
				43°09'00"N 1°57'19"W

^a Indicates whether samples have been collected in artificial shelters (Yes) or elsewhere (No).

^b Indicates the exact position of the localities where the samples were collected.

Materials and methods

Sample collection

We searched for faeces of the Pyrenean desman in the basins of the Urumea and Oria rivers, in the Basque Country (Northern Iberian Peninsula), between November 2015 and April 2016, either by prospecting rock crevices and roots, or by building artificial shelters (Table 1) specially designed for this species (Appendix 1; González-Esteban *et al.*, 2018). A total of 24 droppings were preserved in 98% ethanol and frozen at -80°C.

Table 2. Details of the five primer sets used in this study.

Custom name	Primer names	Forward primer(s) (5'-3')	Reverse primer(s) (5'-3')	Length (bp) ^a	Source
Brandon-Mong	F:LepF1	ATCAACCAATCATAAAGA	CGTGGAAWGCTATATC	218	Brandon-Mong <i>et al.</i> , 2015
	R:MLepF1_Rev	TATTGG (25bp)	WGGTG (21bp)		
Gillet	F:LepF1 (modified)	ATTCHACDAAYCAYAARGA	ACTATAAAARAAAATYTD	133	Gillet <i>et al.</i> , 2015
	R:EPT-long-univR (modified)	YATYGG (25 bp)	AYAADGCRTG (29 bp)		
Leray	F:mICOintF	GGWACWGGWTGAACWG	TAICYTCIGGRTGICCR	313	Leray <i>et al.</i> , 2013
	R:jgHCO2198	TWTAYCCYCC (26 bp)	ARAYCA (26 bp)		
Meusnier	F:Uni-MinibarF1	TCCACTAATCACAARGATAT	GAAAATCATAATGAAGGC	130	Meusnier <i>et al.</i> , 2008
	R:Uni-MinibarR1	TGGTAC (26 bp)	ATGAGC (24 bp)		
Zeale	F:ZBJ-ArtF1c	AGATATTGGAACWTTATATT	WACTAATCAATTWCCAAA	157	Zeale <i>et al.</i> , 2011
	R:ZBJ-ArtR2c	TTATTTTTGG (30bp)	TCCTCC (24bp)		

^a Amplicon size excluding primers (bp = base pair).

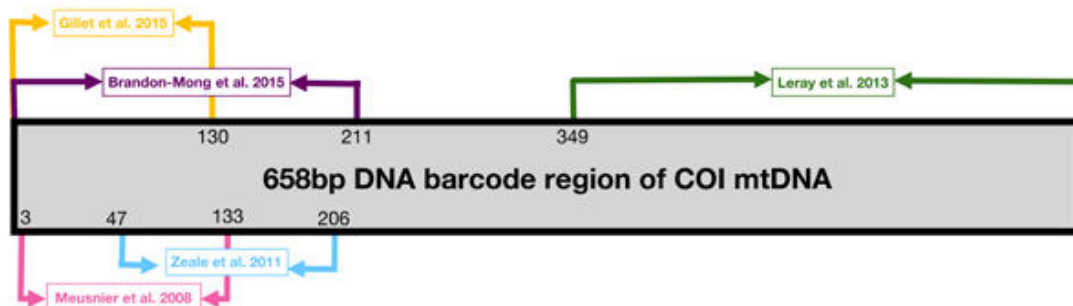


Figure 2. Primer locations. Visual representation of the marker locations in the mitochondrial COI (modified from Brandon-Mong *et al.*, 2015).

This study is part of broader research on the spatial and trophic ecology of the Pyrenean desman, which met local legal requirements and was approved by the Ethics Committee for Animal Welfare of the University of the Basque Country (Ref. CEBA/M20/2016/022). No specific permissions were required for the activities carried out in this study, as samples were not invasively collected and did not involve manipulation of endangered or protected species.

Selection of universal primers

We selected five primer sets (Table 2) aiming at a broad taxonomic coverage of potential prey species. These primers amplify fragments of varying lengths within the COI region (Fig 2), currently used as the standard animal barcode region, which has a well-documented reference database.

DNA extraction, library preparation and NGS sequencing

DNA was extracted using the Qiagen Powerfecal DNA kit (Qiagen Iberia, S.L. Madrid), following the manufacturer guidelines. Subsequently, DNA was PCR amplified from extracts using the five primer sets (Table 2), at the Analytical Services (SGIker) of the University of the Basque Country, UPV/EHU. Samples were purified and a second reaction was performed to index each amplified product and attach Illumina adaptors using the Illumina Nextera v2 kit. Amplifications with Zeale, Gillet, Leray and Meusnier primer sets were performed with the Qiagen Multiplex PCR Kit protocol (Qiagen Iberia, S.L. Madrid, using 12.5 μ L Qiagen 2X (1X final), 1.25 μ L forward primer (10 μ M; 0.5 μ M final), 1.25 μ L reverse primer (10 μ M; 0.5 μ M final), 8 μ L H₂O and 2 μ L DNA, in a final volume of 25 μ L. Amplification with Brandon-Mong primer set was performed with 12.5 μ L KAPA HIFI 2X (1X final), 2.5 μ L forward primer (10 μ M; 1 μ M final), 2.5 μ L reverse primer (10 μ M; 1 μ M final), 5.5 μ L H₂O and 2 μ L DNA, in a final volume of 25 μ L. Each primer set had its own PCR program, modified from the reference to the used reactive, as indicated in the S1 Table. Once amplified, PCR outputs were sequenced in a *Illumina MiSeq NGS platform* (sequencing of 2x300 bp paired-end reads) with the *MiSeq Reagent Kit v3 (600 cycle)*, following the manufacturer instructions.

Bioinformatic analyses

Paired-end reads were merged using USEARCH (Edgar, 2010; Edgar & Flyvbjerg, 2015), demultiplexed by primers, adapter and primer sequences

were removed, and reads were quality- and length-filtered using CUTADAPT (Martin, 2011). Then, singletons were removed and the remaining sequences were screened for chimeras using USEARCH. UPARSE algorithm (Edgar, 2013) was used to cluster sequences into Operational Taxonomic Units (OTUs) at a 97% similarity threshold. Finally, Genbank nt database was used to assign taxonomy to OTUs using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Species level assignments were performed when query sequences matched reference sequences above 98% similarity and 75% overlap (Clare *et al.*, 2013). If query sequences matched more than one species in the database, the hit with the longest alignment length was selected. Besides, as a rule, only hits with evaluate below $1e^{-20}$ were accepted (Vesterinen *et al.*, 2013) to make sure that the match did not occur by chance.

Subsequent analyses were performed taking into account the occurrence of identified prey taxa (the number of pellets that a taxon was found in) (Shiel *et al.*, 1997). Primer outputs were also tested to see whether any of the OTUs built from them could also identify the predator itself, i.e. the Pyrenean desman.

Data analysis

To study the overall effect of primers on variation in species composition of diet, we performed a permutational multivariate ANOVA using *adonis* with 999 random permutations in *vegan* 2.4-6 package (Oksanen *et al.*, 2016) for R version 3.4.3 (R Core Team, 2017). A Jaccard distance measure was used to calculate dissimilarities between samples. We performed NMDS in *vegan* 2.4-6 package for R to visualize dissimilarities in species composition among samples. Pairwise differences in species composition between primers were also tested using the function *pairwise.perm.manova* in package *RVAideMemoire* 0.9-69-3 for R (Hervé, 2014). The variation in species composition within primer sets (i.e. the homogeneity) was also tested using the *betadisper* and *permutest* functions with 999 permutations in package *vegan* 2.4-6 for R (Oksanen *et al.*, 2016). Pairwise differences in homogeneity between primer sets were analysed using Tukey's HSD test in package *vegan* 2.4-6 for R (Oksanen *et al.*, 2016).

Results

The sequencing output differed considerably among primer sets, both in the amount of sequence reads and in the number of rough (total) OTUs (Fig 3). Gillet primers yielded the highest numbers of reads and rough OTUs, followed by Leray (S2 Table). Brandon-Mong, Gillet and Leray primers identified the desman

itself, but only Gillet and Leray did so in all faecal samples; no other predator whose scats could be mistaken was identified. All five primers identified the most common prey taxa (namely *Baetis* sp., *Hydropsyche* sp., *Odontocerum* sp. and *Psychoda* sp.) but differed considerably in the less abundant prey groups (Fig 4 and S3 Table). Quantitatively, Gillet yielded the largest list of taxonomically assigned OTUs (19.15% of the OTUs), as well as the highest occurrence values. Zeale and Leray primers followed in rough OTUs and assignments (with the 38.5% and the 7.81% of the OTUs assigned, respectively), whilst Brandon-Mong and Meusnier primers showed the lowest efficiency, with less rough OTUs, and only 15.79% and 7.12% of them assigned, respectively. Taking into account the number of taxa assigned, Gillet primer set was the most efficient amplifying DNA of Mollusca, Annelida and arthropods such as Diptera, Ephemeroptera, Plecoptera, Trichoptera or Arachnida (S3 Table); Meusnier primer set was the most efficient amplifying Salmonids, whereas Zeale set was the best amplifying Coleoptera. Although the detected occurrence was

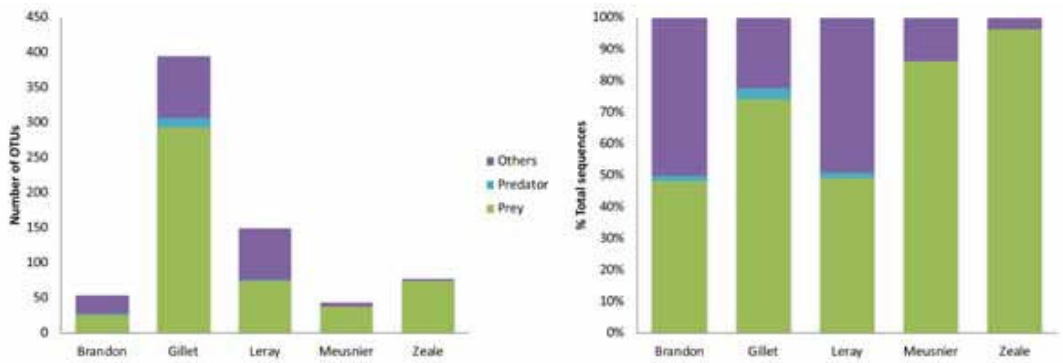


Figure 3. Species-level taxonomic assignment of OTUs obtained for each primer set. Number of OTUs and % of total sequences obtained.

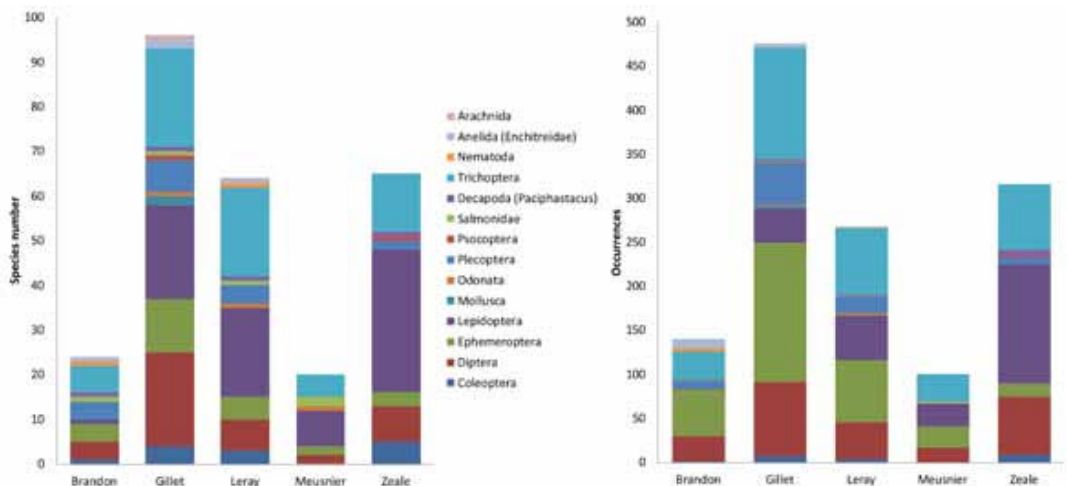


Figure 4. Number of OTUs assigned to species and their occurrences for each primer set. Results taking into account high taxonomical levels.

lower, Leray primer set was also quite efficient amplifying Trichoptera and Coleoptera. Comparing Gillet with Zeale and Leray separately, Gillet primer set provided more information on Mollusca and arthropods such as Diptera, Ephemeroptera, Plecoptera, Trichoptera and Arachnida than Zeale, whereas the reverse occurred for Coleoptera, Psocoptera and Decapoda. Gillet primer sets outperformed Leray primers for Mollusca, Annelida and arthropods such as Coleoptera, Diptera, Ephemeroptera, Plecoptera, Psocoptera, Trichoptera and Arachnida, while Leray yielded slightly higher values for Nematoda.

Primer choice had statistically significant effects on the resulting diet composition ($F_{(4,115)} = 15.712$; $R^2 = 0.353$; $p = 0.001$). This was clearly illustrated by the NMDS (Fig 5), where samples were clustered in 4 main groups: Zeale, Meusnier, Brandon-Mong and Leray+Gillet. There were significant overall and pairwise differences in species composition between primer sets (overall test: $F = 10.425$; $p < 0.001$; pairwise tests: all $p = 0.001$), but samples amplified with Zeale

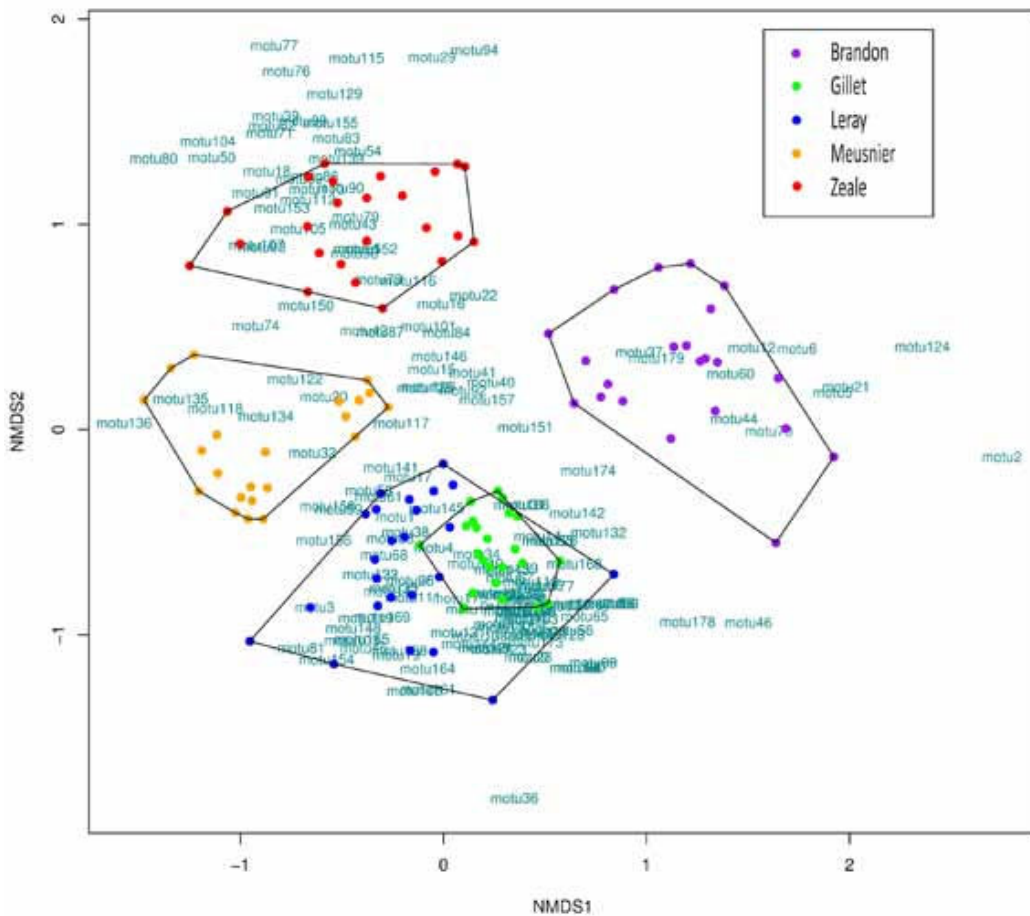


Figure 5. NMDS ordination of samples. Stress = 0.193; $k = 2$; non-metric fit $R^2 = 0.963$. Dots represent individual desman samples and colours different primer sets. OTUs are represented with green letters. More distant dots indicated a more different prey composition.

and Leray or Gillet differed the most in terms of species composition, whereas Leray and Gillet primer sets showed rather similar composition, although Leray seemed to yield higher variability. Moreover, differences in species composition were lowest between Meusnier and Gillet primer sets ($p = 0.029$), followed by Zeale and Meusnier ($p = 0.013$) and Gillet and Brandon-Mong ($p = 0.003$). On the contrary, the highest differences were found between Zeale and Gillet ($p < 0.001$), followed by Leray and Gillet ($p = 0.001$) (Fig 6).

We optimized the potential prey species identified by accumulating the amplification outputs of the five primer sets. Gillet provided the highest prey identifications on its own (Fig 7). When combining Gillet with the other sets, the

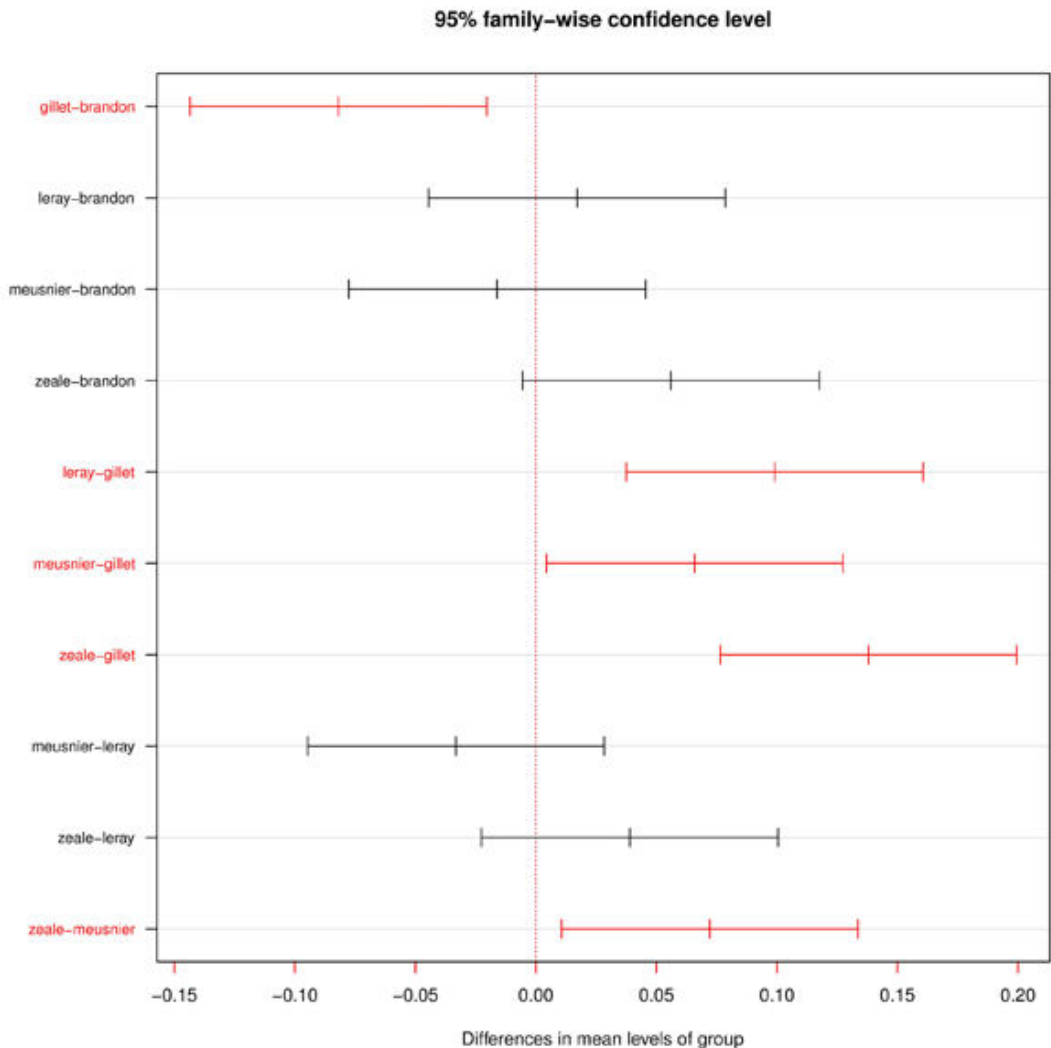


Figure 6. Tukey's HSD plot for all the comparisons between primer sets. Red colour represents significant differences ($p < 0.05$). The plot shows the differences in mean levels of groups where the largest difference in homogeneity was between Gillet and Zeale, followed by Gillet and Leray.

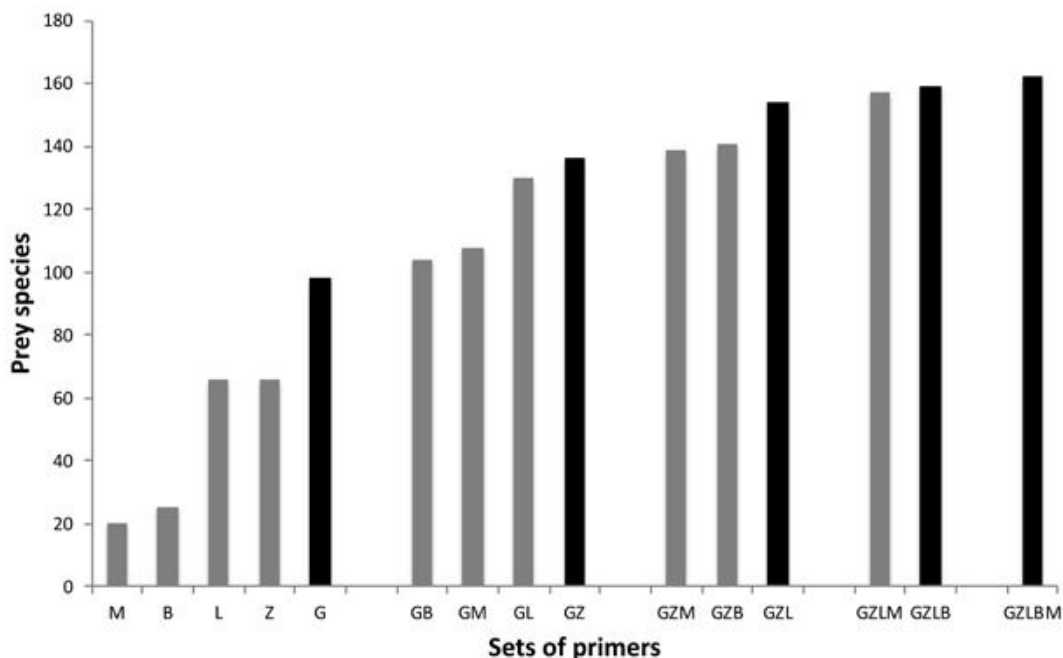


Figure 7. Number of prey species accumulations obtained by different and progressive primer combinations. Progressive primer combinations (one+one, two+one, and so on) were selected according to the highest species accumulation values (black columns) obtained in previous combination-level. B refers to Brandon-Mong primers, G to Gillet, L to Leray, M to Meusnier and Z to Zeale.

identified species number increased most with Zeale (a total of 129 prey species or accurate taxonomic groups, 37.2% more than only using Gillet). Subsequent combinations yielding the highest increases in species numbers were obtained by adding up Leray first, followed by Brandon-Mong and Meusnier primer sets, in this order, with quantitatively smaller increases in each step.

Discussion

Our study shows that the selection of molecular markers—even primers targeting different sections of the same gene—considerably affect the characterization of diet. This is particularly important in generalist predators with a phylogenetically diverse diet. Although some prey taxa were consistently detected by all five primer sets used—likely the ones most frequently consumed—, the resulting picture of dietary composition depended on the primer set considered. Our results confirmed those by Alberdi *et al.* (2018) in bats, who had also observed contrasting performance of four primer sets targeting two different genetic markers (the COI and the 16S).

Primer performance differed not only quantitatively, in the total amounts of sequence reads, rough and taxonomically assigned OTUs, and numbers of identified putative prey specific taxa, but also qualitatively, in the proportion of the main phylogenetic groups each primer yielded. These differences may be due to the specific affinity of each primer set to amplify certain taxa, due to the amplicon length, or to the degeneration degree of each primer as well.

Specifically, since longer sequences of DNA resist digestion worse (Deagle *et al.*, 2006), the primer sets targeting longer DNA fragments are less efficient in detecting prey from faeces. The integrity of a sequence of a given length may be related to various factors such as differences in digestion among tissues, the time under digestion, retention time, etc., and this variation differs among prey species (reviewed in Clare, 2014). This is partially consistent with our case study, where Gillet primers, targeting almost the shortest fragment of COI gene (Table 1), yielded the highest output in terms of sequence reads, OTUs, and potential prey detected as well. On the contrary, Leray primers, which targeted a similarly broad taxonomic range but with much longer amplicons, retrieved less fragments of DNA and were unable to amplify some taxa that Gillet primer pairs did recover. Nevertheless, primers targeting almost the same stretch length—Gillet and Meusnier, for instance—performed unequally as well, both in terms of quantitative and qualitative data. This may be a consequence of the different degrees of degeneration of the primers (Elbrecht & Leese, 2017). In fact, Gillet and Leray primers, showing the highest degeneration degree (Table 2), are the most successful in terms of OTUs gathered after the PCR, but many of them could not be assigned to any known taxa (only 19.15% and 7.8% of them, respectively), suggesting that they would also amplify many other DNA substrates beyond the targeted COI fragment. On the contrary, primers with lesser degeneration—such as Zeale—produced a much smaller amount of OTUs, but a higher proportion of them (38.5%) were assigned to know taxa, showing a higher bond to the barcode region. Consequently, the varying persistence of relatively long DNA sequences after digestion, and the primers used to amplify them, affect the final inferences about the intraspecific variation of individual diets. Therefore, the selection of suitable primer sets for diet analyses is crucial when a wide dietary variation is expected (De Barba *et al.*, 2014).

Recently, a pipeline for the diet analysis of the desman has been published (Hawlitshchek *et al.*, 2018), where DNA metabarcoding of old and fresh faecal samples was implemented with nested PCRs. This methodological contribution adds a pre-amplification step to increase the number of reads corresponding to the target taxa. Nevertheless, authors pointed out that their procedure could lead to an increase in the specificity of the amplification, thus losing some essential prey sequences. Our results suggest that such nested PCR

would further increase primer bias, multiplying the effect of the two primer sets used. Hawlitschek *et al.* (2018) also proposed that primer cocktails or pools of amplification products of more than one primer pair should be used in future studies to improve the amplification success of the target group. That is precisely what the present work tested and showed to be true.

Regarding the desman trophic ecology, two recent metabarcoding studies provided new data: Biffi *et al.* (2017a), employing only the Gillet primer set, described the desman as “more generalist than previously thought” as a consequence of its diverse summer diet, mostly based on Ephemeroptera and Plecoptera in the North of Pyrenees; Hawlitschek *et al.* (2018), working in the north-western Iberian Peninsula with Meusnier primers and nested PCR, found that Ephemeroptera and Diptera were the most abundant prey groups. Our data, although carried out in a different area and more limited in sample size, also identified these taxa as relevant components of the desman winter diet. However, the number of species and their occurrence rates largely depended on the primer set considered. To which extent do the results of these studies show methodological differences rather than regional variations in desman prey availability? We can hardly tell. Our results suggest the characterization of the diet of desmans—or any other animal—using a single primer set to be prone to serious biases. These will weaken ecological conclusions such as predator-prey interactions or prey selection patterns, which may be key for implementing conservation measures.

A species might be characterised as generalist due to the overall consideration of many individual-level specialist diets (Araujo *et al.*, 2011) or due to the capacity of individuals to forage on a wide diversity of food items (Clare *et al.*, 2011). When analysing the diet of a generalist predator, the choice of primer sets may condition the interpretation of its trophic ecology and specialization. The way foraging habits are perceived can in turn affect the interpretation of community dynamics and ecosystem functioning (Bolnick *et al.*, 2011), and thus, any biased description of diet can lead managers to misinterpret food chain structures and to take wrong conservation decisions (Bolnick *et al.*, 2003; Devictor *et al.*, 2010). For instance, trophic relationships have been described with molecular techniques for bats (Burgar *et al.*, 2013; Mata *et al.*, 2016; Krauel *et al.*, 2018), birds (Gerwing *et al.*, 2016; Ando *et al.*, 2018), rodents (Lopes *et al.*, 2015) and invertebrates such as snails (Boyer *et al.*, 2013), among others. It is essential to invest in accurate trophic and spatial ecology studies to obtain detailed knowledge about the trophic requirements of a species. Our results stress the importance of combining different primer sets to detect the widest range of potential prey species and to avoid losing essential information. Methodological decisions affect the assessment of trophic requirements of any animal, as well as management measures based on this assessment (Kohn &

Wayne, 1997).

Additionally, when working with endangered and elusive animals it is essential to unambiguously identify the source of faecal samples collected. So far, molecular tools have been useful to provide accurate dietary description from scats in a wide range of predators such as bats (Vesterinen *et al.*, 2013; Arrizabalaga-Escudero *et al.*, 2018), carnivores (Farrel *et al.*, 2000; Shehzad *et al.*, 2012), pinnipeds (Casper *et al.*, 2007), birds (Marchesi *et al.*, 2002) and the desman itself (Biffi *et al.*, 2017a). In the present study desman was identified from faeces with 3 of the tested primer sets, namely Leray, Gillet and Meusnier. However, only Leray and Gillet primer sets successfully identified the desman in all analysed faecal samples, confirming the lower performance of Meusnier to identify Chordata (Meusnier *et al.*, 2008). In general, in diet studies involving insectivorous predators with non-unequivocally identifiable faeces, at least one of the selected primers should be able to amplify DNA of the predator, reducing identification mistakes.

Our results suggest that the diet of Pyrenean desman can be characterised by combining multiple primers, but primer selection must also consider data accuracy and costs. In terms of cost-effectiveness, Gillet and Zeale primer sets would be the best combination to identify the widest taxonomic range of prey, as well as the desman itself. This combination would further improve stepwise by sequentially adding Leray, Brandon-Mong and Meusnier primers, from highest to lowest enrichment. Nevertheless, it must also be taken into account that to combine primers the sequences they amplify must not differ too much in length, to avoid biases towards the smallest amplicons in some NGS sequencing procedures. Thus, in our case the combination of Gillet and Zeale primers would likely result in less problems than the combination of Gillet and Leray, as fragment amplicons with Leray are double in size.

Overall, the present study shows that using different sub-regions in a specific marker yields contrasting results, and highlights the ecological relevance of considering several primer sets to characterize the prey spectrum of generalist predators. In the end, when taking conservation measures for threatened species the methodological procedure could be crucial. For instance, the diet of an endangered animal yields key information about its ecological role and its biology, information that can be essential to protect it. Thus, in diet metabarcoding studies it is necessary to test and assess different primer combinations. Trophic and habitat requirements are key factors in the conservation of many endangered species (Sinclair *et al.*, 2006; Sutherland, 2000). Given that results on diet depend so much on the combination of primers used, testing and assessing different primer combinations is necessary in diet metabarcoding studies.

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Supporting information

Table S1. Details of PCR conditions for the five primer sets.

PCR stages	Zeale	Gillet	Leray	Meusnier	Brandon-Mong
Initialization	95 °C, 10 min	95°C, 15min	95°C, 10 min	95°C, 10 min	95°C, 2 min
Denaturation	16 cycles:	40 cycles:	16 cycles:	5 cycles:	25 cycles:
	95 °C, 30 seg	94 °C, 30 seg	95 °C, 10 seg	95 °C, 1 min	95 °C, 15 seg
	61 °C, 30 seg	45 °C, 45 seg	62 °C (-1 °C per cycle), 30 seg	46 °C, 1 min	45 °C, 30 seg
	72 °C, 30 seg	72 °C, 30 seg	72 °C, 60 seg	72 °C, 30 seg	72 °C, 3 min
Annealing	24 cycles:		25 cycles:	35 cycles:	
	95 °C, 30 seg		95 °C, 10 seg	95 °C, 1 min	
	53 °C, 30 seg		46 °C, 30 seg	53 °C, 1 min	
	72 °C, 30 seg		72 °C, 60 seg	72 °C, 30 seg	
Extension	72 °C, 10 min	72 °C, 10 min	72 °C, 10 min	72 °C, 10 min	72 °C, 10 min

Table S2. Gross sequence reads, sieved sequence reads and number of OTUs obtained with each primer set.

	Brandon-Mong	Gillet	Leray	Meusnier	Zeale
Gross sequence reads	3754874	12667575	4364497	5050701	3110300
Sieved sequence reads	557978	11788479	1668749	4120090	2231688
Rough (Total) OTUs	342	2063	1920	604	200
Taxonomically assigned OTUs	54	395	150	43	77

Table S3. Number of species identified (N.Sp.) number of occurrences (Occ.) in each taxonomic group, for each primer set.

Taxonomic group	Brandon-Mong		Gillet		Leray		Meusnier		Zeale	
	N.Sp.	Occ.	N.Sp.	Occ.	N.Sp.	Occ.	N.Sp.	Occ.	N.Sp.	Occ.
Annelida (Enchitreidae)	1	10	2	4	1	1	0	0	0	0
Arachnida	0	0	1	1	0	0	0	0	0	0
Coleoptera	1	1	4	7	3	3	0	0	5	8
Decapoda (Paciphastacus)	1	1	1	2	1	2	0	0	1	7
Diptera	4	29	21	84	7	43	2	17	8	67
Ephemeroptera	4	53	12	159	5	70	2	24	3	15
Lepidoptera	1	1	21	39	20	51	8	25	32	135
Mollusca	0	0	2	2	0	0	0	0	0	0
Nematoda	1	4	0	0	1	1	0	0	0	0
Odonata	0	0	1	2	1	2	1	1	0	0
Plecoptera	4	9	7	47	4	19	0	0	2	7
Psocoptera	0	0	1	1	0	0	0	0	1	2
Salmonidae	1	1	1	1	1	1	2	2	0	0
Trichoptera	6	31	22	127	20	75	5	31	13	75
Total	24	140	96	476	64	268	20	100	65	316

Chapter 4



It is the ambience, not the menu.
Prey availability does not drive
habitat selection by the endangered
Pyrenean desman

Publication

An article based on this chapter is under review in the journal *Aquatic Conservation: Marine and Freshwater Ecosystems*:

Esnaola, A., Mata, V., Arrizabalaga-Escudero, A., González-Esteban, J., Larrañaga, A., Rebelo, J., Elosegi, A., & Aihartza, J. (2018). It is the ambience, not the menu. Prey availability does not drive habitat selection by the endangered Pyrenean desman. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Abstract

1. The Pyrenean desman (*Galemys pyrenaicus*) is an endangered stream-dwelling insectivore endemic to the Iberian Peninsula and the north of the Pyrenees. It favours riffles over slow habitats such as runs or pools, yet it is still unclear whether habitat preference is based on prey availability or on other factors.

2. Desman diet and prey selection were analysed along the pristine Elama and the slightly anthropized Leitzarán streams (the Basque Country).

3. 94 faeces were metabarcoded per stream to identify consumed prey and characterized prey availability from 10 Surber samples taken at each habitat type (riffles, runs and pools) per stream. Invertebrates were sorted and identified, the biomass of each taxon was calculated, and their DNA was also extracted, PCR amplified and sequenced to build a reference database.

4. Prey availability and diet varied between streams. Desmans positively selected shredders and invertebrates that live on the substrate, and selected against taxa that live in fine sediment, taxa with hard shells or highly mobile. The diet of desmans was more selective in Elama, the stream with better ecological status. Food availability did not differ among habitats, and therefore does not explain the preference of desmans for riffles; rather, mechanical constraints to deal with buoyancy and physical habitat heterogeneity were hypothesized as the reason behind the preference.

5. Our results suggest that promoting the formation of riffles in streams can improve the situation of some desman populations living in degraded habitats.

Keywords

behaviour, endangered species, feeding, invertebrates, mammals, metabarcoding, river, stream

Introduction

Understanding the trophic ecology and foraging strategies of endangered animals is important for predicting their population dynamics and developing effective conservation programmes (Sinclair *et al.*, 2006). Since foraging behaviour largely depends on the composition, abundance and distribution of food resources (e.g. Waggit *et al.*, 2018), the relationship between habitat and food availability is a key issue in conservation biology (e.g. Goiti *et al.*, 2008; Karanth, *et al.*, 2004; Sutherland & Green, 2004).

The Pyrenean desman (*Galemys pyrenaicus*, É. Geoffroy Saint-Hilaire, 1811, Eulipotyphla, Talpidae, Fig. 1) is a semi-aquatic insectivorous generalist mammal that lives in mountain streams. It is endemic to the northern Iberian Peninsula and the Pyrenees, but its distribution area has been severely reduced during the last decades, being currently listed as Vulnerable by the IUCN (Fernandes *et al.*, 2008). It has a strong preference for fast-flowing riffles over slower runs or pool habitats (Chapter 2; Esnaola *et al.*, 2018b), although it is unknown whether this preference is based on prey availability or on other factors, *i.e.*, whether it selects its foraging areas (the restaurant) as a function of the ambience (*i.e.* physical characteristics) or the menu (*i.e.* available prey). The lack of information on desman foraging ecology limits the capacity to adequately manage this species (MAGRAMA, 2013; Némoz & Bertrand, 2008).



Figure 1. The Pyrenean desman (*Galemys pyrenaicus*) in the Elama Stream. Photo by Jorge González-Esteban.

Visual identification of prey remains in desman faeces or stomach contents (e.g. Bertrand, 1992; Casti n & Gos lbez, 1995; Santamarina, 1993) reported that they feed on a broad spectrum of benthic invertebrates. More recently, DNA metabarcoding of faeces (Bohmann *et al.*, 2014), allowed identifying prey, including soft-bodied animals, to species level. Several authors have applied these methods to desman faeces. Biffi *et al.* (2017a) and Biffi *et al.* (2017b), in the north of the Pyrenees, identified 156 prey genera from 91 families and 30 orders. Subsequently, Hawlitschek *et al.* (2018) identified a total of 140 prey genera in the Iberian Peninsula. These studies confirmed that desmans mostly eat Ephemeroptera, Plecoptera, Trichoptera and Diptera, although these results can be affected by primer bias (Chapter 3; Esnaola *et al.*, 2018a; Pi nol *et al.*, 2019) and other methodological constraints (e.g. Oehm *et al.*, 2011).

To test whether desman preference for riffles is determined by food availability or other factors, prey availability was characterized in three microhabitats (riffles, runs or pools) and compared it to the diet determined from desman faeces, collected simultaneously with the radio-tracking study of habitat selection performed by Esnaola *et al.* (2018a; 3. Kapitulu ). The main hypothesis was that desmans prefer riffles because of higher prey availability therein (Dewson *et al.*, 2007). To test the flexibility of the desman diet, the study was carried out in two streams with different ecological status. The study also aimed to test which are the prey selected by the desman. The results from this work should provide important information for conservation managers and guide future management actions to better protect the threatened Pyrenean desman populations.

Materials and Methods

Study area

The study was conducted in two mountain streams in the northern Iberian Peninsula (Basque Country; Fig. 2). Both streams are protected under the EU Natura 2000 network and both are in good ecological status according to the EU Water Framework Directive (Council of the European Communities, 2000). Nevertheless, they contrast in the environmental pressures they are subjected to. Elama is a 2nd order headwater stream draining an uninhabited basin of 1.415 ha over schist that has been managed as a strict nature reserve since 1919, resulting in extensive cover of beech and oak forests (Castro, 2009). Currently there is no extractive activity in the Elama basin. On the other hand, Leitzaran is a 4th order stream draining a basin of 12.402 ha over limestone, slate and sandstones. Contrasting with Elama, in the headwaters of Leitzaran

Stream there are two towns totalling 3150 inhabitants, but then the stream enters a long, uninhabited valley ca. 25 km in length where forest activities and diversion hydropower schemes are the main human activities (Izagirre *et al.*, 2013).

The research was carried out in the same reaches and periods as the radiotracking survey published by Esnaola *et al.* (2018b; Chapter 2), on a 4 km-long section of Elama Stream (from 1°48'36"W 43°12'40"N to 1°48'4"W 43°11'14"N; 330 m mean altitude; 7.08 m mean width) and a 10 km-long section of Leitzarán Stream (from 1°57'26"W 43°8'57"N to 1°56'9"W 43°6'55"N; 290 m mean altitude; 12.42 m mean width). The difference between section lengths was due to the lower density of desmans in Leitzarán.

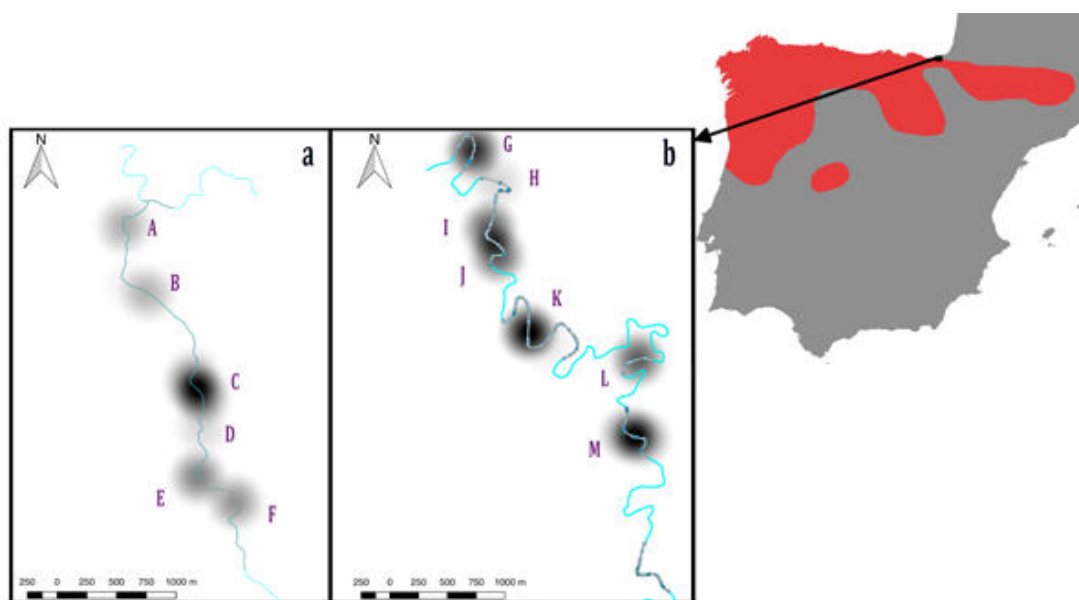


Figure 2. Study area: (a) Elama and (b) Leitzarán streams. Faecal samples were clustered in sampling sites (A-M) according to a heat map of their occurrence along the prospected stream stretches. The distribution of the Pyrenean desman in the Iberian Peninsula is displayed in red (Fernandes *et al.*, 2008).

Desman diet

We searched for faeces of the Pyrenean desman along both streams (Fig. 2) in September and October 2016, by building artificial shelters specially designed for this species (Appendix 1; González-Esteban *et al.*, 2018). A total of 188 droppings (94 per stream) were collected and stored in tubes containing 98% ethanol and frozen at -80 °C until DNA extraction.

DNA extraction from desman faecal samples, PCR amplification and high throughput sequencing (HTS) were performed as described in Esnaola *et al.* (2018a; Chapter 3), combining the use of both Gillet (Gillet *et al.*, 2015) and Zeale (Zeale *et al.*, 2011) primer sets to identify the broadest range of consumed prey species and the predator itself. To build a custom database, DNA was also extracted from the invertebrates collected in both streams (see below). DNA was extracted, amplified and sequenced at the Analytical Services (SGIker) of the University of the Basque Country, UPV/EHU. More methodological details are given in Supporting information 1 and 2 (S1 Table).

Diet results were expressed in frequency of occurrence (FO) and percentage of occurrence (PO) of each prey taxa for each stream separately. FO of a prey taxon represents the proportion of scats where it was detected and PO the number of occurrences of each taxon divided by the total number of occurrences of all taxa, in percentages (Deagle *et al.*, 2019).

Prey availability

Aquatic macroinvertebrates were sampled in September and October 2016 with a Surber net (30 cm x 30 cm; 1 mm mesh) across the three habitat types (Overton *et al.*, 1997) identified in a previous radiotracking study (Chapter 2; Esnaola *et al.*, 2018b): namely “riffles” with fast, turbulent water, uneven surface level, and white water; “runs” with close to laminar flow and even depth; and “pools” with slow flow on riverbed depressions. Ten samples were collected per habitat and per stream, making a total of 60 samples. These were stored in plastic jars and preserved with 70% ethanol until identification.

In the laboratory, macroinvertebrates were sieved with a 2 mm mesh, sorted, counted, measured and identified under a dissecting microscope. Taxonomic resolution was genus or species level for most taxa, with the exception of some Diptera, Coleoptera, Oligochaeta, Hirudinidae and Nematoda, which were identified at family or order level (Supporting information 2, S2 and S3 Table). Prey biomass (gAFDMm⁻², or grams of ash-free dry mass per square metre) was calculated from size-mass regressions (Supporting information 2, S4 Table).

Statistical analyses

Sampling completeness was assessed for both diet and invertebrate availability by building taxa accumulation curves using the ‘iNEXT’ online platform (Chao *et al.*, 2016).

Differences between streams in dietary composition were analysed using Permutation based MANOVA (PerMANOVA; Anderson, 2001) at both species and family level, and taxa driving these differences were identified with a Correspondence Analysis (CA). PerMANOVA was implemented using the function *procD.lm* of the *geomorph* package for R (Adams *et al.*, 2017).

Prey availability was compared between streams at three levels: potential prey availability (including all sampled invertebrates), availability of consumed prey (only taxa found in diet) and availability of positively selected prey (only taxa that were positively selected, i.e. that occurred more frequently in diet than in invertebrate samples, with a threshold of 20% difference). Taxa richness of potential prey availability were compared using ANOVA, considering taxa at species and family level. The density (individuals m⁻²), biomass (gAFDMm⁻²) and average body mass (gAFDM) of each availability measure were also compared with ANOVA, after normalizing by log₁₀ transformation. Average body mass (gAFDM) per prey taxa was calculated dividing biomass by density (Flores *et al.*, 2011).

To assess prey selection, potential prey availability, consumed prey availability and positively selected prey availability were compared with the FO of each taxon in diet. For that, prey availability was characterized in three ways: 1) total prey availability (the least conservative analysis); 2) total prey availability weighted by the mean available habitat surface in desman home range in each stream (based on radiotracking data from Esnaola *et al.*, 2018b; Chapter 2); and 3) total prey availability weighted by the mean desman activity per habitat type and per stream (the most conservative analysis; based on radiotracking data from Esnaola *et al.*, 2018b; Chapter 2). These methods were compared with PerMANOVA with taxa at species level. Prey selection was estimated for each stream and for both streams together, showing the difference in FO of each taxon between diet and availability. To do so, prey availability was also expressed in frequency of occurrence (FO) of each prey taxa for each stream, representing the proportion of Surber samples where each prey taxa was detected in each stream. A threshold of 20% difference was set to consider positive or negative selection of taxa.

Positively selected prey taxa were used to have a more conservative approach of prey availability, looking for differences between habitats that could explain the spatial ecology of the desman (preference for fast habitats, Esnaola *et al.*, 2018b; Chapter 2). Estimations of available density, biomass and average body mass of prey were done for both streams and values compared with ANOVA.

The significance level of the tests was set at $p < 0.05$. Statistical analyses were performed using R 3.4.3 (R Core Team, 2014).

Results

DNA sequences

DNA was successfully extracted and amplified from all samples, with the exception of one faecal sample from Leitzaran that did not produce enough reads during sequencing. This way, a total of 94 faeces from Elama and 93 from Leitzaran were analysed. In total, 4896 OTUs were obtained with Gillet primer set and 844 OTUs with Zeale primer set. From them, 64 Gillet and 75 Zeale OTUs passed the filters for diet analysis and were assigned to 41 taxa (23 families) with Gillet and 46 (33 families) with Zeale. Both primer sets shared 13 families. Gillet amplified several OTUs corresponding to the desman itself in all the samples, confirming that all the faeces belonged to this species.

Diet

Taxa accumulation curves reached an asymptote and sample completeness was over 95%, confirming that the sample size used (number of faecal samples) was enough to describe the diet of desmans in both Elama and Leitzaran streams (Supporting information 2, Fig. S1).

Diet was mostly composed of Insecta and Malacostraca (FO being 100% and 93% respectively), with Ephemeroptera, Diptera, Amphipoda and Trichoptera the most common orders (FO 99%, 94%, 92% and 88%, respectively) (Fig. 3; Table 1; Supporting information 2, S5. Table). The most frequently consumed prey families were Heptageniidae, Gammaridae, Baetidae and Simuliidae in both streams (FO 94%, 92%, 91% and 90%, respectively), and the most common genera (FO >70%) were *Baetis*, *Ecdyonurus* and *Echinogammarus* in Elama and *Baetis*, *Echinogammarus* and *Epeorus* in Leitzaran.

Dietary composition differed significantly between streams, both at the species ($F = 58.24$; $df = 1$; $p = 0.001$) and family level ($F = 16.88$; $df = 1$; $p = 0.001$). Odontoceridae, Perlidae, Leptophlebiidae and Astacidae contributed the most to these differences (CA graph dimension 1, Supporting information 2, Fig. S2), with the first three mostly occurring in Elama and the last one in Leitzaran (Fig. 4).

Prey availability

Taxa accumulation curves with abundance and presence-absence data reached an asymptote and sample completeness was over 80%, confirming that the sample size used (number of Surber samples) was enough to describe prey availability in both Elama and Leitzarán streams (Supporting information 2, Fig. S3 and Fig. S4).

Invertebrate taxa richness was higher in Leitzarán than in Elama (Fig. 5), the differences being statistically significant at both the species ($F = 4.41$; $df = 1$;

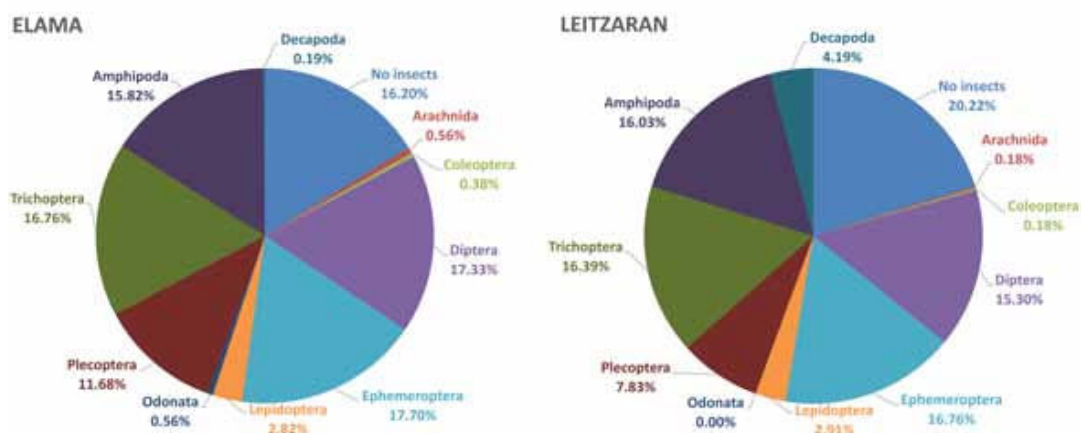


Figure 3. Pyrenean desman diet composition pie charts. Percentage of occurrence (PO) of each prey group, in both streams.

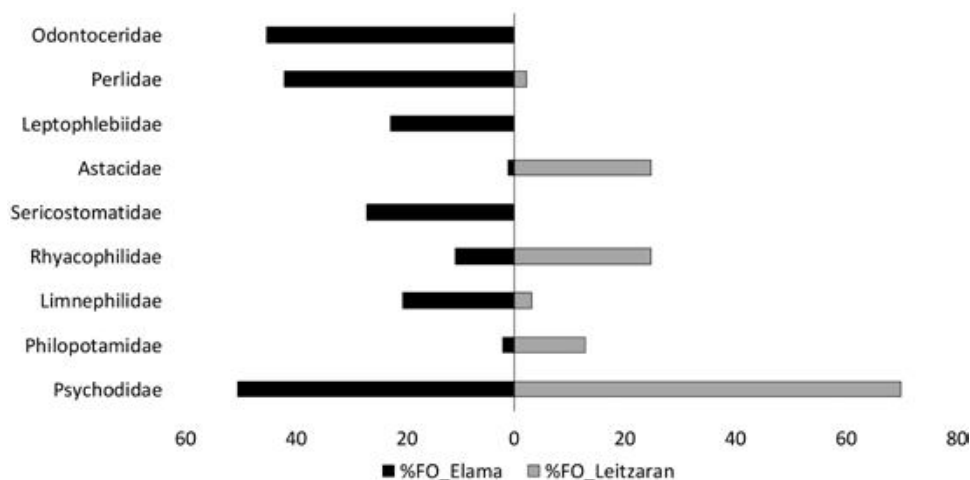


Figure 4. Frequency of occurrence (FO) of the prey families that most influenced dietary differences between both streams. These taxa contributed more than 1% to the first dimension of the CA, which separates sites belonging to each stream. Taxa were ordered from the highest (top) to the lowest contribution values (bottom).

Table 1. Comparison between the present study and previous dietary studies. Results expressed in FO. Taxa present in less than 5% of the faeces in all studies were removed. Primers: G = Gillet *et al.*, 2015; Z = Zeale *et al.*, 2011; F = Folmer *et al.*, 1994; M = Meusnier *et al.*, 2008. The data used in the analyses of the present study, that is the data obtained through Gillet and Zeale primers, have been highlighted in bold.

Class	Order	Family	Primers	Present study									Hawliits-
				Elama			Leitzarlan			Pyrenees	Pyrenees	Peninsula	check
				G	Z	G+Z	G	Z	G+Z	G	G	F and M	<i>et al.</i> ,
				North			North			North			2018
Arachnida			0	3	3	1	0	1	2	8	0		
Clitellata	Haplotaxida	Lumbricidae	0	0	0	0	0	0	1	6	0		
Diplopoda			0	0	0	0	0	0	4	22	0		
	Glomerida		0	0	0	0	0	0	2	16	0		
		Doderiidae	0	0	0	0	0	0	0	8	0		
		Glomeridae	0	0	0	0	0	0	2	11	0		
	Polydesmida		0	0	0	0	0	0	1	7	0		
Insecta			100	100	100	100	100	100	100	93	100		
	Coleoptera		1	1	2	1	1	1	14	14	56		
		Carabidae	0	1	1	0	0	0	7	1	0		
		Scirtidae	1	0	1	0	0	0	6	8	0		
	Diptera		16	100	98	12	99	90	46	66	100		
		Anthomyiidae	0	0	0	0	0	0	3	5	0		
		Blephariceridae	0	0	0	0	0	0	1	7	0		
		Chironomidae	13	51	53	10	41	44	15	42	66		
		Empididae	1	3	3	0	9	9	0	2	0		
		Limoniidae	0	10	10	0	4	3	1	2	0		
		Mycetophilidae	0	2	2	0	6	5	0	4	0		
		Psychodidae	2	51	50	0	76	70	3	8	0		
		Simuliidae	1	96	94	2	94	86	15	18	0		
		Stratiomyidae	0	0	0	0	0	0	11	1	0		
		Tipulidae	0	7	6	0	0	0	5	4	0		
	Ephemeroptera		100	43	100	99	60	99	87	71	100		
		Baetidae	87	0	87	95	0	95	57	42	0		
		Ephemerellidae	2	1	3	0	0	0	37	2	0		
		Ephemeridae	4	0	4	0	0	0	0	8	0		
		Heptageniidae	98	41	98	90	60	90	59	61	0		
		Leptophlebiidae	22	2	22	0	0	0	5	1	0		
	Lepidoptera		0	16	16	0	19	17	1	3	0		
		Erebidae	0	7	6	0	7	6	0	1	0		

Table 1. (continuation).

Class	Order	Family	Present study						Biffi	Biffi	Hawliits-	
			Elama			Leitzaran			Pyrenees	Pyrenees	check	
			Primers	G	Z	G+Z	G	Z	G+Z	G	G	et al.,
												et al.,
		Geometridae	0	7	6	0	11	10	0	2	et al.,	
	Neuropteroida		0	0	0	0	0	0	0	0	2018	
	Orthoptera		0	0	0	0	0	0	7	0	NW	
		Anostostomatidae	0	0	0	0	0	0	7	0	Iberian	
	Plecoptera		62	21	66	33	44	46	85	53	Peninsula	
		Leuctridae	22	21	28	30	44	45	30	9	F and M	
		Nemouridae	4	0	4	1	0	1	49	26		
		Notonemouridae	0	0	0	0	0	0	6	0		
		Perlidae	41	0	41	2	0	2	26	33		
		Perlodidae	0	0	0	0	0	0	33	5		
		Pteronarcyidae	0	0	0	0	0	0	5	6		
	Trichoptera		61	75	87	75	46	88	64	64		
		Hydropsychidae	48	27	51	73	7	73	47	54		
		Limnephilidae	2	20	20	0	4	3	19	15		
		Odontoceridae	0	46	45	0	0	0	3	12		
		Philopotamidae	0	2	2	6	13	13	0	4		
		Polycentropodidae	0	4	4	0	6	5	1	0		
		Rhyacophilidae	0	11	11	0	27	25	7	7		
		Sericostomatidae	27	0	27	0	0	0	2	5		
Malacostraca			89	1	89	95	27	97	18	23		
	Amphipoda		89	0	89	95	0	95	17	21		
		Gammaridae	89	0	89	95	0	95	17	21		
	Decapoda	Astacidae	0	1	1	0	27	25	0	1		
		Number of samples	94	92	94	93	85	93	287	184	41	

$p = 0.041$) and family level ($F = 6.59$; $df = 1$; $p = 0.013$). Differences between habitats and between habitats within stream were not significant.

The density of potential prey ($F = 28.60$; $df = 1$; $p < 0.001$), their biomass ($F = 7.62$; $df = 1$; $p = 0.008$) and their average body mass ($F = 52.31$; $df = 1$; $p < 0.001$) differed significantly between streams, with higher density and biomass in Leitzaran and higher average body mass in Elama (Fig. 6a-c).

Differences among habitats and among habitats within each stream were not significant. On the other hand, density ($F = 28.6$; $df = 1$; $p < 0.001$), biomass ($F = 7.81$; $df = 1$; $p = 0.007$) and average body mass ($F = 73.85$; $df = 1$; $p < 0.001$) of consumed prey also differed significantly between streams, density being higher in Leitzarán and biomass and average body mass higher in Elama (Fig. 6d-f). Again, differences among habitats and among habitats within each stream were not significant.

Prey selection

No significant differences were found in prey availability when using any of the tested methods, i.e. comparing the total availability with the availability weighed by the available habitat and the availability weighed by desman activity in each habitat ($F = 0.07$; $df = 2$; $p = 0.932$), so data weighed by desman activity was used to analyse prey selection.

Combining both streams, desmans positively selected Simuliidae and Psychodidae (Diptera), Baetidae and Heptageniidae (Ephemeroptera), Odontoceridae (Trichoptera) and Gammaridae (Crustacea) (Table 2). On the other hand, they negatively selected Planorbidae (Gastropoda), Goeridae (Trichoptera), Perlidae (Plecoptera) and Athericidae (Diptera). Additionally, in Elama they also positively selected Hydropsychidae (Trichoptera), Limnephilidae (Trichoptera), Leptophlebiidae (Ephemeroptera), Chironomidae (Diptera) and Leuctridae (Plecoptera), whilst negatively Rhyacophilidae (Trichoptera) and

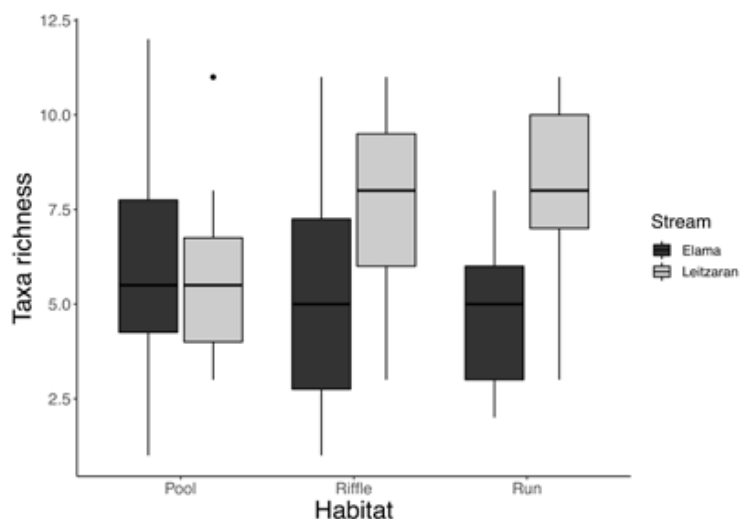


Figure 5. Taxa richness of potential prey per habitat and stream. The confidence limits were represented with the “1.5 rule”: the whiskers extend to the most extreme data point which is no more than [1.5] times the length of the box away from the box.

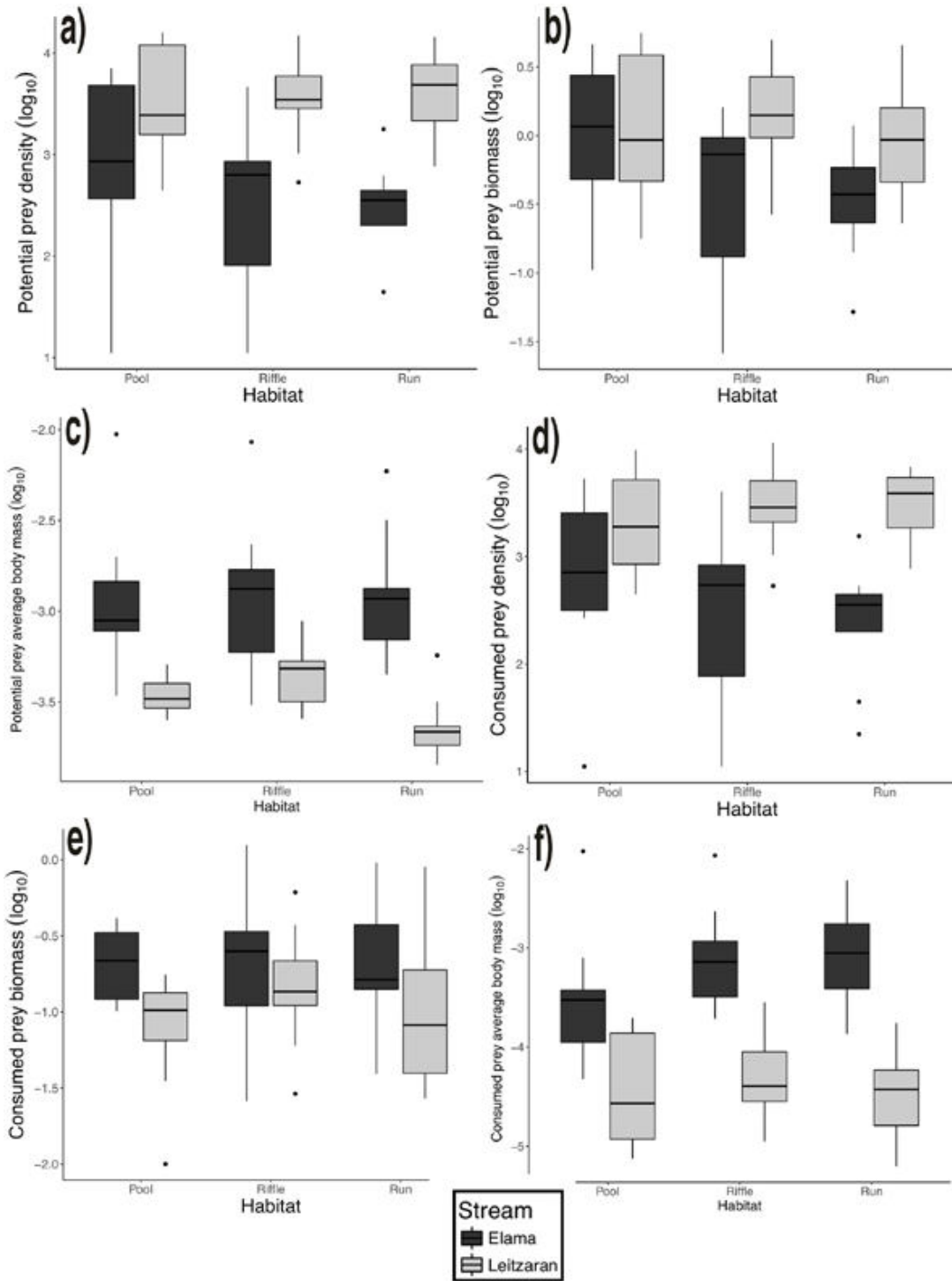


Figure 6. Potential and consumed prey density (a, d), biomass (b, e) and average body mass (c, f) of each habitat in each stream. The confidence limits were represented with the “1.5 rule”: the whiskers extend to the most extreme data point which is no more than [1.5] times the length of the box away from the box.

Table 2. Diet selection at the family level for each stream and for both streams together. Diet was expressed in frequency of occurrence (FO). Average availability of each taxon (Avail.) was weighted by average activity of desmans (see explanation in the text) and expressed also in FO. Dif is the difference between both metrics. Positive and negative selections are highlighted in bold.

Family	Elama			Leitzaran			Total		
	Diet (FO)	Avail. (FO)	Dif	Diet (FO)	Avail. (FO)	Dif	Diet (FO)	Avail. (FO)	Dif
Simuliidae	0.936	0.064	0.87	0.860	0.115	0.75	0.898	0.089	0.81
Psychodidae	0.500	0.000	0.50	0.699	0.000	0.70	0.599	0.000	0.60
Baetidae	0.872	0.064	0.81	0.946	0.804	0.14	0.909	0.434	0.48
Heptageniidae	0.979	0.679	0.30	0.903	0.678	0.23	0.941	0.679	0.26
Odontoceridae	0.447	0.000	0.45	0.000	0.000	0.00	0.223	0.000	0.22
Gammaridae	0.894	0.445	0.45	0.946	0.960	-0.01	0.920	0.702	0.22
Hydropsychidae	0.511	0.241	0.27	0.731	0.716	0.02	0.621	0.478	0.14
Limnephilidae	0.202	0.000	0.20	0.032	0.000	0.03	0.117	0.000	0.12
Leptophlebiidae	0.223	0.000	0.22	0.000	0.015	-0.01	0.112	0.007	0.10
Geometridae	0.064	0.000	0.06	0.097	0.000	0.10	0.080	0.000	0.08
Astacidae	0.011	0.000	0.01	0.247	0.099	0.15	0.129	0.049	0.08
Chironomidae	0.532	0.335	0.20	0.441	0.498	-0.06	0.486	0.416	0.07
Erebidae	0.064	0.000	0.06	0.065	0.000	0.06	0.064	0.000	0.06
Leuctridae	0.277	0.073	0.20	0.452	0.547	-0.10	0.364	0.310	0.05
Mycetophilidae	0.021	0.000	0.02	0.054	0.000	0.05	0.038	0.000	0.04
Noctuidae	0.043	0.000	0.04	0.022	0.000	0.02	0.032	0.000	0.03
Empididae	0.032	0.064	-0.03	0.086	0.000	0.09	0.059	0.032	0.03
Syrphidae	0.000	0.000	0.00	0.043	0.000	0.04	0.022	0.000	0.02
Lonchopteridae	0.011	0.000	0.01	0.032	0.000	0.03	0.021	0.000	0.02
Scatopsidae	0.000	0.000	0.00	0.032	0.000	0.03	0.016	0.000	0.02
Linyphiidae	0.032	0.000	0.03	0.000	0.000	0.00	0.016	0.000	0.02
Cordulegastridae	0.032	0.003	0.03	0.000	0.000	0.00	0.016	0.002	0.01
Aturidae	0.000	0.000	0.00	0.011	0.000	0.01	0.005	0.000	0.01
Diptera	0.000	0.000	0.00	0.011	0.000	0.01	0.005	0.000	0.01
Gyrinidae	0.000	0.000	0.00	0.011	0.000	0.01	0.005	0.000	0.01
Carabidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Chloropidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Oniscidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Pediciidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Scirtidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Glossosomatidae	0.011	0.003	0.01	0.000	0.000	0.00	0.005	0.002	0.00
Philopotamidae	0.021	0.064	-0.04	0.129	0.084	0.05	0.075	0.074	0.00
Rhyacophilidae	0.106	0.322	-0.22	0.247	0.030	0.22	0.177	0.176	0.00
Lumbriculidae	0.000	0.000	0.00	0.000	0.001	0.00	0.000	0.001	0.00
Dytiscidae	0.000	0.000	0.00	0.000	0.003	0.00	0.000	0.001	0.00
Asellidae	0.000	0.003	0.00	0.000	0.000	0.00	0.000	0.002	0.00
Leptoceridae	0.000	0.003	0.00	0.000	0.000	0.00	0.000	0.002	0.00

Table 2. (continuation).

Family	Elama			Leitzaran			Total		
	Diet (FO)	Avail. (FO)	Dif	Diet (FO)	Avail. (FO)	Dif	Diet (FO)	Avail. (FO)	Dif
Ephemerellidae	0.032	0.040	-0.01	0.000	0.000	0.00	0.016	0.020	0.00
Nemouridae	0.043	0.067	-0.02	0.011	0.000	0.01	0.027	0.033	-0.01
Cucurllionidae	0.000	0.000	0.00	0.000	0.015	-0.01	0.000	0.007	-0.01
Sphaeriidae	0.000	0.003	0.00	0.000	0.015	-0.01	0.000	0.009	-0.01
Rhagionidae	0.000	0.040	-0.04	0.000	0.001	0.00	0.000	0.021	-0.02
Sericostomatidae	0.266	0.316	-0.05	0.000	0.000	0.00	0.133	0.158	-0.03
Nematoda	0.000	0.064	-0.06	0.000	0.000	0.00	0.000	0.032	-0.03
Tipulidae	0.064	0.131	-0.07	0.000	0.000	0.00	0.032	0.065	-0.03
Lepidostomatidae	0.000	0.073	-0.07	0.000	0.000	0.00	0.000	0.037	-0.04
Planariidae	0.000	0.000	0.00	0.000	0.084	-0.08	0.000	0.042	-0.04
Hirudinidae	0.000	0.000	0.00	0.000	0.099	-0.10	0.000	0.049	-0.05
Hydrobiidae	0.000	0.000	0.00	0.000	0.100	-0.10	0.000	0.050	-0.05
Ephemeridae	0.043	0.006	0.04	0.000	0.149	-0.15	0.021	0.078	-0.06
Elmidae	0.000	0.000	0.00	0.000	0.115	-0.11	0.000	0.057	-0.06
Hydrophilidae	0.000	0.131	-0.13	0.000	0.000	0.00	0.000	0.065	-0.07
Naididae	0.000	0.000	0.00	0.000	0.196	-0.20	0.000	0.098	-0.10
Lumbricidae	0.000	0.040	-0.04	0.000	0.296	-0.30	0.000	0.168	-0.17
Polycentropodidae	0.043	0.006	0.04	0.054	0.446	-0.39	0.048	0.226	-0.18
Limoniidae	0.096	0.391	-0.30	0.032	0.099	-0.07	0.064	0.245	-0.18
Planorbidae	0.000	0.090	-0.09	0.000	0.354	-0.35	0.000	0.222	-0.22
Goeridae	0.021	0.381	-0.36	0.000	0.197	-0.20	0.011	0.289	-0.28
Perlidae	0.415	0.616	-0.20	0.022	0.418	-0.40	0.218	0.517	-0.30
Athericidae	0.043	0.292	-0.25	0.000	0.548	-0.55	0.021	0.420	-0.40

Limoniidae (Diptera). Besides, in Leitzaran Rhyacophilidae (Trichoptera) were also positively selected, while Naididae and Lumbricidae (Oligochaeta) and Polycentropodidae (Trichoptera) were negatively selected.

Summing up the FO in diet of all positively, opportunistically and negatively selected taxa (Table 2), they averaged 4.49%, 2.83% and 0.25% respectively. Percentages were 6.37, 0.88 and 0.57 for Elama, and 2.71, 4.43 and 0.08 for Leitzaran.

For positively selected prey taxa, there were significant differences between streams in terms of density ($F = 33.1$; $df = 1$; $p < 0.001$), biomass ($F = 4.80$; $df = 1$; $p = 0.033$) and average body mass ($F = 38.14$; $df = 1$; $p < 0.001$). Available density was higher in Leitzaran, but available biomass and average body mass were higher in Elama (Fig. 7a-c). Differences among habitats and among habitats within each stream were not significant.

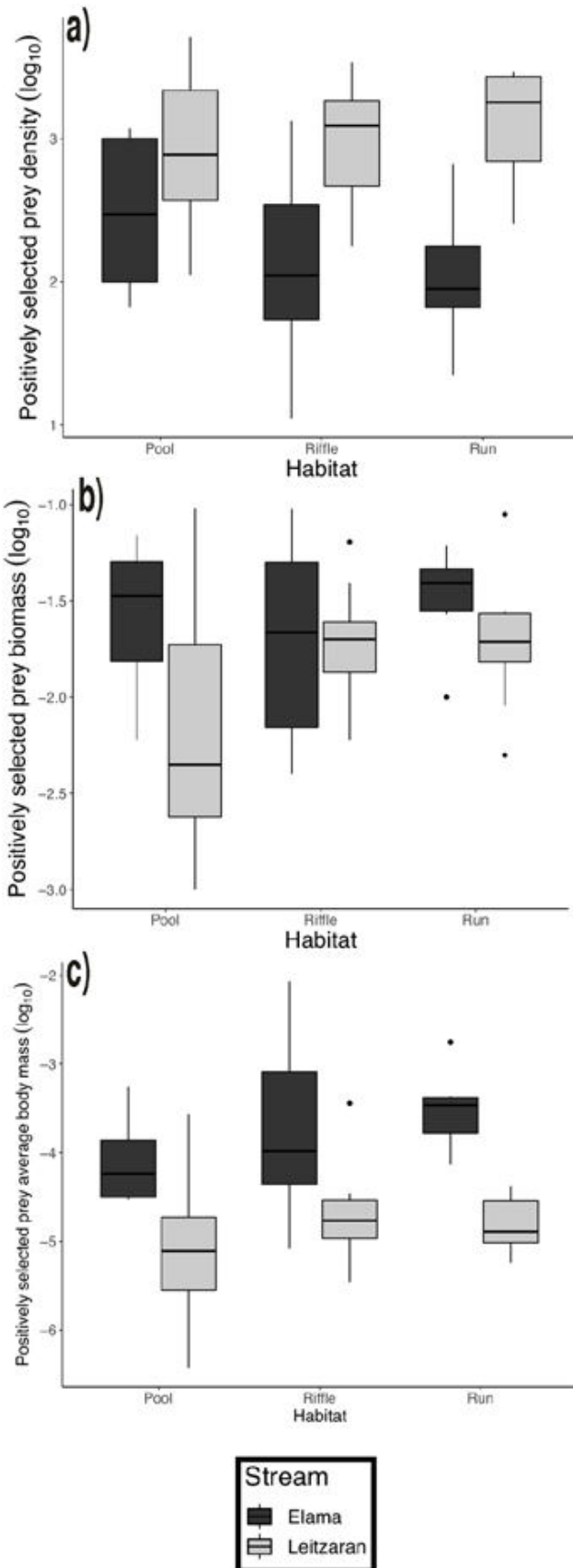


Figure 7. Positively selected prey density (a), biomass (b) and average body mass (c) of each habitat in each stream. The confidence limits were represented with the “1.5 rule”: the whiskers extend to the most extreme data point which is no more than [1.5] times the length of the box away from the box.

Discussion

This study completes the first research on the foraging requirements of the Pyrenean desmans that addresses simultaneously the spatial ecology of two populations (Chapter 2; Esnaola *et al.*, 2018b), their diet and prey availability in their foraging areas. The major finding was that prey availability does not explain desman habitat selection.

Diet

Desmans are specialist rheophilic predators with strong preference for riffle habitat (Chapter 2; Esnaola *et al.*, 2018b). On the other hand, as previously described, the results depict desmans as generalist foragers preying upon a wide variety of prey: 41 species, 23 families and 9 orders of arthropods were identified in their diet.

Traditional morphology-based diet studies of prey remains in guts, stomachs or faeces described desmans as feeding mainly on Trichoptera, Ephemeroptera, Plecoptera, Crustacea (Amphipoda) and Diptera (Bertrand, 1992; Castián & Gosálbez, 1995; Santamarina & Guitián, 1988), and less frequently on Coleoptera, Annelida and Odonata (Puisségur, 1935; Richard & Viallard, 1969; Santamarina, 1992). Similarly, in the present work desmans mostly fed on Ephemeroptera –Heptagenidae and Baetidae–, Amphipoda –Gammaridae–, Diptera –Simuliidae– and Plecoptera, their selection differing between streams –particularly regarding trichopteran Rhyacophilidae–. The species-level identification of consumed prey achieved by molecular methods allows more detailed characterization of desman diet. It is worth noting that molecular methods confirmed the minor importance of soft-bodied prey groups, which could have passed unnoticed in morphological studies. Thus, potential prey such as Clitellata, Nematoda and Amphibians appeared with very low frequency (Biffi *et al.*, 2017a; Chapter 3; Esnaola *et al.*, 2018a). On the other hand, freshwater crayfish (*Pacifastacus*) was a frequent prey in Leitzarán, which had not been reported before for the desman.

Regarding the five studies recently published on desman diet using DNA metabarcoding (Biffi *et al.*, 2017a,b; Chapter 3; Esnaola *et al.*, 2018a; Gillet *et al.*, 2015; Hawlitschek *et al.*, 2018), although methodological differences (Alberdi *et al.*, 2019) limit robust comparisons, there are some interesting aspects to consider. First, a lower number of prey taxa than Hawlitschek *et al.* (2018), Biffi *et al.* (2017a) and Biffi *et al.* (2017b) was found, probably because the bioinformatic procedures in the present work were more conservative, as

OTUs with less than 1% of reads were discarded (i.e. 98.7% of Gillet OTUs and 91% of Zeale OTUs) to avoid secondary predation, false positives and chimeras (Alberdi *et al.*, 2018, 2019; De Barba *et al.*, 2014; Sheppard *et al.*, 2005). Thus, most of the rare taxa detected by Biffi *et al.* (2017a) and Biffi *et al.* (2017b) were most likely filtered out by the procedure of this work. Besides, some of the most frequent (FO>25%) prey groups identified in this study were probably underestimated by Biffi *et al.* (2017a) and Biffi *et al.* (2017b) due to primer bias: in the present study dipterans Psychodidae, Simuliidae, and maybe Chironomidae, trichopterans Odontoceridae, Phylopotamidae, Polycentropodidae or Rhyacophilidae, plecopterans Leuctridae, and Astacidae or Lepidoptera were mainly amplified by Zeale primers (Table 1), confirming the importance of primer choice in metabarcoding studies (Elbrecht & Leese, 2017; Chapter3; Esnaola *et al.*, 2018a). On the other hand, there were some contrasts among the study sites, as well as with those studied by Biffi *et al.* (2017a), Biffi *et al.* (2017b) and Hawlitschek *et al.* (2018), which surveyed much broader areas (Table 1). These contrasts probably reflect differences in faunal composition, consequence of differences in local conditions, although this point cannot be assessed as other authors have not described prey availability. Whatever the reason for differences, it is worth noting that desman diet in Elama resembles more closely than in Leitzaran the diets described by Biffi *et al.* (2017a) and Biffi *et al.* (2017b) in the north of the Pyrenees. Speculation about the reasons for this contrast could be done, but it might reflect the poorer status of the Leitzaran stream as a consequence of intense forest plantations and water diversions for hydropower (CHC, 2018).

Prey selection

The estimated general prey selection patterns coincided with previous studies, albeit with small differences. First, desmans seem to positively select conspicuous invertebrates that live on top of the substrate, such as Simuliidae, Baetidae and Heptageniidae. In this line, Bertrand (1992) also speculated that desmans would positively select benthic and rheophilic prey. Second, they seem to positively select shredding invertebrates linked to coarse organic matter, such as Gammaridae, Odontoceridae, Leptophlebiidae, Limnephilidae and Leuctridae. Casti n and Gos lbez (1995) found negative selection towards Gammarids, which they attributed to their sclerified tegument, but the results of the present study contradict their observation. This probably reflects a preference of desmans for accumulations of leaf litter, where the density of invertebrates, especially large shredders, is higher than in other type of substrata (Flores *et al.*, 2017). Third, desmans negatively select soft-bodied taxa inhabiting within fine sediments, such as Athericidae, Limoniidae, Naididae and Lumbricidae. Fourth, they negatively select invertebrates with

very hard shells, such as Goeridae or Planorbidae, which might reflect a smaller interest in food items hard to process. Molluscs were also selected against by Santamarina (1992). And fifth, desmans negatively selected predators such as Perlidae, Polycentropodidae and Rhyacophilidae. The reason for this negative selection is not very clear, but it could be related to defence/escape strategies exhibited by these predators. Nevertheless, these are tentative interpretations and they cannot be generalised, as some prey with similar characteristics (e.g. Psychodidae) have been positively selected. Remarkably, selection interacted with location as Rhyacophilidae were negatively selected in Elama and positively in Leitzarar. The study of biological and ecological traits of prey could shed some light into this selection pattern.

Location vs selection

In Elama, desmans seemed to positively select taxa related to coarse organic matter (2nd group), which did not happen in Leitzarar. We suggest that the habitat complexity of Elama favoured the retention sites of coarse organic matter (jams), where habitat stability, surface and food resources for invertebrates increased, a higher biomass of prey is hoarded (Flores *et al.*, 2013), and desmans had in average prey with higher body mass, that is, more profitable food, available. When the profitability of the main prey falls below a critical threshold, a generalist predator is expected to switch to alternative more profitable prey items (Křivan, 1996). Therefore, it seems that desmans may opt for other prey in Leitzarar Stream because the preferred ones were lacking, widening their trophic niche to face poorer availability (Araujo *et al.*, 2011).

Choosing a restaurant: the ambience or the menu?

In a previous study on the foraging ecology of the desman, riffles were reported to be their preferred habitat in both study areas (Chapter 2; Esnaola *et al.*, 2018b), and hypothesized it to be a consequence of higher prey availability there. The present work, though, showed no significant inter-habitat differences in prey availability, neither for consumed, nor for positively selected prey. These results raise the question of why do desmans forage preferentially in riffles. It is likely that the fast water current there make it easier for desmans to overcome their natural buoyancy, and crawl on the bottom using their strong claws to take hold (Richard, 1986).

Conclusions

The present study provides new information about desman foraging habits, key for adequate management policies. The results of the present work show desmans to be habitat specialist but generalist predators, which feed upon a wide range of prey, adapting their preferences to local changes in availability. Desmans prefer prey that appear in organic jams and become more opportunistic in less favourable situations. It seems that enhancing channel heterogeneity and the storage of organic matter by increasing the abundance of dead wood (Flores *et al.*, 2013) can be a positive management action to improve desman habitats. Their strong preference for riffles seems to respond to mechanical reasons, as no differences between habitats in terms of prey availability were found. Consequently, management of stream flow and its effect on riffle availability may be key issues for desman conservation.

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Supporting information

Supporting information 1

USEARCH (Edgar, 2010; Edgar & Flyvbjerg, 2015) was used for general sequence processing. Briefly, paired-end reads were aligned and demultiplexed by primers, adapter and primer sequences were removed, and finally, sequences were collapsed into haplotypes. Then, singletons, chimeras, and PCR putative errors were removed using the 'unnoise3' command. Finally, an OTU table was generated by matching raw reads to the remaining haplotypes with a 100% pairwise-identity. The process was carried out independently for each primer.

Finally, Genbank nt (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), BOLD (<http://www.boldsystems.org/>) and personal databases were used to assign taxonomy to OTUs. Species level assignments were performed when query sequences matched reference sequences above 98% pairwise affinity and above 75% query cover, following Clare *et al.* (2013). Besides, as a rule only hits with e-value below $1e^{-20}$ were accepted (Vesterinen *et al.*, 2013) to make sure that the match was not been given by chance. OTUs occurring in less than 3 samples, representing less than 1% of the reads in each sample (Mata *et al.*, 2016), or matching taxa that could not be eaten by desmans (Brose *et al.*, 2006) were removed from analysis, in order to avoid false positives. Subsequent analyses were performed considering the frequency of occurrence (FO) of identified prey taxa (the number of pellets a taxon was found in; Shiel *et al.*, 1997). Reads were checked as well to see whether DNA of the predator itself, i.e. the Pyrenean desman, was also identified in the faecal samples.

The extraction of insects DNA was done with an isopropanol manual extraction method, based on Gilbert *et al.* (2007), Pfeiffer *et al.* (2004) and Aljanabi and Martinez (1997). Extracted DNA was amplified with Gillet and Zeale primers using the same conditions as for the diet. After purification, quantification and library preparation steps, DNA amplicons were sequenced in the *Illumina MiSeq NGS platform*. Insect DNA sequences were aligned and manually edited with the software Geneious 8.0.5 (Kearse *et al.*, 2012). Aligned sequences were checked in Genbank nt and BOLD databases.

Supporting information 2

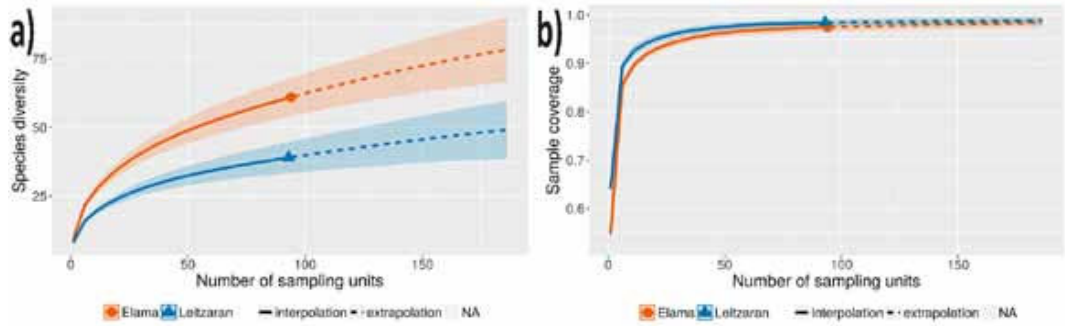


Figure S1. Species accumulation curves for desmans' dietary data: species diversity (a) and sample coverage (b) graphs for Elama and Leitzaran.

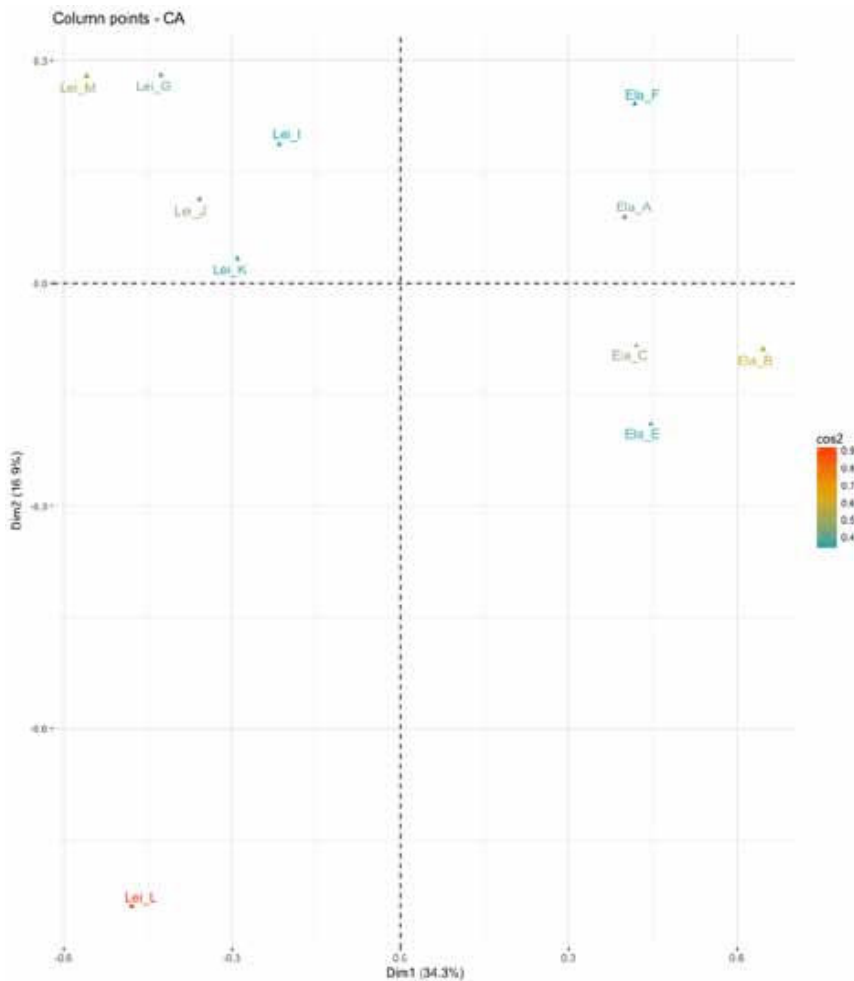


Figure S2. Position of sampling sites in the first factorial plane of a Correspondence Analysis (CA) on the diet composition of the Pyrenean desman (prey family level). Ela referred to sites in Elama and Lei to Leitzaran. Sites D and H were removed from the CA given the small number of samples.

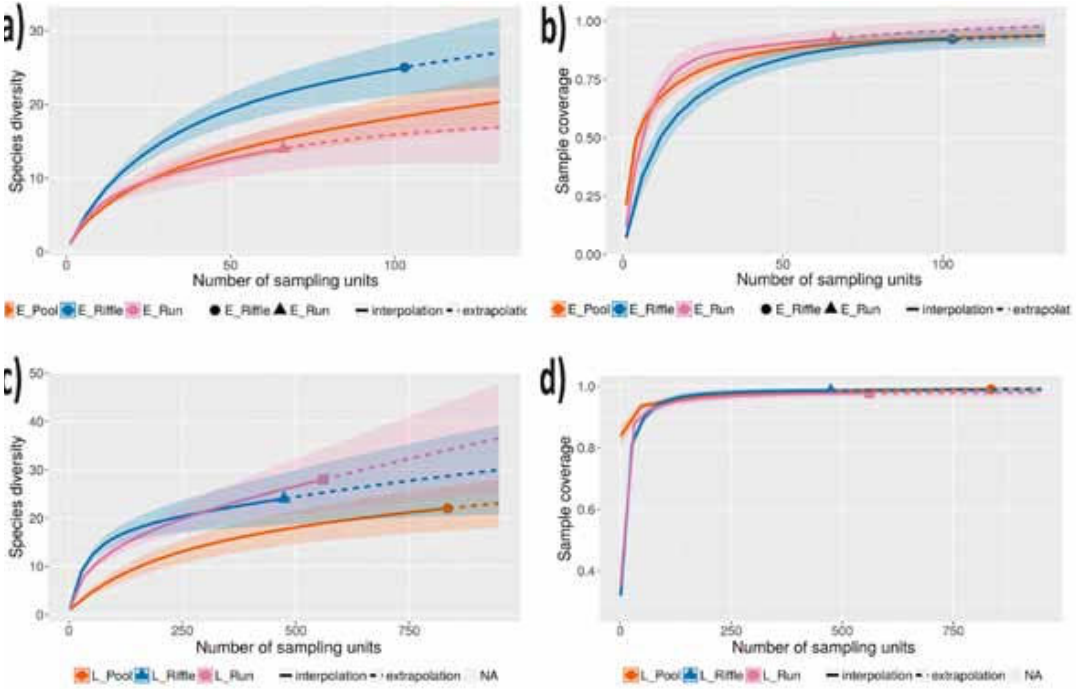


Figure S3. Species accumulation curves for prey availability abundances data: species diversity (a, c) and sample coverage (b, d) graphs, (a, b) for Elama and (c, d) for Leitzaran.

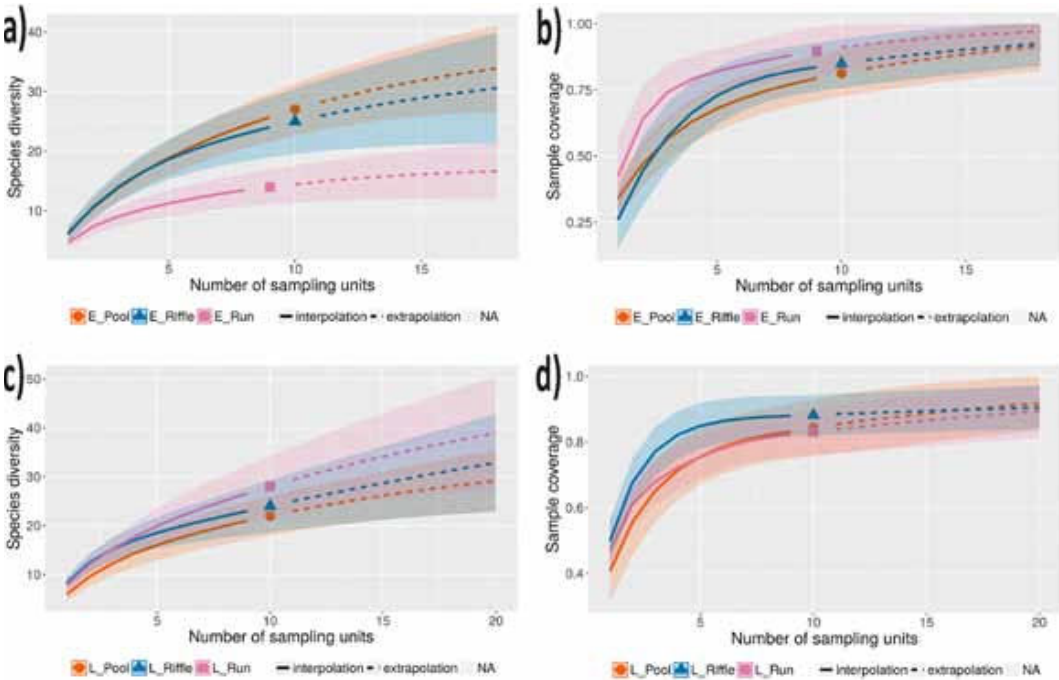


Figure S4. Species accumulation curves for prey availability presence-absence data: species diversity (a, c) and sample coverage (b, d) graphs, (a, b) for Elama and (c, d) for Leitzaran.

Table S1. Details of the primer sets used in this study and PCR conditions for each primer set. “Length (bp)” refers to the amplicon size excluding primers.

Custom name	Primer names	Forward primer(s) (5'-3')	Reverse primer(s) (5'-3')	Length (bp)*	Source	PCR stages
Gillet	F:LepF1	ATTCHACDAAYCAYAAR	ACTATAAAAAAPAAAAATYTD	133	Gillet <i>et al.</i> , 2015	Initialization: 95 °C, 15 min Denaturation (40 cycles): 94 °C, 30 seg 45 °C, 45 seg 72 °C, 30 seg Extension: 72 °C, 10 min
	(modified) R:EPT-long-univR (modified)	GAYATYGG (25 bp)	AYAAADGCRTG (29 bp)			
Zeale	F:ZBU-ArtF1c	AGATATTGGAACWTTAT	WACTAATCAATTWCCAA	157	Zeale <i>et al.</i> , 2011	Initialization: 95 °C, 10 min Denaturation (16 cycles): 95 °C, 30 seg 61 °C, 30 seg 72 °C, 30 seg Annealing (24 cycles): 95 °C, 30 seg 53 °C, 30 seg 72 °C, 30 seg Extension: 72 °C, 10 min
	R:ZBU-ArtR2c	ATTTTATTTTGG (30bp)	ATCCTCC (24bp)			

Table S2. Total prey availability in Elama: abundances in each sample (Surber net) of each habitat.

Taxa	Riffle										Run										Pool																		
	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	01	02	03	04	05	06	07	08	09	10										
<i>Ancylius fluviatilis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aselus aquaticus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix ibix</i>	0	0	0	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i> sp	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Berosus</i> sp	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	0	0	0	1	0	0	9	1	8	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chrysopilus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Climoceridae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dinocras cephalotes</i>	0	4	1	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ecdyonurus</i> sp	1	0	0	0	0	0	0	1	0	1	0	1	1	2	4	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Echinogammarus cf aquilifer</i>	1	2	0	0	1	0	0	1	2	0	0	0	0	4	1	0	0	2	0	0	0	0	0	2	16	0	0	3	4	2	0	0	0	0	0	0	0	0	0
<i>Eiseniella tetraedra</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epeorus</i> sp	0	0	1	3	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera danica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera ignita</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma boltoni</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydropsyche dinarica</i>	0	0	1	0	0	0	4	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydropsyche instabilis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepidostoma hirtum</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leuctra</i> sp	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limoniidae	0	0	0	1	0	0	1	0	2	0	1	0	2	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nematoda	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oecetis testacea</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S2. (continuation)

Taxa	Riffle										Run										Pool									
	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10
<i>Oesimus monedula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	6	0	0	4	0	0	0	0
<i>Perla</i> sp	0	2	4	2	0	4	1	1	0	0	1	3	4	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	2	0
<i>Philopotamus</i> sp	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
<i>Protonemura pirenaica</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Protonemura vandeli</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Rhithrogena</i> sp	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila adjuncta</i>	0	1	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila meridionalis</i>	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila obelix</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila tristis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Sericostoma</i> sp	0	0	0	0	0	0	1	0	0	0	0	0	3	1	1	4	1	4	0	8	4	32	1	1	17	21	18	3	8	0
<i>Silo</i> sp	0	1	0	0	0	1	0	0	1	0	1	1	1	2	0	0	1	0	1	0	0	3	0	0	0	0	0	0	0	0
Simuliidae	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sphaerium</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tipulidae	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table S3. Total prey availability in Leizaran: abundances in each sample (Surber net) of each habitat.

Taxa	Riffle										Run										Pool										
	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10	
<i>Ancylus fluviatilis</i>	2	0	0	0	2	0	0	1	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	3	0	0	0	0	
<i>Atherix ibix</i>	0	3	0	1	0	1	1	5	3	0	0	0	0	0	1	0	0	0	1	0	2	1	0	0	0	0	0	0	0	0	
<i>Aulodrilus pluriseta</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Baetis</i> sp	4	7	10	1	0	5	2	3	11	3	2	0	0	6	0	3	0	0	0	0	0	0	1	1	1	0	1	0	0	0	
Chironomidae	4	5	0	0	0	0	11	0	0	2	51	44	21	3	35	4	94	8	32	25	6	81	100	12	18	167	33	103	13	231	
<i>Chrysopilus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cucurionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Ecdyonurus</i> sp	0	0	0	2	0	0	0	1	2	0	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	2	0	0	0	
<i>Echinozammarus longisetosus</i>	17	38	17	2	39	25	25	19	59	19	9	2	0	0	9	11	1	4	2	25	0	0	0	0	0	0	1	0	2	1	0
<i>Eiseniella tetraedra</i>	0	0	2	0	0	0	1	2	0	0	4	0	0	0	0	0	2	0	0	6	0	0	0	0	0	0	0	0	0	0	
<i>Embolecephalus velutinus</i>	0	0	0	0	0	0	0	0	0	1	0	1	0	4	0	4	1	4	3	0	0	0	0	1	0	0	0	0	0	2	
<i>Epeorus</i> sp	0	1	0	0	1	0	1	1	6	7	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Ephemera danica</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	1	2	1	0	1	0	1	0	0	0	0	0	1	
<i>Goera pilosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Hirudinae	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Hydropsyche dinarica</i>	0	1	2	0	0	0	3	4	3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Hydropsyche siltalai</i>	0	4	1	1	2	0	0	4	5	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Leuctra</i> sp	4	7	0	0	0	0	1	1	5	0	14	13	0	9	10	4	5	0	1	14	2	1	4	2	0	1	0	1	1	0	
<i>Limnius</i> sp	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
<i>Limnodrilus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
Limoniidae	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lumbriculidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table S3. (continuation)

Taxa	Riffle										Run										Pool									
	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10	01	02	03	04	05	06	07	08	09	10
<i>Oulimnius</i> sp	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pacifastacus leniusculus</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Perla</i> sp	1	4	3	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Philopotamus</i> sp	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Planaria</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Platambus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	
<i>Polycentropus</i> sp	1	9	0	0	1	0	0	0	1	0	6	1	0	1	1	4	0	0	3	4	0	0	0	1	2	1	3	1	0	
<i>Potamopyrgus antipodarum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	1	0	0	0	
<i>Rhyacophila meridionalis</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Rhyacophila relicta</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Silo</i> sp	0	0	1	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Simuliidae	0	0	0	0	0	0	0	0	0	10	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
<i>Sphaerium</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Stylodrilus</i> sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	1	1	2	
Tubificinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	2	0	

Table S4. References used to calculate prey biomass (gAFDMm⁻²) of different taxa.

Reference	Taxa
Burgherr and Meyer (1997)	Cucurlionidae, Dytiscidae, Elmidae (<i>Limnius</i> sp.), Hydropsychidae
Larrañaga <i>et al.</i> (2009)	Gammaridae
Meyer (1989)	Ancyliidae, Athericidae, Baetidae, Chironomidae, Elmidae (<i>Oulimnius</i> sp.), Empididae, Ephemerellidae, Glossosomatidae, Goeridae, Heptageniidae, Leuctridae, Limoniidae, Nemouridae, Perlidae, Rhagionidae, Rhyacophilidae, Sericostomatidae, Simuliidae
Benke <i>et al.</i> (1999)	Cordulegastridae, Ephemeridae, Lepidostomatidae, Leptoceridae, Leptophlebiidae, Philopotamidae, Planariidae, Polycentropodidae, Sphaeriidae, Tipulidae
Stoffels <i>et al.</i> (2003)	Hydrobiidae, Oligochaeta
Baumgärtner and Rothaupt (2003)	Asellidae

Table S5. Diet table: taxa in desman's diet and their occurrences in each stream (with each primer set –G = Gillet, Z = Zeale– and with both primer sets –T = total–). In red, taxa that occur in more than 50% of the samples, and in orange taxa that occur in more than 30% of the sample.

Pylum	Class	Order	Family	Species	Elama			Leitzaran		
					G	Z	T	G	Z	T
Arthropoda	Crustacea	Malacostraca	Oniscidae	<i>Oniscus asellus</i>	1	0	1	0	0	0
Arthropoda	Arachnida	Araneae	Aturidae	<i>Aturidae</i> sp	0	0	0	1	0	1
Arthropoda	Arachnida	Trombidiformes	Linyphiidae	-	0	3	3	0	0	0
Arthropoda	Insecta	Coleoptera	Carabidae	-	0	1	1	0	0	0
Arthropoda	Insecta	Coleoptera	Gyrinidae	-	0	0	0	0	1	1
Arthropoda	Insecta	Coleoptera	Gyrinidae	<i>Orectochilus villosus</i>	0	0	0	1	1	1
Arthropoda	Insecta	Coleoptera	Scirtidae	<i>Elodes marginata</i>	1	0	1	0	0	0
Arthropoda	Insecta	Diptera	-	-	0	0	0	0	1	1
Arthropoda	Insecta	Diptera	Athericidae	<i>Atherix ibix</i>	0	4	4	0	0	0
Arthropoda	Insecta	Diptera	Chironomidae	-	12	47	50	8	31	36
Arthropoda	Insecta	Diptera	Chironomidae	<i>Barbadocladus</i> sp	0	0	0	0	5	5
Arthropoda	Insecta	Diptera	Chironomidae	<i>Brillia bifida</i>	0	0	0	1	0	1
Arthropoda	Insecta	Diptera	Chloropidae	-	0	1	1	0	0	0
Arthropoda	Insecta	Diptera	Empididae	<i>Wiedemannia rhychops</i>	1	0	1	0	0	0
Arthropoda	Insecta	Diptera	Empididae	<i>Wiedemannia zetterstedti</i>	0	3	3	0	8	8
Arthropoda	Insecta	Diptera	Limoniidae	-	0	1	1	0	3	3
Arthropoda	Insecta	Diptera	Limoniidae	<i>Eloeophila</i> sp	0	8	8	0	0	0
Arthropoda	Insecta	Diptera	Lonchopteridae	-	0	1	1	0	3	3
Arthropoda	Insecta	Diptera	Mycetophilidae	-	0	0	0	0	5	5
Arthropoda	Insecta	Diptera	Mycetophilidae	<i>Gnoriste apicalis</i>	0	2	2	0	0	0
Arthropoda	Insecta	Diptera	Pediciidae	-	0	1	1	0	0	0
Arthropoda	Insecta	Diptera	Psychodidae	<i>Psychoda erminea</i>	2	47	47	0	65	65
Arthropoda	Insecta	Diptera	Scatopsidae	-	0	0	0	0	3	3
Arthropoda	Insecta	Diptera	Simuliidae	-	1	88	88	2	80	80
Arthropoda	Insecta	Diptera	Syrphidae	<i>Heringia</i> sp	0	0	0	0	4	4

Table S5. (continuation - 1)

Pylum	Class	Order	Family	Species	Elama			Leitzaran		
					G	Z	T	G	Z	T
Arthropoda	Insecta	Diptera	Tipulidae	-	0	6	6	0	0	0
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Alainites muticus</i>	51	0	51	0	0	0
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Baetis</i> sp	70	0	70	88	0	88
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Centroptilum luteolum</i>	1	0	1	0	0	0
Arthropoda	Insecta	Ephemeroptera	Baetidae	<i>Procloeon pennulatum</i>	0	0	0	1	0	1
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	<i>Ephemerella ignita</i>	0	1	1	0	0	0
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	<i>Torleya major</i>	2	0	2	0	0	0
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	<i>Ephemera danica</i>	4	0	4	0	0	0
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	-	0	1	1	0	0	0
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Ecdyonurus</i> sp	91	18	91	56	0	56
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Electrogena lateralis</i>	7	0	7	0	0	0
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Epeorus</i> sp	47	26	51	79	51	79
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	<i>Rhithrogena</i> sp	9	0	9	0	0	0
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	<i>Habrophlebia eldae</i>	20	0	20	0	0	0
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	<i>Habrophlebia lauta</i>	4	2	4	0	0	0
Arthropoda	Insecta	Lepidoptera	Erebidae	<i>Herminia tarsicrinalis</i>	0	6	6	0	6	6
Arthropoda	Insecta	Lepidoptera	Geometridae	-	0	1	1	0	1	1
Arthropoda	Insecta	Lepidoptera	Geometridae	<i>Idaea degeneraria</i>	0	1	1	0	1	1
Arthropoda	Insecta	Lepidoptera	Geometridae	<i>Parectropis similaria</i>	0	2	2	0	0	0
Arthropoda	Insecta	Lepidoptera	Geometridae	<i>Trichopteryx carpinata</i>	0	4	4	0	7	7
Arthropoda	Insecta	Lepidoptera	Noctuidae	<i>Deltote pygarga</i>	0	4	4	0	1	1
Arthropoda	Insecta	Lepidoptera	Noctuidae	<i>Spodoptera exigua</i>	0	0	0	0	1	1
Arthropoda	Insecta	Odonata	Cordulegastridae	<i>Cordulegaster</i> sp	3	0	3	0	0	0
Arthropoda	Insecta	Plecoptera	Leuctridae	<i>Leuctra</i> sp	21	19	26	28	37	42
Arthropoda	Insecta	Plecoptera	Nemouridae	<i>Protonemura meyeri</i>	0	0	0	1	0	1
Arthropoda	Insecta	Plecoptera	Nemouridae	<i>Protonemura pirenaica</i>	1	0	1	0	0	0
Arthropoda	Insecta	Plecoptera	Nemouridae	<i>Protonemura vandeli</i>	3	0	3	0	0	0
Arthropoda	Insecta	Plecoptera	Perlidae	-	1	1	1	0	0	0
Arthropoda	Insecta	Plecoptera	Perlidae	<i>Dinocras cephalotes</i>	5	0	5	0	0	0
Arthropoda	Insecta	Plecoptera	Perlidae	<i>Perla</i> sp	36	0	36	2	0	2
Arthropoda	Insecta	Trichoptera	Glossosomatidae	<i>Glossosoma boltoni</i>	1	1	1	0	0	0
Arthropoda	Insecta	Trichoptera	Goeridae	<i>Silo</i> sp	0	2	2	0	0	0
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche dinarica</i>	45	25	48	16	6	19
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche instabilis</i>	1	0	1	1	0	1
Arthropoda	Insecta	Trichoptera	Hydropsychidae	<i>Hydropsyche siltalai</i>	0	0	0	65	0	65
Arthropoda	Insecta	Trichoptera	Limnephilidae	-	0	17	17	0	3	3
Arthropoda	Insecta	Trichoptera	Limnephilidae	<i>Chaetopteryx villosa x fusca</i>	1	0	1	0	0	0
Arthropoda	Insecta	Trichoptera	Limnephilidae	<i>Halesus radiatus</i>	1	1	1	0	0	0
Arthropoda	Insecta	Trichoptera	Odontoceridae	<i>Odontocerum albicorne</i>	0	42	42	0	0	0
Arthropoda	Insecta	Trichoptera	Philopotamidae	<i>Chimarra marginata</i>	0	0	0	0	3	3
Arthropoda	Insecta	Trichoptera	Philopotamidae	<i>Philopotamus</i> sp	0	2	2	6	8	9
Arthropoda	Insecta	Trichoptera	Polycentropodidae	<i>Polycentropus</i> sp	0	4	4	0	5	5

Table S5. (continuation - 2)

Pylum	Class	Order	Family	Species	Elama			Leitzaran		
					G	Z	T	G	Z	T
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	<i>Rhyacophila fasciata</i>	0	1	1	0	0	0
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	<i>Rhyacophila meridionalis</i>	0	9	9	0	0	0
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	<i>Rhyacophila relictata</i>	0	0	0	0	23	23
Arthropoda	Insecta	Trichoptera	Sericostomatidae	<i>Sericostoma</i> sp	25	0	25	0	0	0
Arthropoda	Malacostraca	Amphipoda	Gammaridae	-	0	0	0	2	2	2
Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Echinogammarus cf aquilifer</i>	84	0	84	0	0	0
Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Echinogammarus longisetosus</i>	1	0	1	88	0	88
Arthropoda	Malacostraca	Decapoda	Astacidae	<i>Pacifastacus leniusculus</i>	0	1	1	0	23	23
Total samples					93	92	94	94	85	93

Chapter 5



Trait-based dietary characterization
of the Pyrenean desman
(*Galemys pyrenaicus*,
Insectivora, Talpidae)

Abstract

Traditionally, diet selection has been assessed by comparing the taxonomic composition of consumed versus available food. However, taxonomic adscription is irrelevant to predators, who may base their selection in other characteristics instead, of which prey biological traits could give clues. To understand the criteria driving the food preferences of the threatened Pyrenean desman (*Galemys pyrenaicus*), we performed a trait-based analysis of prey selection in two contrasting streams, one in excellent conservation status, and the other affected by diversion hydropower schemes. To do that, 94 desman faeces were sampled and metabarcoded at each stream to identify consumed prey. Prey availability was measured from Surber samples taken in three main habitat types: pools, runs, and the highly preferred riffles. To infer trait preference, we compared available and consumed invertebrates by looking at nine biological traits. Desman diet and main prey selection pattern were very similar in both streams despite differences in prey availability between streams and among habitats. Desmans positively selected prey with potential size of **2-8 mm**, prey living on the surface of the benthos, agile prey, prey with **some** body flexibility and prey living **clustered**. The rest of preferred traits (**fast** flow, boulder-size substrate, etc.) were linked to riffle habitats. Almost all positively selected trait categories were more abundant in riffles. Overall, our data outlined the importance of riffles as a foraging habitat for the Pyrenean desman, although it is unclear whether this reflects higher availability of preferred prey or mechanical constraints on its hunting habits.

Keywords

Aquatic mammal, DNA metabarcoding, functional traits, feeding behaviour, macroinvertebrates, management

Introduction

Conservation of endangered species is often limited by lack of detailed knowledge about their biology, including factors that determine their distribution, habitat, or diet requirements (Morrison *et al.*, 2006). Diet studies traditionally relied on visual identification of prey remains in predator faeces and guts, but are being replaced by new molecular tools such as DNA metabarcoding (Bohmann *et al.*, 2014; Sheppard & Harwood, 2005; Taberlet *et al.*, 1999). Whatever the technique, most studies analyse diet from the point of view of taxonomic composition, although taxonomic adscription may be irrelevant for consumers, who likely select prey depending on other characteristics such as their abundance, detectability, catchability, profitability or nutritional quality (Almenar *et al.*, 2013; Symondson, 2002). Selectivity is inferred when prey consumption significantly differs from availability (Manly *et al.*, 2002), although the latter is difficult to measure and often not equivalent to prey counts, as other factors influence prey detectability, accessibility, etc. (Backwell *et al.*, 1998; Holmes & Schultz, 1988). Predator sensory capabilities constrain the potential prey upon which “active prey selection” may act (Siemers & Guttinger, 2006), and then, other factors such as prey catchability, evasive or defensive behaviour or taste (De Crespín & Usseglio-Polatera, 2002; Spitz *et al.*, 2014; Stephens & Krebs, 1986) are likely to affect the predator foraging strategy. Some of these biologically relevant characteristics are reflected in prey functional traits (Schmitz, 2017), which could thus yield essential clues about the factors that drive predator foraging preferences.

Although rarely used for insectivorous mammals, trait-based approaches may allow researchers to detect prey selection patterns in species erroneously thought to be opportunistic based on taxonomic studies (Spitz *et al.*, 2014), or conversely, unveil a broad functional trophic spectrum in species previously considered strict prey specialists (Arrizabalaga-Escudero *et al.*, 2019). Therefore, trait-based approaches can greatly improve our knowledge of relationships between species and their environments (Luck *et al.*, 2012), and even mark a breakdown in foraging ecology (Spitz *et al.*, 2014). Although prey trait studies have some limitations (Cesar & Frid, 2012; Kremer *et al.*, 2017; Poff *et al.*, 2006), the way traits characterize prey communities are likely more relevant for the predator than taxonomic adscription.

The Pyrenean desman (*Galemys pyrenaicus*, É. Geoffroy Saint-Hilaire, 1811, Insectivora, Talpidae) is a semi-aquatic insectivorous generalist mammal that lives in cold clean mountain streams. It is endemic to the northern Iberian Peninsula and the Pyrenees, but its distribution area has been severely reduced during the last decades, being currently listed as Vulnerable in the Red List

Categories by the IUCN (Fernandes *et al.*, 2008). The desman shows a strong preference for fast-flowing riffles over slower runs or pools (Chapter 2; Esnaola *et al.*, 2018b), although benthic invertebrates, the main prey for the desman, are not more abundant there (Chapter 4). This raises the question of why do desmans show such a strong preference for riffles, and one potential explanation is that, despite overall abundance being similar, the prey types favoured by desmans are disproportionately more abundant there. This question calls for a trait-based approach to unveil desman preferences, what could offer key information for effective conservation measures.

Here, we present results of a study in which we analysed desman diet by DNA metabarcoding and compared it to prey availability at the habitat scale in two contrasting streams, to see whether prey selection changes with streams conservation status. The aims of our study were to (1) identify the prey traits that drive diet selection by the Pyrenean desman, (2) analyse whether trait preference can explain desman habitat selection, and (3) assess whether preferred traits differ between the two streams. The main hypothesis was that desmans would prefer large slow moving prey living in clusters on the surface of the streambed, without defence mechanisms. A second hypothesis was that these preferences drive the use of habitats by the desman and explain the higher preference for riffles in the stream with lower conservation status.

Materials and methods

Study area

This study was conducted in 2016, together with a radiotracking study (Chapter 2; Esnaola *et al.*, 2018b) in two streams in the northern Iberian Peninsula (the Basque Country). Elama is a second-order headwater stream draining an uninhabited basin of 1,415 ha over granite and schist in Artikutza estate, which has been managed as a strict Nature Reserve since 1919, having no extractive activity at present and being almost totally covered of beech and oak forests (Castro, 2009). Riffles and runs are similarly available in the study area (45% each), being pools the scarcest habitat (Chapter 2; Esnaola *et al.*, 2018b). Leitzaran is a fourth-order stream draining a basin of 12,402 ha over limestone, slate, and sandstone. Contrasting with Elama, in the headwaters of Leitzaran there are two towns totalling 3,150 inhabitants, but then the stream enters a long, uninhabited valley approximately 25 km in length where forestry and hydropower diversion schemes are almost the only human activities (Izagirre *et al.*, 2013). In Leitzaran runs are dominant (60%) and pools are again the

least abundant habitat (Chapter 2; Esnaola *et al.*, 2018b). Although these streams experience different environmental pressures and Elama has a better conservation state, both are in good ecological status according to the EU Water Framework Directive (Council of the European Communities, 2000), and both belong to the EU Natura 2000 network of protected areas (Council of the European Communities, 1992).

Sampling was carried out on the same stretches and periods as the radiotracking work published by Esnaola *et al.* (2018b; Chapter 2), on a 4-km section of Elama stream (from 43°12'40"N, 1°48'36"W to 43°11'14"N, 1°48'4"W; mean altitude, 330 m; mean width 7.08 m) and on a 10-km section of Leitzarán stream (from 43°8'57"N, 1°57'26"W to 43°6'55"N, 1°56'9"W; mean altitude, 290 m; mean width, 12.42 m). Section lengths were different because of the greater difficulty in trapping desmans in Leitzarán.

Desman diet

We searched for faeces of the Pyrenean desman in Elama and Leitzarán streams in September and October 2016, using artificial shelters specially designed for this species (Appendix 1; González-Esteban *et al.*, 2018). A total of 188 droppings (94 per stream) were collected and stored in tubes containing 98% ethanol and frozen at -80 °C until DNA extraction.

DNA extraction from desman faecal samples, PCR amplification and high throughput sequencing (HTS) were performed as described in Esnaola *et al.* (2018a; Chapter 3). In short, DNA was extracted from faeces using *Qiagen Powerfecal DNA kit* (Qiagen Iberia, S.L. Madrid), following the manufacturer guidelines and fragments of the COI barcode region were subsequently PCR amplified using LepF1/EPT-long-univR (modified following Gillet *et al.*, 2015) and ZBJ-ArtF1c/ZBJ-ArtR2c primer pairs (Zeale *et al.*, 2011). Samples were purified and a second reaction was performed to index each amplified product and attach Illumina adaptors using the Illumina Nextera v2 kit. Sequencing was performed on the *Illumina MiSeq NGS platform* (sequencing of 2x300 bp paired-end reads) with the *MiSeq Reagent Kit v3 (600 cycle)*, following the manufacturer instructions. USEARCH (Edgar, 2010; Edgar & Flyvbjerg, 2015) was used for general sequence processing and Genbank nt (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), BOLD (<http://www.boldsystems.org/>) and personal databases to assign taxonomy to OTUs (Operational Taxonomic Units) with BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). This work was performed at the Analytical Services (SGIker) of the University of the Basque Country, UPV/EHU. Results were expressed in frequency of occurrence (FO, the proportion of scats where each prey taxon was detected).

Prey availability

Aquatic macroinvertebrates were sampled with a Surber net (30 cm x 30 cm; 1 mm mesh) across three habitat types (Overton *et al.*, 1997): “riffles” with fast, turbulent water, uneven surface level, and white water; “runs” with close to laminar flow and even depth; and “pools” with slow flow on riverbed depressions. Ten samples were collected per habitat and stream in randomly selected locations, making a total of 60 samples. These were stored in plastic jars and preserved with 70% ethanol until identification.

In the laboratory, macroinvertebrates were sieved with a 2 mm mesh, sorted, counted and identified under a dissecting microscope. The 2 mm mesh was chosen because, following Brose *et al.* (2006) predator-prey body size relationships, prey smaller than 2 mm were not expected to be consumed by desmans. Taxonomic resolution reached genus level for most taxa, with the exception of some Diptera, Coleoptera, Oligochaeta, Hirudinidae and Nematoda, which were identified at family or order level.

Data analysis

Sampling completeness was assessed for both diet and invertebrate availability by building species accumulation curves using the ‘iNEXT’ online platform (Chao *et al.*, 2016). These reached an asymptote and sample completeness was over 95%, confirming that the sample size used was appropriate to describe the diet of desmans in both streams (see Chapter 4).

Trait selection was inferred from 9 traits potentially affecting desman prey selection. Among these, 4 (depth preference, current velocity preference, substrate preference and preference of exposure to the flow; Table 1) were likely influenced by the type of habitat (riffle, pool or run), and the other 5 (type of mobility/attachment to substrate, agility, aggregation tendency, maximal potential body size and body flexibility; Table 1) were considered to be independent. Each trait had 2-5 categories, with affinity scores (from 0 to 5) of each taxon for each category expressed by means of fuzzy coding (Chevenet *et al.*, 1994; Sánchez-Hernández, 2014; Table S1).

Affinities were transformed into relative affinities by dividing each value by the sum of affinities for each trait of each taxon. For taxonomic levels with no trait data (e.g. Chironomidae), the average value of all the lower taxonomic levels was computed (see Sánchez-Hernández, 2014; Table S1). Taxa with no data at

all (annelids, lepidopterans and some terrestrial coleopterans, all of them rarely consumed; see Chapter 4) were excluded from the analyses (Table S1).

For the analysis a “taxa x traits” array was built both for diet and availability, which was weighed by means of frequency of occurrence (FO) in diet and the abundance of the individuals for availability in the benthos. These values were summed and transformed into relative proportions in diet for each stream (building a “stream x traits” array), and in availability for each sample category (building a “sample category x traits” array, where sample categories were: Elama riffle, Elama run, Elama pool, Leitzaran riffle, Leitzaran run and Leitzaran pool).

Table 1. Traits and their categories used to characterize desman prey. Trait categories, originally described by De Crespín & Usseglio-Polatera (2002), have been renamed and reclassified as shown here. Data compilation: Sánchez-Hernández (2014).

Trait	Abbreviation	Categories	Sánchez-Hernández's original categories
Depth	DEP1	Hyporheic	Hyporheic 'burrower' + Hyporheic 'interstitial'
	DEP2	Epibenthic	Epibenthic erosional + Epibenthic depositional
	DEP3	Water column	Water column
Current velocity	CUV1	Slow	Still / Slow (0-25 cm/s)
	CUV2	Moderate	Moderate (25-75 cm/s)
	CUV3	Fast	Fast (>75 cm/s)
Substrate	SUS1	Blocks	Blocks (>256 mm)
	SUS2	Gravel-Cobble	Fine gravel / Gravel-Cobble (2-256 mm)
	SUS3	Fine sediment	Silt (0.001-0.2 mm) + Sand-Silt (0.2-2 mm) + Mud
	SUS4	Macrophytes-Roots	Bryophytes + Other macrophytes + Roots
	SUS5	Litter	Litter, organic detritus
Flow exposure	FLE1	Protected	Protected
	FLE2	Exposed	Exposed
Mobility/attachment to substrate	MAS1	Swimmer	Swimmer
	MAS2	Crawler	Crawler / walker + Crawler / slider
	MAS3	Attached	Permanently attached + Temporarily attached
Agility	AGI1	None	None
	AGI2	Weak	Weak
	AGI3	High	High
Aggregation tendency	AGT1	High	High
	AGT2	Weak	Weak
Potential size	POS1	≤ 2	≤ 2 mm
	POS2	2-8	> 2-4 mm + > 4-8 mm
	POS3	8-32	> 8-16 mm + > 16-32 mm
	POS4	> 32	> 32 mm
Body flexibility (including cases/tubes)	BOF1	None	None (<10°)
	BOF2	Weak	Weak (10-45°)
	BOF3	High	High (> 45°)

Traits of prey availability and desman diet were described for both streams (and for each of the three habitats for availability) with proportions of each trait category. Differences in weighed trait category values for prey availability between streams and among habitats were analysed with ANOVA by permutation using the function *Imp* of the *ImPerm* package for R (Wheeler & Torchiano, 2016). We fitted stream, habitat, trait-category and second order interactions to the models by permutation.

A multivariate analysis was performed looking for similarities between diet and availability data, and between streams and among habitats. For that, a fuzzy principal component analysis (FPCA; using the *prep.fuzzy.var* and *dudi.fpca* functions from the *ade4* package for R) was performed taking into account all the categories of all the traits together. FPCA is a robust modification of principal component analysis (Cundari *et al.*, 2002). Finally, a redundancy analysis (RDA, with a Hellinger distance matrix and the functions *rda* and *anova.cca* from the *vegan* package for R) was used to assess significance of stream, habitat and diet vs. availability in explaining the variation of trait categories. We show the relative importance of each trait explaining the overall differences between the two streams, the three habitats and between diet and availability by means of inertia values.

Then, desman prey selection was considered by means of diet-to-availability ratios. As desman faeces could not be linked to specific habitats, we did not consider separately the different habitats in this part of the study. Instead, the “taxa x traits” array of availability data was multiplied by taxonomic abundance values previously weighed by the relative habitat surface in the studied section of each stream. Weighing the abundance in the benthos by the habitat surface we take into account the contribution of the habitat to the total availability of prey at the reach level. After that, a $\log_{10}(\text{diet}/\text{availability})$ ratio was calculated for each trait category and sample, dividing the diet value of each stream by each of the availability replicates. To deal with the zeros in availability when computing the ratios, we added a constant to every value (half of the minimum value in the database) before calculating the ratio. Finally, the average of the ratio with its confidence interval was calculated for each stream. This ratio served to test whether desmans select (positively or negatively) specific prey trait categories. To test if the selection of trait categories was stream dependent an ANOVA by permutation was performed to test for similarities for the affinities of trait categories between the two studied streams, using the function *Imp* of the *ImPerm* package for R (Wheeler & Torchiano, 2016). Again, stream, habitat, trait-category and second-order interactions to the models by permutation were included in the models. The significance level of all tests was set at $p < 0.05$. All analyses were performed using R 3.4.3 (R Core Team, 2014).

Results

Traits in diet

The relative contribution of the various trait categories to desman diet was very similar in both streams (Figure 1). Regarding the preferred depth trait, desmans mainly consumed *epibenthic* (73.3% in average), followed by *hyporheic* (15.3%) and *water column* prey (11.3%). Differences were negligible among traits related to current velocity (*moderate* 35.0%, *slow* 34.8%, *fast* 30.2%). Regarding substrate categories, desmans mainly consumed prey associated to *gravel-cobble* (31.1%) and *macrophytes-roots* (29.8%), followed by *blocks* (19.5%), *fine sediment* (11.1%) and finally, *litter* (8.4%). The flow exposure trait showed little differences (53.2% for *exposed* vs. 46.8% for *protected*), whereas regarding the mobility/attachment to substrate, *crawler* prey were consumed much more frequently (70.5%) than the rest of the categories. Prey with *no agility* were the least frequent by a large margin (4.2% vs. 52.9% for *weak* and 42.8% for *high* agility, respectively), whereas prey with *high* aggregation tendency were more frequent in diet (64.6%) than those with *weak* tendency (35.4%). In terms of potential size, desmans mainly consumed *medium-sized* prey (*8-32 mm*, 64.3%, followed by *2-8 mm* 33.6%), desmans very rarely consumed prey with *large* potential size (> 32 mm, 2.1%), and almost never prey with potential size ≤ 2 mm. Finally, the body flexibility trait showed the highest relative contribution for *high* flexibility (49.0%), followed by *weak* (34.7%) and *none* (16.2%) categories.

Prey availability

Regarding the abundance (availability) of invertebrates classified by trait categories, there were interesting differences among habitats, as well as between streams (Figure 2). *Epibenthic* fauna was most abundant in all habitats and streams (66.3% in average), followed by *hyporheic* (22.3%) and *water column* fauna (11.4%); the proportion of depth preferences changed significantly among the studied habitats (pseudo-F = 28.25, df = 4, $p < 0.001$), *epibenthic* fauna being statistically most abundant in riffles, whereas *hyporheic* fauna was most abundant in pools. The interaction between trait categories and streams was significant (pseudo-F = 57.67, df = 2, $p < 0.001$), the *epibenthic* fauna being more abundant in Elama than in Leitzaran.

The overall availability of current velocity trait categories was highest for *slow* (42.3%), followed by *moderate* (33.8%) and *fast* (23.9%). It changed markedly

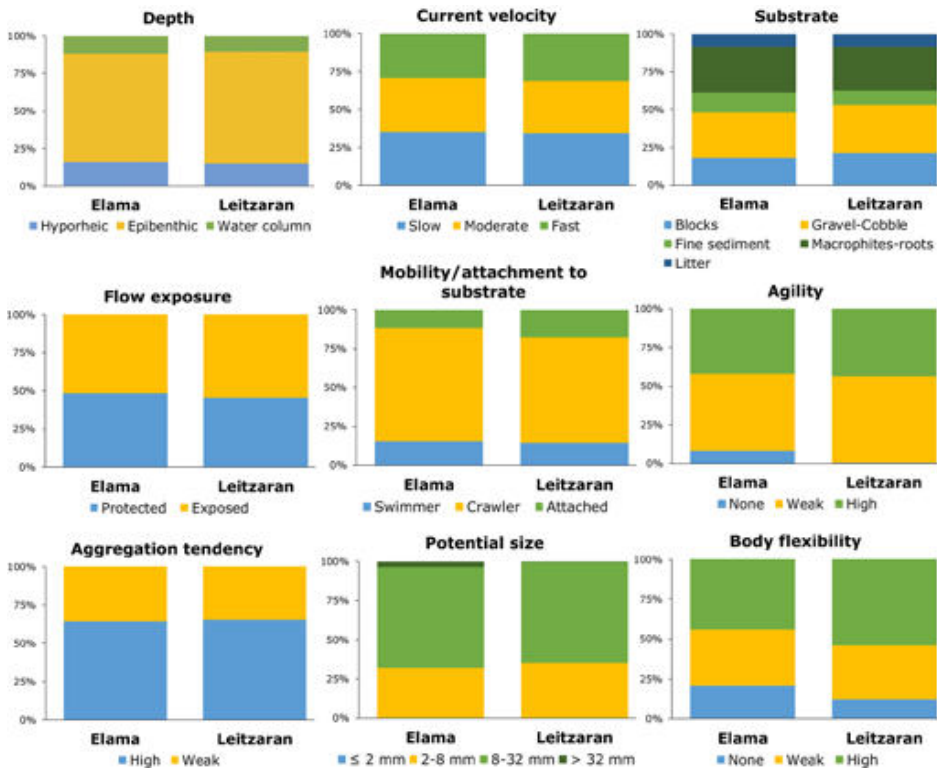


Figure 1. Relative contribution of each trait category in desman diet in Elama and Leitzarlan streams.

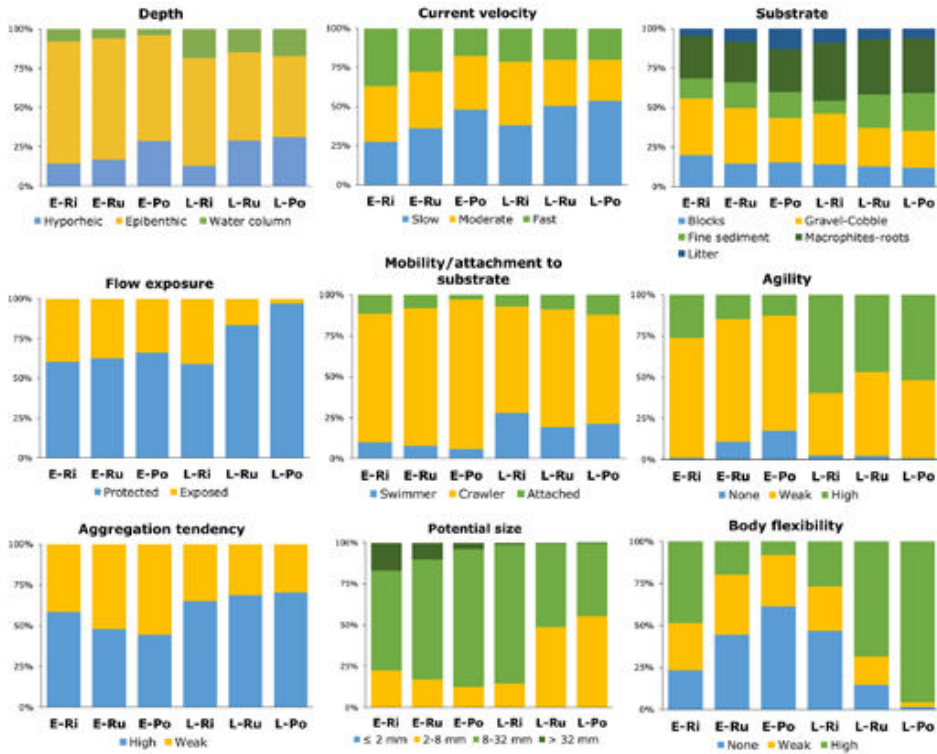


Figure 2. Relative contribution of each trait category to prey availability in the three habitats (Ri = riffles, Ru = runs and Po = pools) and the two streams (E = Elama and L = Leitzarlan).

between habitats (pseudo-F = 23.42, df = 4, $p < 0.001$), showing an increase in the **fast** category from pools to runs and riffles, whereas the opposite pattern was found for the **slow** category (Figure 2). The interaction between trait categories and streams was also significant (pseudo-F = 18.90, df = 2, $p < 0.001$), the **slow** category being more abundant in Leitzarán, where riffles were less abundant.

Overall, the most available invertebrates were those associated to **macrophytes-roots** (30.9%), followed by **gravel-cobble** (29.9%), **fine sediment** (16.4%), **blocks** (14.7%) and **litter** (8.1%). The interaction between trait categories and streams was significant (pseudo-F = 29.52, df = 4, $p < 0.001$), being **fine sediment** and **macrophytes-roots** higher in Leitzarán. At the habitat level the interaction between trait categories and habitats was significant (pseudo-F = 15.28, df = 8, $p < 0.001$), so fauna associated to **gravel-cobble** and **blocks** were most abundant in riffles (33.9% and 17.1%, respectively) and fauna associated to **fine sediments** and **litter** were most abundant in pools (20.2% and 9.6%, respectively).

Regarding flow exposure, the **protected** category was most abundant in all habitats and streams (71.3% in average). In this case there were significant differences between streams (pseudo-F = 51.04, df = 1, $p < 0.001$), being **protected** invertebrates more abundant in Leitzarán, and the interaction between trait categories and habitats was also significant (pseudo-F = 27.69, df = 2, $p = 0.006$). The **exposed** category had the highest values in riffles (40.4%) and the lowest in pools (18.5%).

With respect to the mobility/attachment trait categories, the **crawler** category had the highest abundance values (76.3% in average), being followed by **swimmer** (15.3%) and **attached** (8.4%) categories. The interaction between trait categories and streams was significant (pseudo-F = 77.46, df = 2, $p < 0.001$), the **crawler** category being more abundant in Elama. The interaction between trait categories and habitats was also significant (pseudo-F = 8.96, df = 4, $p = 0.043$), showing a light increase of **crawler** and **swimmer** categories and a decrease of **attached** category from riffles to runs and pools.

Most invertebrates (58.8% in average) had **weak** agility, being followed by **high** (35.3%) and **none** (5.9%) categories. The interaction between trait categories and streams was significant (pseudo-F = 157.90, df = 2, $p < 0.001$), **high** agility having higher values in Leitzarán. The interaction between trait categories and habitats was also significant (pseudo-F = 12.67, df = 4, $p < 0.001$), the **high** category having the highest values in riffles (42.9%), **weak** in runs (62.7%) and **none** in pools (9.2%).

Invertebrates with *high* aggregation tendency were the most abundant in all habitats and streams (59.2% in average). The interaction between trait category and stream was statistically significant (pseudo-F = 184.44, df = 1, $p < 0.001$), the *high* aggregation tendency being more abundant in Leitzarar. However, the interaction between trait categories and habitats was not significant (pseudo-F = 2.60, df = 2, $p = 0.086$).

In terms of potential size, the *8-32 mm* potential size category was the most abundant in all habitats and streams (66.1% in average), being followed by *2-8 mm* (28.3%), *> 32 mm* (5.5%) and *≤ 2 mm* (0.0%). The interaction between trait categories and streams was statistically significant (pseudo-F = 37.62, df = 3, $p < 0.001$), invertebrates *smaller than 8 mm* being more abundant in Leitzarar. The interaction between trait categories and habitats was also significant (pseudo-F = 9.79, df = 6, $p < 0.001$), as an increase of invertebrates *smaller than 8 mm* and a decrease of invertebrates *bigger than 8 mm* was observed from riffles to runs and pools.

Finally, in respect to body flexibility, the overall availability was highest for *high* category (44.5%), followed by *none* (32.0%) and *weak* (23.5%). The interaction between trait categories and streams was significant (pseudo-F = 45.27, df = 2, $p < 0.001$), as invertebrates with *high* flexibility had higher values in Leitzarar. The interaction between trait categories and habitats was also significant (pseudo-F = 5.10, df = 4, $p < 0.001$), *high* category being highest in pools (51.9%) and *none* and *weak* in riffles (35.1% and 31.7%, respectively).

Trait selection

In the fuzzy PCA performed with all data (diet and availability, Figure 3) body flexibility, flow exposure and agility showed the highest inertia values (> 0.020 ; Table S2), the first axis explained the 58.4% of the total variance, and the second 16.5%. Differences between streams were smaller for diet than for availability. The FPCA mostly showed differences between streams (Axis1) and among habitats (Axis2). Most remarkably, desman diet in both streams appeared next to each other, closer to the availability in riffles and furthest from the availability in pools. Also, the ellipses that covered habitat-scale availability were larger and overlapped more for Elama than for Leitzarar, showing that heterogeneity in availability within habitats was larger but differences among habitats smaller in the best-preserved stream. A redundancy analysis showed differences to be significant between streams ($F = 34.16$; df = 1; $p = 0.001$; 32.8% of the variance explained) and among habitats ($F = 6.48$, df = 2, $p = 0.001$; 12.4%), but not between diet and availability ($F = 1.01$, df = 1, $p = 0.348$; 1.0%) (Table S3).

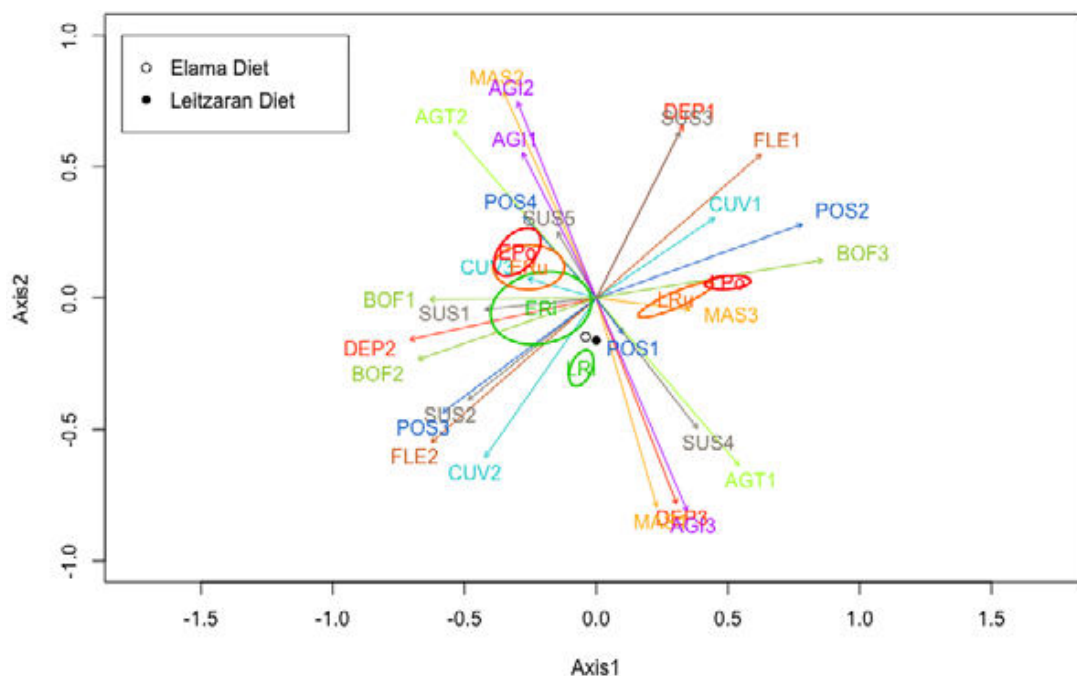


Figure 3. FPCA of the invertebrate traits in diet and availability in the stream. Points represent diet, ellipses the availability of each habitat (ERi = Elama riffle; ERu = Elama run; EPo = Elama Pool; LRi = Leitzarzan riffle; LRu = Leitzarzan run; LPo = Leitzarzan pool; where riffles are represented in green, runs in orange and pools in red) and arrows the trait categories (see abbreviations in Table 1). Each trait has been represented with a different colour. Note larger ellipses but larger overlap for Elama than for Leitzarzan stream.

Regarding prey selection, diet-to-availability ratios showed that desmans positively selected prey associated with the *water column* and *epibenthic* prey, areas of *fast* water (> 75 cm/s), *blocks* and habitats *exposed* to water flow (Figure 4). According to prey characteristics, they positively selected prey that were 2-8 mm long, prey with *some* body flexibility (*weak* or *high*), either *swimmers* or with mechanisms to get *attached* to the substrate, *highly* agile prey and prey with a *strong* tendency to form aggregations.

On the other hand, desmans negatively selected prey associated with *slow* water velocities, *fine* substrates and areas *protected* from the water flow (Figure 4), prey *smaller than 2 mm*, *crawlers*, and prey with *no* tendency to aggregate.

Although the general pattern was the same for both streams (Figure 4), differences in selection ratios between streams were significant for the traits depth ($p < 0.001$), flow exposure ($p = 0.004$), mobility/attachment to substrate ($p < 0.001$), agility ($p < 0.001$) and potential size ($p < 0.001$). Interactions in selection ratios between trait categories and streams were also significant for all the traits (all $p < 0.001$).

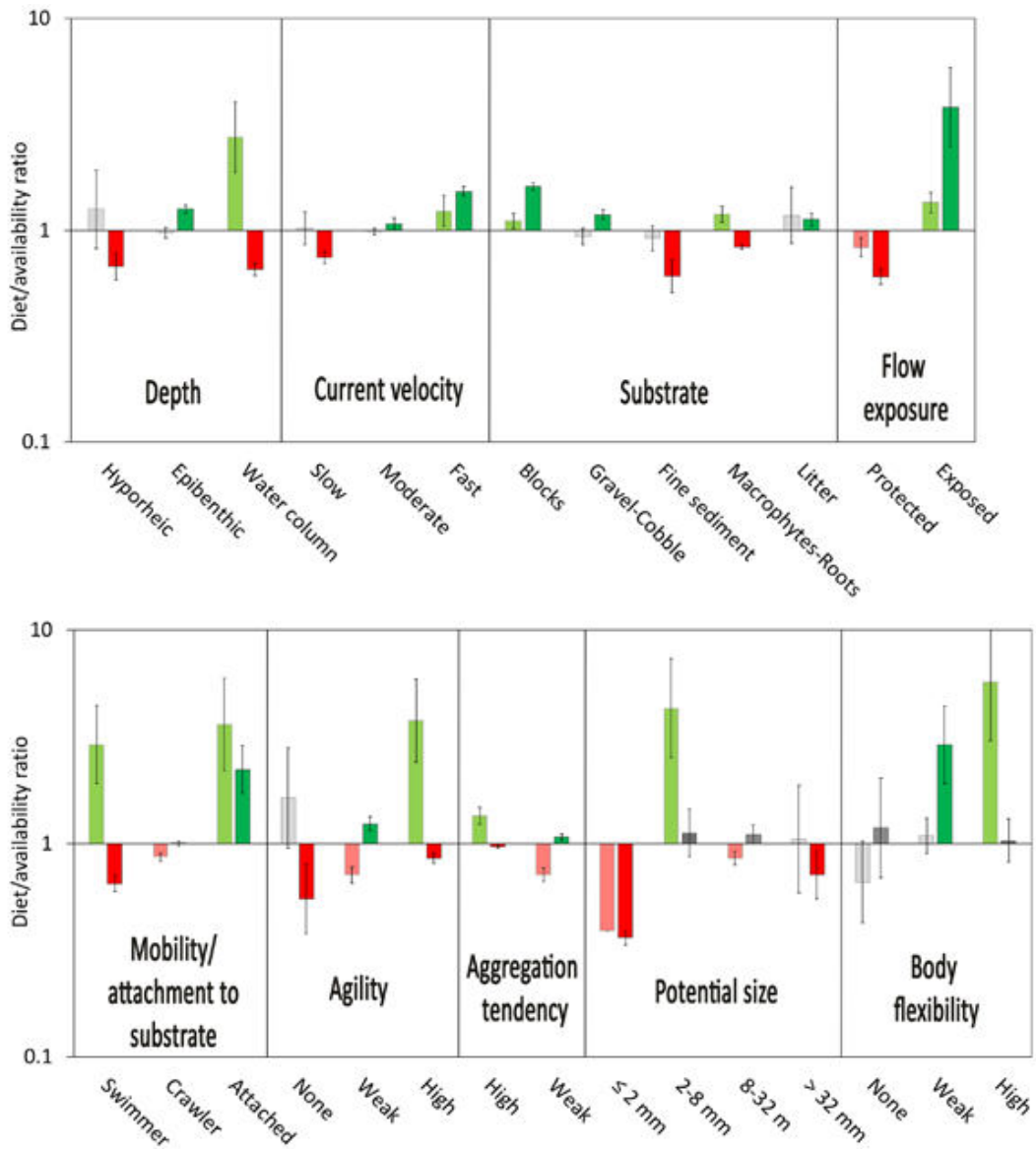


Figure 4. Selection of trait categories: positive (green), negative (red) and no selection (grey). Colours are lighter for Elama and darker for Leitzar.

Discussion

This study provides a prey trait analysis as a functional approach to understand the mechanisms involved in predator-prey relationships of the Pyrenean desman, a generalist insectivore. The traits herein studied help to better explain the desman foraging decisions than the taxonomic adscription of prey.

Traits in diet

Desmans are specialist rheophile predators with strong preference for riffle habitats (Chapter 2; Esnaola et al., 2018b). Taxonomic studies of their diet have reported desmans to mainly feed on Trichoptera, Ephemeroptera, Plecoptera, Crustacea (Amphipoda), and Diptera (Bertrand, 1992; Castién & Gosálbez, 1995; Santamarina & Guitian, 1988), and less frequently on Coleoptera, Annelida and Odonata (Puisségur, 1935; Richard & Viallard, 1969; Santamarina, 1992). Some of these authors noted that desmans vary diet with season (e.g. Santamarina, 1993), although most of them agreed defining them as generalist foragers preying upon a variety of prey (e.g. Biffi *et al.*, 2017a,b; Hawlitschek *et al.*, 2018). Differences among studies may derive from factors such as differences in number of samples, geographical regions or methodology.

In our previous taxonomic approach (Chapter 4) we found that desmans in Elama and Leizaran had a diet similar to that previously described. Our trait-based study shows that desmans' mostly consumed food type are **crawling epibenthic** prey —Psychodidae, Baetidae, Heptageniidae, Rhyacophilidae and Gammaridae—, prey living in **gravel-cobble** or **macrophytes-roots** substrata —Simuliidae, Baetidae, Heptageniidae and Gammaridae— (although macrophytes other than mosses are virtually absent from Elama and Leizaran streams), prey of **8-32 mm** potential size — Heptageniidae, Odontoceridae, Rhyacophilidae and Gammaridae —, and more often prey with **high** rather than **low** aggregation tendency —Simuliidae, Baetidae, Odontoceridae and Gammaridae— (Table S1). Besides, desmans showed almost no differences in prey regarding their traits for current velocity, ate prey with **some** exposure to water flow, ate few prey **without** body flexibility (such as Limnephilidae) and ate almost no prey **without** agility (such as molluscs). As for the body flexibility trait, desmans showed some plasticity, consuming prey with either **high**—mostly Psychodidae, Simulidae, Baetidae—, **weak** —e.g. Heptageniidae—, or **none** body flexibility —e.g. Gammaridae—.

The trait-based diet description and the multivariate analysis performed (FPCA) were consistent in both streams, thus indicating a clear pattern in the desman diets, unaffected by the conservation status of the streams. These results, thus, highlight the consistency in the foraging behaviour of this animal and the importance of the methodology used to characterize prey (taxonomy vs. biological traits). For instance, the use of traits allowed to show the characteristics shared by prey from different taxonomic groups, and at the same time, allowed to distinguish species grouped in the same taxonomic groups.

Trait selection

Measuring the diet in FO could overestimate prey that are consumed little but often, and underestimate others that are consumed rarely but in large quantities (Deagle *et al.*, 2019). Given this methodological issue, we tried to collect a large number of samples to avoid sampling bias as much as possible (Mata *et al.*, 2019).

Desmans prefer prey with depth preference for *water column* and *epibenthic* zone, prey in *fast* facies with *blocks* as substrate, prey *exposed* to flow, prey with potential size of *2-8 mm*, *highly* agile prey, prey *with* some flexibility, *clustered* prey, and prey that are either *swimmers* or have mechanisms to get *attached* to the substrate. Some highly consumed taxa such as *Alanites*, *Baetis* or *Simuliidae* (Table S1) share many of these traits. Desmans negatively selected prey living in *slow* water velocities, in *fine* substrates, *protected* from the flow, prey with potential size *below 2 mm*, prey *without* tendency to aggregate, and *crawlers*. Although no prey taxa shared all these trait categories, *Atherix*, *Eleophila*, *Prionocera* and *Wiedemannia* (Table S1) were examples of taxa with some of these traits and likely driving these preferences. Thus, these results partially refuted our first hypothesis, that desmans would positively select large, slow prey. On the other hand, as expected, desmans positively selected prey with at least certain body flexibility (without cases/tubes) and prey with *high* tendency to aggregate, while they negatively selected prey usually found in *fine* substrates. Our results also highlighted that they prefer prey with depth preference for *water column* and *epibenthic* zone, prey living in areas of *fast* flowing water, with substrates of *blocks*, and *swimmers* or *with* hold-down mechanisms.

Regarding the positively selected trait categories, these can be classified in two groups. On the one hand, some of them are directly related to riffles: being *exposed* to water flow, *attached* to the substrate, *blocks* as substrate and *fast* flowing waters are categories expected of invertebrates living in riffles. These trait categories seem not to directly drive desman choices; rather, they seem to be a consequence of the habitat selection by desmans. As desman preferentially forage in riffles, their most abundant prey show adaptations to life in riffles as well, irrespective of their effect on profitability or catchability for desmans. The remaining trait categories should offer better insight on desman prey preferences: prey with preference for *water column* and *epibenthic* zones (such as *Baetis*, *Epeorus* or *Ecdyonurus*), with potential size of *2-8 mm* (such as *Leuctra*, *Psychoda* or *Simuliidae*), *highly* agile (such as *Alanites* or *Echinogammarus*), *with* some body flexibility (*weak* or *high*) (such as *Ecdyonurus*, *Epeorus*, *Hydropsyche* or *Psychoda*), prey that live *clustered*

(such as *Hydropsyche*, *Leuctra*, *Odontocerum* or *Simuliidae*) and prey that are *swimmers* (such as *Alanites*, *Baetis* or *Echinogammarus*). The preference for *water column* and *epibenthic* zone prey, as opposed to *hyporheic* ones, seems easy to understand, as digging into the streambed must be energetically costly. The selection preferences for potential prey size are harder to interpret, as desmans showed negative selection for the largest prey, those that seem energetically more profitable. On the other hand, the negative selection of prey *smaller than 2 mm* follows the energetic criteria by Brose *et al.* (2006), who stated that the consumer-resource \log_{10} body-mass ratio in that case should not exceed the value of 6. It is noteworthy that we sampled invertebrates with a 2 mm-mesh net, that would capture a disproportionately low amount of invertebrates smaller than 2 mm, and still their frequency was even lower in diet than in our samples, thus showing a strong negative preference by the desman. Prey agility seems a counterintuitive criterion for desman preference, likely linked to other traits such as the depth preference or being exposed to water flow. Regarding body flexibility, the selection by desmans seems to reflect their overall avoidance of prey with strong body covers (cases, shells, etc.), harder to manipulate and eat (Rychlik & Jancewicz, 2002), and thus, likely less profitable (Bertrand, 1992). Anyway, some studies showed that the selection for Trichopterans is not conditioned by the presence of the cases, since desmans select both positively and negatively Trichopterans with (such as Goeridae, Lepidostomatidae, Limnephilidae and Odontoceridae; *none* body flexibility) or without cases (such as Hydropsychidae, Polycentropodidae, Philopotamidae and Rhyacophilidae; *high* body flexibility; Biffi *et al.*, 2017b; Chapter 4; Santamarina, 1992, 1993). The same has been observed in dippers (Ormerod & Tyler, 1991; Santamarina, 1990; Taylor & O'Halloran, 1997, 2001), which share to some extent the habitat and trophic preferences with desmans. The consumption of Trichopterans with cases by desmans may be related to the force with which the case adheres to the substrate, since Trichopterans with more fixed cases (such as Glossosomatidae and Goeridae) are less consumed than those less fixed (such as Limnephilidae, Odontoceridae and Sericostomatidae; Chapter 4; Vieira-Lanero, 2000).

It should be noted that prey selection does not depend on any single trait. The positive selection of highly aggregated prey is consistent with the foraging behaviour described by radiotracking in the study area, where desmans spent long bouts in specific riffles —hot spots— before moving elsewhere (Chapter 2; Esnaola *et al.*, 2018b). In the case of desman prey, aggregation tendency seems a consequence of individuals looking for similar conditions, such as Simuliids gathering in high densities on the upper part of boulders, where they find optimal hydraulic conditions for filtering. In these situations, any desman finding such a patch would gain much energy at a low cost by foraging intensively on it. Finally, the positive selection of *swimmer* prey by desmans

seems to reflect their preference for taxa such as Gammarids, which are the right size, appear on the surface of the bottom, and are relatively slow movers (Tachet *et al.*, 2002).

Based on taxonomic morphological data, Bertrand (1992) concluded that desmans consume benthic, rheophile and medium to large body sized fauna, and suggested that desmans prefer prey with high intrinsic energy value (Trichoptera without cases, such as Hydropsychidae, Rhiacophilidae and Philopotamidae) and with low sclerification. Moreover, he mentioned that they rarely consume Trichoptera with cases, which has been refuted by other studies (e.g. Biffi *et al.*, 2017a,b; Santamarina & Guitian, 1988; Santamarina, 1992, 1993). Although the energy value of prey has not been addressed in the present work, Esnaola *et al.* (Chapter 4) confirmed the positive selection of Amphipoda and some groups of Trichoptera, either with (such as *Odontocerum* and Limnephilidae) or without (such as *Hydropsyche*) cases. The “low sclerification” described by Bertrand fits the positive selection of weak or high body flexibility we observed. Accordingly, Santamarina (1992, 1993) also reported soft-bodied taxa as positively selected prey —vs. molluscs, as negatively selected ones—. Hawlitschek *et al.* (2018) and Biffi *et al.* (2017b), based on observations from their taxonomic studies, also suggested that invertebrates with hard bodies and hard cases were negatively selected. However, prey with *no* body flexibility were opportunistically consumed in our work, namely *Glossossoma*, *Protonemura* and *Sericostoma*, although it is possible that methodological constraints (FO in scats vs. abundances in benthos, number of samples, etc.) affect our selection ratios.

Selected traits and riffles

Esnaola *et al.* (Chapter 4) detected no differences in total prey availability among habitats that could explain the strong preference of desmans for riffles, and thus, suggested that such a preference would respond to other —likely mechanical—reasons. Here, instead, when looking at prey traits, differences in availability between habitats became evident for some traits. Nevertheless, many of the preferred trait categories were adaptations to live in riffles, likely without further advantage for desmans. Therefore, those characteristics would hardly explain habitat selection by this species, and they would instead most likely be a consequence of it. Alternatively, to assess to which extent prey availability could explain desman habitat selection, we checked whether trait categories related with prey profitability were more abundant in riffles. According to our results, on average, all preferred prey categories were slightly more abundant in riffles, except the *high* body flexibility. *High* body flexibility was just positively selected in Elama, where it was more abundant in riffles,

whereas *weak* flexibility was the positively selected category in Leitzaran, where it also was more abundant in riffles.

These results could lead us to accept our second hypothesis and to conclude that desmans select riffles for the food in them. However, differences in availability among categories were small and varied between streams. In addition, the fact that the preferred prey type is more abundant in riffles can hardly prove that this habitat is selected for the food it harbours. There can be other ecomorphological, functional, or behavioural constraints to deal with buoyancy and physical habitat heterogeneity (Richard, 1986) or other unknown reasons behind that preference. Almenar *et al.* (2013) observed in an insectivorous species that the foraging patch choice fitted a hierarchical sequence, driven first by the species morphological specialisations and ability to hunt in certain spaces, then by detectability of prey on specific areas and finally by the relative abundance of prey. The likelihood of analogous stepwise decision-making processes, together with interrelations among traits at habitats and local scales, blur the lines between causes and consequences of desmans foraging.

Differences between streams

Desmans basically selected the same prey traits in both streams, although with certain differences, probably owing to the contrasting prey availability between streams and habitats. The FPCA showed that desmans consumed prey with virtually identical traits in both streams, despite contrasting availability, thus highlighting the trophic specialization of the species.

Verifying our last hypothesis, trait ellipses for availability in Elama (in the FPCA graph) were larger and overlapped with each other, whereas they were smaller and did not overlap in Leitzaran. The higher similarity among habitats in Elama helps to understand the reported null selection by desmans for runs in Elama (Chapter 2; Esnaola *et al.*, 2018b), as in this stream riffles are more similar to runs in terms of prey availability (Figure 3).

Conclusions

Desmans have almost identical diets in terms of traits even in streams that differ in conservation status and in prey availabilities, as a consequence of their selective foraging behaviour. Despite differences in prey traits among habitats, and the preferred traits being more abundant in riffles in general, we were not

able to conclude whether desmans select riffles for the food in them or for their ability to hunt in this habitat. As a whole, this research highlights the importance of riffles as foraging habitats of this rheophile species and results suggest that conservation and restoration of its preferred habitat will be key for effective conservation. Since discharge affects riffle availability, the management of stream flows is a key issue for desman conservation.

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Supporting information

Table S2. The inertia value of each trait in the FPCA.

Trait	Inertia
Body flexibility (including cases/tubes)	0.0651
Flow exposure	0.0316
Agility	0.0264
Potential size	0.0196
Aggregation tendency	0.0145
Depth	0.0104
Mobility/attachment to substrate	0.0090
Current velocity	0.0083
Substrate	0.0030

Table S3. ANOVA of the RDA of the global analysis in trait-level.

	Df	Variance	F	p-value
Diet vs. Availability	1	0.0006	1.01	0.348
Stream	1	0.0215	34.16	0.001
Habitat	2	0.0081	6.48	0.001
Residual	56	0.0352		

Chapter 6



General discussion

The Pyrenean desman: a living fossil

The Pyrenean desman has been described as a relict species (Rümke, 1985) adapted to live in fast flowing mountain streams (Charbonnel *et al.*, 2015). Furthermore, taking into account its restricted distribution and its monophyly (Arribas, 2004; Cabria *et al.*, 2006), the Pyrenean desman can be considered a living fossil. Together with other rheophilic species (e.g. the white-throated dipper *Cinclus cinclus*, Hourlay *et al.*, 2008, the Pyrenean brook salamander *Calotriton asper*, Valbuena-Urena *et al.*, 2013), they lived in glacial refugia during the last glaciation, after which they expanded again in the Iberian Peninsula and the Pyrenees (Gómez & Lunt, 2007; Igea *et al.*, 2013). The habitats that desmans occupy today are these that most resemble conditions that likely were more widespread in the past, constituting a perfect example of species that would have presumably occurred at lower elevations and more widespread during the colder glacial periods (Ohlemüller *et al.*, 2008). Desmans are highly specialized to live in biotopes characterized by fast currents of cold water harbouring a specific food. However, the desman is now suffering a setback in its total distribution area due to the human activity (e.g. water diversion) and climate change (Araújo *et al.*, 2012; Charbonnel *et al.*, 2016; Ohlemüller *et al.*, 2008). The rheophilic areas that desmans need are declining, having less and less habitable stream sections available. In that context, the high specialization of this mammal makes the preservation of its specific habitats key for conservation.

Spatial ecology and habitat

First radiotracking of desman began in the eighties and showed that desmans have a bimodal activity rhythm, with one short bout of diurnal activity and one principal bout of nocturnal activity (Stone, 1985, 1987a). More recent works revealed that nocturnal foraging may also show two or three activity peaks (Melero *et al.*, 2014), and that desmans usually share shelters with other individuals, independently of sex, age, or number of conspecifics sharing the home range (Melero *et al.*, 2012), which cast doubt on their supposed territorial behaviour. These studies provided novel information on desman ecology, but they did not address its habitat preferences. Since the last work of Melero *et al.* (2014) no other radiotracking study had been carried out.

During the development of this PhD study some authors modeled desman habitat requirements. First, based on desman faeces, Charbonnel *et al.* (2015) found out that desmans presence was affected by flow variation, substrate

heterogeneity and presence of shelters, and concluded that the flow is the main factor driving habitat occupation by the desman. Second, Biffi *et al.* (2016) concluded that desmans habitat use is driven by riverbed and riverbank characteristics, showing a preference for riffles, heterogeneous shelters and river substrates, abundant emerging structures, low amounts of fine sediment and high proportion of rocks and low proportion of earth in the riverbank. Third, Charbonnel *et al.* (2016) found that desman distribution is mainly influenced by hydrological and climatic variables, although other biotic and/or abiotic factors such as habitat fragmentation, water and substrate pollution, invasive species, local habitat degradation, riparian vegetation changes, presence of competitors or predators and changes in macroinvertebrate communities could also affect the contraction of the Pyrenean desman range. Fourth, Quaglietta *et al.* (2018) concluded that the extinction rate across two studied periods (1993-1996 and 2014-2015) was 63.5%, and showed a strong range contraction into headwater streams. They were unable to explain the changes observed, but highlighted the importance of headwater streams as refugia against anthropogenic stressors such as climate change and invasive species. Finally, Biffi *et al.* (2019) showed a negative impact of human pressure, ammonium concentration and the presence of the Eurasian water shrew *Neomys fodiens* in desman presence, which showed a high correlation with low conductivity and the presence of the dipper *Cinclus cinclus*. They suggested that activities directly impacting the riverbed could have an adverse effect on Pyrenean desman populations, but concluded that variables not measured by them could drive the desman distribution at the local scale. Furthermore, the moderate response of the desman to the many abiotic and biotic variables suggested the species was more tolerant of environmental conditions than previously thought.

All these studies provided valuable information for better understanding the spatial ecology of the Pyrenean desman, as they described likely key habitat factors in stream stretches occupied by desmans. The faecal presence data they relied on may provide biased results though (see the discussion in Chapter 2). Our study sought to address issues that remained unknown, such as the factors controlling occupancy and the intensity of habitat use and selection at the microhabitat scale. That information is key for understanding which factors define the distribution of the desman at the local scale and to carry out actions for the species conservation.

In this thesis the habitat preference of the desman was studied at the microhabitat level, radiotracking individuals within their home ranges and analysing their use of the space in two contrasting streams. Our results highlighted the importance of the fast-flowing facies (riffles) previously foreseen by some authors (e.g. Biffi *et al.*, 2016), and provided more consistent information on desman habitat preferences. The stronger use of riffles in the stream affected by water

diversion highlights the importance of the flow in the species spatial behaviour. As Richard (1986) said, the Pyrenean desman has a frenetic way of life. We also observed that, as suggested by Melero *et al.* (2012), desmans share their home ranges with a variable number of individuals, showing no pattern in relation to sex or age. The characteristics of the shelters of the desmans we tracked were the same as those previously described (Stone, 1987a), varying between natural crevices between rocks, stone walls, or cavities below the roots of riparian trees. The description of riffles as habitats necessary for the desman and the importance of the availability of shelters within their home ranges are two important aspects to be considered in the future conservation actions. Furthermore, in accordance with Biffi *et al.* (2016), our data encourage management actions that favour aquatic habitats offering heterogeneous substrates and appropriate connectivity corridors.

Although the radiotracking study allowed us to draw sound conclusions about the spatial ecology of the Pyrenean desman, it posed the question of whether such habitat use was related to varying food availability in the habitats or to other reasons (see Richard, 1986).

Trophic ecology

Pyrenean desmans had been characterised as insectivorous a long time ago (Cabrera, 1914; Puissegur, 1935). This knowledge was confirmed by new dietary studies (e.g. Bertrand, 1992; Castián & Gosálbez, 1995; Santamarina, 1992), which reported desmans to prey upon aquatic Dipteran, Ephemeropteran, Plecopteran and Trichopteran larvae and that their diet can vary among seasons and streams. However, these studies were unable to provide fine taxonomic resolution (at genus or species level; Clare, 2014), which is considered of paramount interest when studying the trophic ecology of rare, endangered or cryptic species (e.g. Bohmann *et al.*, 2014; Razgour *et al.*, 2011).

Thus, in this PhD thesis I also aimed at describing in detail the diet of the Pyrenean desman, non-invasively DNA metabarcoding its faeces “for the first time”. However, other authors worked for the same goal, and several metabarcoding studies were published during the course of this thesis. First, Gillet *et al.* (2015) allowed the high-resolution identification of the Pyrenean desman and its prey with a specific primer set. Then, Biffi *et al.* (2017a), in the first complete description of desman diet with DNA metabarcoding, revealed a diverse diet containing a high proportion of rare prey and substantial consumption of terrestrial prey, suggesting a more generalist diet than previously understood. Subsequently, Biffi *et al.* (2017b) reported that the diet of the Pyrenean desman

and the Eurasian water shrew (*Neomys fodiens*) did not overlap much, since desmans ate mainly aquatic prey. Finally, Hawlitschek *et al.* (2018) showed that fresh samples may yield more reads, a higher proportion of target reads, and potentially more OTUs. All these mentioned metabarcoding diet studies, each with its methodological peculiarities, described desman diet in detail, providing valuable information about its trophic ecology. Their and our results have been thoroughly discussed in this thesis (see Chapters 3, 4 and 5), comparing results and addressing biases derived from methodological details, such as choice of primers.

The use of High-Throughput Sequencing methods has been a great advance in trophic ecology studies of species that are rare, small, aquatic, nocturnal, fast-moving, elusive, or that primarily consume soft-bodied organisms (Berry *et al.*, 2017; Boyer *et al.*, 2013; Buglione *et al.*, 2018; Evans *et al.*, 2016; Piñol *et al.*, 2014; Shehzad *et al.*, 2012). However, there is still a lack of methodological standardization in study designs (Deagle *et al.*, 2019; Deiner *et al.*, 2017; Divoll *et al.*, 2018; Zizka *et al.*, 2019), which complicates reproducibility, replicability and comparability of studies (Alberdi *et al.*, 2018, 2019; Mata *et al.*, 2019) and in some cases makes it difficult to arrive at correct ecological conclusions (Andriollo *et al.*, 2019; Zinger *et al.*, 2019). In addition, among others, error detection, poor quality of reference databases, overestimation of rare species and avoiding secondary predation remain a problem (Clare, 2014; Pompanon *et al.*, 2012; Ruppert *et al.*, 2019), and the methodology for quantifying the diet has not yet been developed (Elbrecht *et al.*, 2017; Fonseca, 2018; Piñol *et al.*, 2015, 2019). Anyway, DNA metabarcoding is considered an essential tool to understand species ecology (De Sousa *et al.*, 2019) both for ecological monitoring and for global conservation studies (Ruppert *et al.*, 2019).

Back to desmans, apart from characterizing its diet, some studies analysed desman prey selection at a broad taxonomic level (i.e. Bertrand, 1992; Santamarina, 1992). Giving a step forward, Biffi *et al.* (2017b) compared desman molecular diet composition with prey availability and concluded that the most frequently consumed prey by the desman corresponded to the most abundant taxa in streams. Besides, they suggested which prey characteristics are valuable or avoided by desmans, although still lacking a detailed description of their patterns of trophic preference and prey selection at the local scale. In this context, the present thesis studied the trophic preferences of the desman at the microhabitat level in two contrasting streams (Chapters 4 and 5), simultaneously characterizing habitat use by radio-tracking, desman diet and prey availability in three habitat types (riffles, runs and pools). This complex sampling design allowed us to consistently assess the relationship between the spatial and trophic ecology of desman, analysing whether local-scale habitat use depended on food availability or on other factors. Moreover, the analysis

of prey traits (Schmitz, 2017) provided novel information on desman foraging preferences, highlighting once again their preference for riffles. What is more, the similarity between the prey characteristics in the two studied streams allowed improved description of the desman resource needs (see Chapter 5; Spitz *et al.*, 2014).

To allow an adequate sample collection and storage, I used artificial shelters (Appendix 1; González-Esteban *et al.*, 2018), ensuring that analysed samples were fresh, and therefore less vulnerable to contamination (McInnes *et al.*, 2017; Oehm *et al.*, 2011). I believe that this method should be commonly used in future studies, due to its efficiency in detecting the desman and its advantage in collecting fresh faeces.

The importance of riffles for desman conservation

This thesis underlines the importance of fast-flowing waters and the presence of some specific prey types for the survival of the Pyrenean desman. In short, it depicts the desman as a stenotopic species, limited by its specialization to forage in fast waters and that, if it is to be protected, it will be essential to pay attention to stream hydrology.

The characteristics of the stream flow, such as magnitude, frequency, duration, timing (or predictability), rate of change (or flashiness), etc. are key aspects in the conservation of many riverine species, as they regulate the ecological integrity of flowing water systems (Palmer & Ruhi, 2019; Poff *et al.*, 1997). Regional patterns of river flow are determined by stream size and by geographic variation in climate, geology, topography and vegetative cover (Poff *et al.*, 1997). However, these regimes are altered by human activities such as diversion weirs, for irrigation, hydropower generation, industry and domestic supply, modifying the natural hydrological regime (e.g. Rosenberg *et al.*, 2000), causing ecological changes (Poff & Zimmerman, 2010) and disrupting the dynamic equilibrium between the movement of water and the movement of sediment (Giulivo *et al.*, 2019). These disruptions alter both gross- and fine-scale geomorphic features that constitute habitat for both aquatic and riparian species (Chien, 1985), having direct effects on the aquatic invertebrates (Englund & Malmqvist, 1996; James *et al.*, 2007; McIntosh *et al.*, 2002). In the Basque streams these diversion schemes have had great impact, as in the province of Gipuzkoa alone there are over 900 low dams and weirs in less than 2000 km² (Boletín Oficial del Estado, 2016; Ente Vasco de la Energía, 1995). Most of these structures are legacies of past activities such as mills or iron foundries, and collectively create

large areas of stagnant water, probably of little use for desmans as pools. The compliance of these dams with environmental flow regime has not been enough to maintain the natural dynamics of the streams, and it is thought that these weirs should be taken down where possible, preserving only those currently in use or with heritage value (Chapter 2). These obstacles, together with global climate change, are causing serious alterations in headwater streams and, thus, directly affect the preferred habitat of desmans. In fact, Pyrenean glaciers are suffering major setbacks (Rico *et al.*, 2017), stream temperatures are rising (van Vliet *et al.*, 2013) and there are fewer and fewer Iberian streams that maintain a significant water flow throughout the year (Lorenzo-Lacruz *et al.*, 2013). Currently, all these factors directly affect riffle availability, and thus, the availability of desman habitable space.

There is an increasing desire, supported by national and regional policies and legislation, to conserve or restore the ecological health and functioning of rivers and their associated wetlands for human use and biodiversity. Many organisations are working in this line. For instance, in the Regional Countries of Gipuzkoa and Navarre, the LIFE IREKIBAI project has carried out several restoration actions in streams occupied by desmans, including dam demolition (IREKIBAI, 2017). The main objective of the project regarding the Pyrenean desman was to enhance river connectivity, decreasing population fragmentation and improving desman habitat. However, neither this project nor any other deal with the environmental flow issue: i.e. there is no work that attempts to establish environmental flows taking into account the hydrological needs of the Pyrenean desman. The environmental flow is defined as the minimal flow that contributes to achieve a good ecological status in rivers and allows the functionality and structure of the aquatic ecosystems and the associated terrestrial ones in a sustainable way (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2015). This definition highlights that all sensitive species –not only fish– should be targeted in any environmental flow management policy. The urge to determine the environmental flow strategies required for the conservation of diverse aquatic mammals has been emphasized (e.g. Grant & Temple-Smith, 2003). However, it is not easy to find works that regulate stream flows to answer the ecological needs of such species.

Questions for the future (unknowns)

Focusing first on the aspects that have been studied in this thesis, in terms of the spatial ecology and the habitat of the Pyrenean desman, our study did not allow analysing how the use of the space varies with season or with the sex or the age of individuals. Furthermore, a more detailed characterization of the

habitat of the desman would be of great interest. In the future it will be necessary to see whether habitat use patterns are consistent across geographical areas and stream types. As for the trophic ecology of the desman, although several authors suggested that desman diet undergoes temporal and spatial variations (e.g. Santamarina, 1993), given the effort and cost of detailed studies, and taking in account that the trophic patterns of the desman are guided by their rheophilic behavior, I consider that little would be gained by these studies.

In our study area desmans share habitat with other potential competitors, the Eurasian water shrew (*Neomys fodiens*) and the white-throated dipper (*Cinclus cinclus*) (Arzak *et al.*, 2014; Regla & Arizaga, 2016; Ventura, 2007), and it would be very interesting to analyse their ecological interactions. Water shrews mostly forage on shallow and slow waters, spend less time than desmans in the water (Churchfield, 1985; Mendes-Soares & Rychlik, 2009; Rychlik, 1997), and their diet has little overlap (Biffi *et al.*, 2017b). Thus, it seems they will hardly compete for food. However, dipper foraging behaviour is more similar to that of desmans as they also prefer riffles (Chen & Wang, 2010; Santamarina, 1990), mainly prey on aquatic invertebrates (Taylor & O'Halloran, 1997), and are especially selective in baseflow (Taylor & O'Halloran, 2001). Several studies have shown that dippers feed their young every day (Morrissey *et al.*, 2010). In addition, it is known that they vary their diet depending on their age (Yoerg, 1994, 1998) and sex (Morrissey *et al.*, 2010), and also depending on whether they are in the breeding period (Morrissey *et al.*, 2010) and whether they have to feed themselves or their young (Chiu *et al.*, 2009; Eguchi, 1990). Although variation could also be expected in the case of the desman, so far no study has managed to show if they follow any pattern depending on their age or sex, neither if they feed their brood.

The Pyrenean desman has been known for a long time for its morphological and taxonomic peculiarities. However, as suggested in previous lines, many aspects of its biology are still unknown. There are still many questions to answer, which are key for desman conservation. On the one hand, the question of how flow variation affects desman ecology remains to be answered. Morueta-Holme *et al.* (2010) mentioned global warming, high variability in water discharge and low precipitation as the climatic factors that may limit desman distribution. Williams-Trip *et al.* (2012) emphasized the importance of factors linked to precipitation in some desman distribution models. Predicted changes will reduce stream flow and impact aquatic invertebrates (Cowx *et al.*, 1984; Englund & Malmqvist, 1996; Wood & Petts, 1994). All the studies recently carried out (e.g. Charbonnel *et al.*, 2015, 2016; Biffi *et al.*, 2016; and this PhD study as well) stand out the need to analyse how flow modifications influence desman spatial ecology, to appropriately manage stream flows in protected desman areas. More precisely, it would be necessary to analyse how discharge

reductions influence riffle availability and, based on that, define environmental flows. This could be done following an approach roughly similar to PHABSIM (Physical Habitat Simulation; Bovee, 1982), although necessarily more simple, as it would be almost impossible to define clear habitat preference curves for the desman.

Appropriate management of stream flows and implementation of environmental flows will be key issues for desman conservation, but there are still some other issues to be resolved. For instance, which is the main determinant of the habitat use by the desman? Swimming constraints? Sensory capacity? We still do not know how desmans perceive what is food and what is not while foraging at night in highly turbulent waters. They are almost blind (Trutat, 1891) but have very good sensory receptors in their trunk-like nose and their tongue (Argaud, 1944; Richard & Villard, 1969). Nevertheless, we do not know whether they discover their prey by olfactive detection exhaling/inhaling air-bubbles and smelling their prey underwater, like the Russian desman (*Desmana mochata*; Ivlev *et al.*, 2013), the American water shrew (*Sorex palustris*; Catania *et al.*, 2008), the water shrew and the semi-aquatic star nosed mole (*Sorex palustris* and *Condylura cristata*, respectively; Catania, 2006). Further studies will be needed to explain how desmans detect their prey under water, why they have specific trait-based selection preferences, how they hunt and what are their underwater movement patterns. A deeper knowledge of desman sensorial ecology could also make it possible to design some kind of deterrent devices at the entrances of hydropower diversion canals, which seem to be traps for desmans.

Little is also known about the dispersion pattern of the species, although some studies have shown that movements within the basin are the most usual ones, with rare inter-river movements (Escoda *et al.*, 2017). Escoda *et al.* (2019) suggested that desmans can walk from one basin to another more easily than crossing a large dam. Gillet *et al.* (2016) also reported that desmans can overcome dams. More information is still needed to shed light on these issues, which can be achieved through new techniques such as individual identification from faeces. The shelters specifically designed for desmans during this thesis (see Appendix 1; González-Esteban *et al.*, 2018) will surely be helpful for that kind of studies, making the collection of fresh faeces easier. In addition, hair traps can be installed on them to improve DNA collection, as done with other mammals (e.g. Barja *et al.*, 2016; Roche, 2008). As for the population dynamics, the use of transponders and automatic readers installed in desman shelters (as is done with some bats, e.g. van Harten *et al.*, 2019) could yield additional information.

Recent research showed that the low genetic diversity, the high inbreeding

coefficient and the low effective population sizes leave desmans in risk of extinction in the future (Gillet *et al.*, 2017). Furthermore, desmans have genetically distinct populations associated to past glacial refuges (Querejeta *et al.*, 2016), and this genetic structure should be preserved while trying to conserve the population (Igea *et al.*, 2013; Querejeta *et al.*, 2017). Having observed the fast decline of desman populations in all their distribution area and the problems of consanguinity, it seems that translocations (reintroductions or reinforcements of populations) would be more appropriate than captive breeding for the near future.

Chapter 7



Conclusions

Conclusions

This PhD thesis analyzed the spatial and tropic ecology of the Pyrenean desman and increased knowledge on the factors determining its habitat preferences and foraging decisions. Its main conclusions are as follows:

- 1) The Pyrenean desman positively select riffles over runs and pools, the selectivity being higher in the stream affected by hydropower schemes.
- 2) Combining Gillet and Zeale primer sets resulted the most cost-effective method for non-invasive determination of the diet of the Pyrenean desman; it also allowed identifying the predator itself in the faecal samples.
- 3) Even though they have a highly diverse diet, desmans are more specialist than previously thought. They positively select taxa including Simuliidae and Psychodidae (Diptera), Baetidae and Heptageniidae (Ephemeroptera), Odontoceridae (Trichoptera) and Gammaridae (Crustacea), and negatively taxa such as Planorbidae (Gastropoda), Goeridae (Trichoptera), Perlidae (Plecoptera) and Athericidae (Diptera). Thus, desmans seem to prefer conspicuous invertebrates that live on top of the substrate and shredding invertebrates linked to coarse organic matter, and seem to avoid soft-bodied taxa inhabiting within fine sediments, invertebrates with very hard shell and predators.
- 4) Prey selection differs between the two contrasting streams, desmans being more opportunistic in the least preserved stream.
- 5) Desmans use riffles for foraging, although there are no significant inter-habitat differences in availability of prey, neither for consumed taxa, nor for positively selected taxa.
- 6) The analysis of biological traits of preys showed that desmans positively selected prey that live in the water column and epibenthic zone, in fast facies with blocks as substrate, prey exposed to the flow, prey with potential size of 2-8 mm, highly agile prey with some flexibility, clustered prey, and prey either swimmers or with mechanisms to attach to the substrate. They negatively selected prey living in slow water velocities,

in fine substrates, protected from the flow, prey with the potential size below 2 mm, prey without tendency to aggregate, and crawlers. A first group of traits might simply reflect the prey characteristics necessary to live in riffles, but a second group seem to reflect desman selectivity criteria related to prey profitability.

- 7) All the preferred prey categories, except high body flexibility, were slightly more abundant in riffles. However, differences in availability among categories were small and varied between streams, thus suggesting that the preference for riffles does not lie on the larger abundance of these prey there. Other ecomorphological, functional, or behavioural constraints to deal with buoyancy and physical habitat heterogeneity might lie behind that preference.
- 8) The strong preference for riffles by the desman has important consequences for the management of this endangered species. It suggests that human activities reducing riffle availability, especially water abstraction or water diversion, can be especially detrimental for this species.

Appendix 1



A new sampling method to detect the Pyrenean desman (*Galemys pyrenaicus*)

Publication

An article based on this chapter was published in the journal *Hystrix, the Journal of Mammalogy*:

González-Esteban, J., Esnaola, A., & Aihartza, J. (2018). A new sampling method to detect the Pyrenean desman (*Galemys pyrenaicus*). *Hystrix, the Italian Journal of Mammalogy*, 29(2), 190-194.

Abstract

The Pyrenean desman (*Galemys pyrenaicus*) is a small semi-aquatic mammal whose populations have suffered a severe decline in recent decades. Its conservation requires a monitoring program to quantify changes in their populations and distribution. Scat surveys have usually been carried out for this purpose, but they tend to yield a low success rate, which depends on local environmental conditions such as channel form and lithology. This methodological constraint causes that part of the population goes unnoticed. In this study a new method for the detection of this elusive species is tested. The research has been done in the Basque Country (Northern Iberian Peninsula), in Elama and Leizaran streams, where desmans have been recently recorded. Artificial shelters have been placed, regularly distributed in both streams, offering desmans suitable places to rest and defecate while foraging. The desmans used quickly and repeatedly the artificial shelters, significantly increasing their detection rate. The field identification of scats, based on their shape, colour, size and odour, was subsequently confirmed by DNA analyses with metabarcoding. This new noninvasive method allows obtaining fresh faecal samples of known age, making them available for further studies on genomics, population genetics, dietary studies, reproductive analyses, etc. The low cost of the materials used and the possibility of identifying desman scats after basic training, make this method optimal for synchronic, regional-scale and/or volunteer-based surveys. Thus, the use of artificial shelters results in a substantial improvement over traditional desman scat surveys, and greatly enhance the means for future monitoring of the populations of this endangered species.

Keywords

desman, detection, metabarcoding, sampling method, scats

Introduction

Conservation of endangered species requires to periodically determining their distribution area as well as demographic parameters of their populations. Because of practical constraints, periodic follow-up is usually possible only if there are accurate and relatively cheap detection methods that are easy to implement. The elusive character and nocturnal habits of most mammals forces researchers to mainly rely on their tracks and signs for surveys (Wilson *et al.*, 1996). The tracks of some species are unmistakable, what allows defining survey protocols based on the search of traces (e.g., Helle *et al.*, 2016), although in environments where tracks are hard to detect faeces can offer a suitable alternative. Faeces can be useful to monitor a species if they can be unequivocally assigned to that species and if the animal deposits them in a predictable way in places accessible to the researcher. Nowadays techniques in molecular genetics allow correct identification of the species that produced a scat (e.g., Janecka *et al.*, 2008; Ruiz-González *et al.*, 2008; Gillet *et al.*, 2015; Walker *et al.*, 2016), although these methods require expensive procedures and specialized technicians, thus making it difficult to intensively survey large geographic areas. Direct identification by observation is a much-preferred option, when available. A good example is the survey of Eurasian otter (*Lutra lutra*), which is periodically performed in Europe (Mason, 1986). These surveys, generally performed at a regional scale, involve a large number of volunteers who are previously trained to acquire the necessary skills. The distinct characteristics of its scats (form, smell, colour, specific layout on the ground) make it possible to get enough reliable observations as to outline the distribution of the otter in a wide area (in the order of thousands of km²) in a short time frame.

The Pyrenean desman (*Galemys pyrenaicus*) is a semi-aquatic mammal of 60-70 g. It usually seeks shelter in natural crevices between rocks, in stone walls, or below the roots of riparian trees; usually there are no outwardly visible signs of the shelters from the surface (Stone, 1987a). It feeds mainly on freshwater invertebrates (Santamarina and Guitián, 1988; Biffi *et al.*, 2017a; Hawlitschek *et al.*, 2018) and, as the otter, lays its faeces on emerging structures in the stream channels (mainly rocks, logs and roots). Desman scats can unequivocally be assigned to the species when fresh, by their smell, colour, shape and size (Nores, 1992), but when they get older their scent vanishes, their colour changes and if they get dry they disintegrate easily, what makes them more prone to misidentification with other small mammals such as *Neomys* sp. or *Rattus* sp., among others (see Gillet *et al.*, 2015). Despite these problems, scat surveys have been used to monitor desman populations through their distribution area (Nores, 1992; Bertrand, 1992; Queiroz *et al.*, 1998). Sometimes these surveys

yield a low success rate (low number of scats per surveyed reach) (González-Esteban *et al.*, 2011; González-Esteban, 2014; Charbonnel *et al.*, 2015), which also depends on channel form and lithology of the streams. In reaches with limited favourable substrata such as emerging rocks and logs, desmans defecate in burrows or in holes below overhanging banks, out of the reach of the surveyors (González-Esteban *et al.*, 2003a). Therefore, some populations are not detected in routine surveys. There has been some discussion on how to correct these false absences, but the problem still remains unsolved (Charbonnel *et al.*, 2014).

The Pyrenean desman is protected under the Bern Convention (Appendix II) and the EU Conservation of natural habitats and of wild fauna and flora Council Directive (Annexes II and IV) (Council of the European Communities, 1992). Its distribution area has been severely reduced during the last decades (Charbonnel *et al.*, 2016; Quaglietta *et al.*, 2018) and it is currently listed as a vulnerable species in the Red List Categories by the IUCN (Fernandes *et al.*, 2008). The problems above outlined about detection of signs of presence — namely the varying success rate, the high cost and the risk of false negatives — prevented detailed large-scale-surveys of the desman. Consequently, its current distribution area is only partially known and no global survey has hitherto been conducted. This could become extremely serious since at least five genetically discrete conservation units have been identified for this species (Querejeta *et al.*, 2016), which should have specific management approaches. Here we show a new detection method that can help solving the main limitations of the current surveys, making desman detection faster, cheaper, and reducing the risk of false absences.

Materials and methods

This study was carried out as part of a broader research on the spatial and trophic ecology of the Pyrenean desman conducted in two mountain streams in the northern Iberian Peninsula: Elama Stream (330 m mean altitude; 7 m mean width; 0.7m³/s mean flow) and Leitzaran Stream (290 m mean altitude; 12 m mean width; 4.6m³/s mean flow) (Fig. 1, 2). Both are protected under the EU Natura 2000 network, both are in good ecological status according to the EU Water Framework Directive (Council of the European Communities, 2000), and the presence of Pyrenean desmans has been known for at least 30 years in both (Chapter 2; Esnaola *et al.*, 2018b; Nores, 1992).

These streams show contrasting environmental pressures. Elama is a second-order headwater stream draining an uninhabited basin of 1415 ha over granite

and schist that has been managed strictly as a nature reserve since 1919, resulting in extensive cover of beech and oak forests (Castro, 2009). At present there is no extractive activity in the Elama basin. On the other hand, Leitzarán is a fourth-order stream draining a basin of 12402 ha over limestone, slate, and sandstones. Unlike in Elama, in the headwaters of Leitzarán there are two towns totalling 3150 inhabitants, after which the stream enters an uninhabited valley, approximately 25 km in length, where forestry and hydropower diversion schemes are the main human activities (Izagirre *et al.*, 2013). The characteristics of the channel are different in both streams: in Elama riffles and runs are similarly available (45%), whereas runs are dominant in Leitzarán (60%). Pools are the least abundant habitat in both streams (10%). Both streams have emerging blocks and logs in the riverbed and in the riverbank. These elements are more abundant in Leitzarán, providing more depositional zones (places to deposit their excrements) to the inhabiting desmans. The vegetation cover is scarce in both streams, mostly herbaceous and shrubby, being easily accessible to the observer.

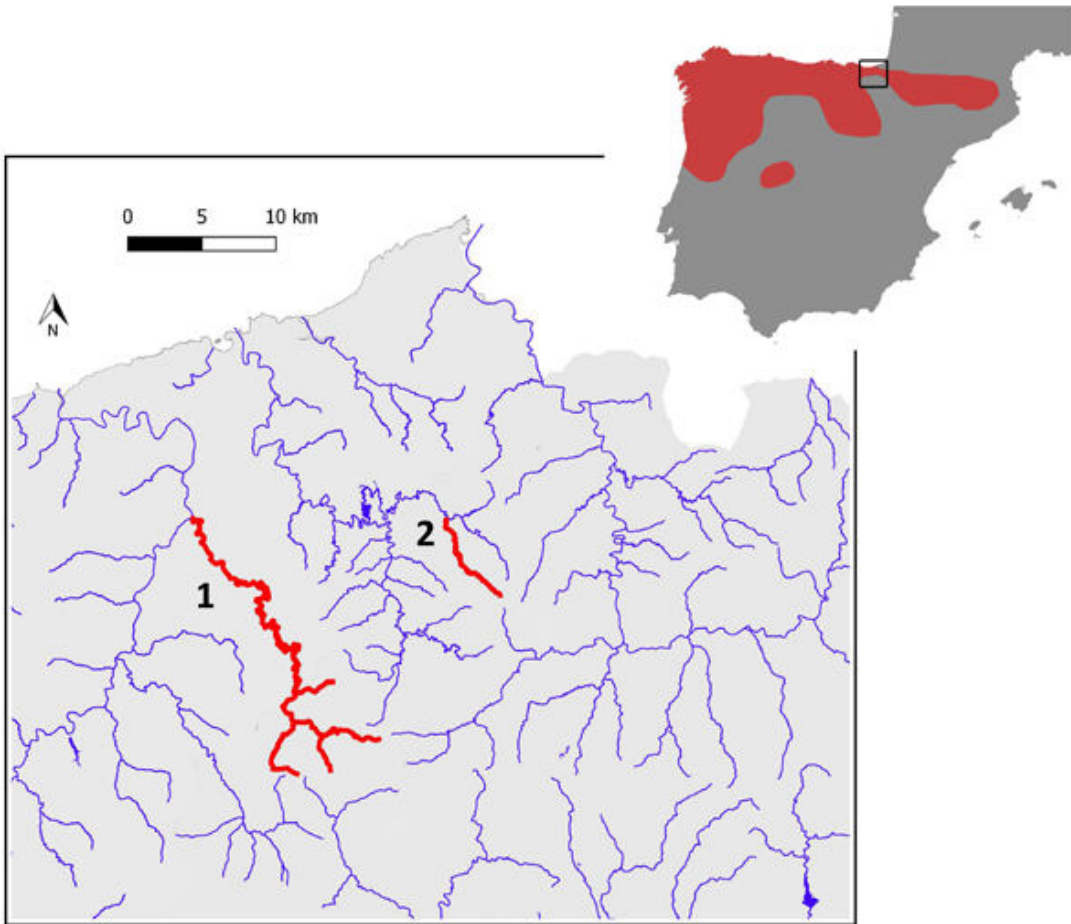


Figure 1. Study sites: (1) Leitzarán and (2) Elama Streams, in the northern Iberian Peninsula.

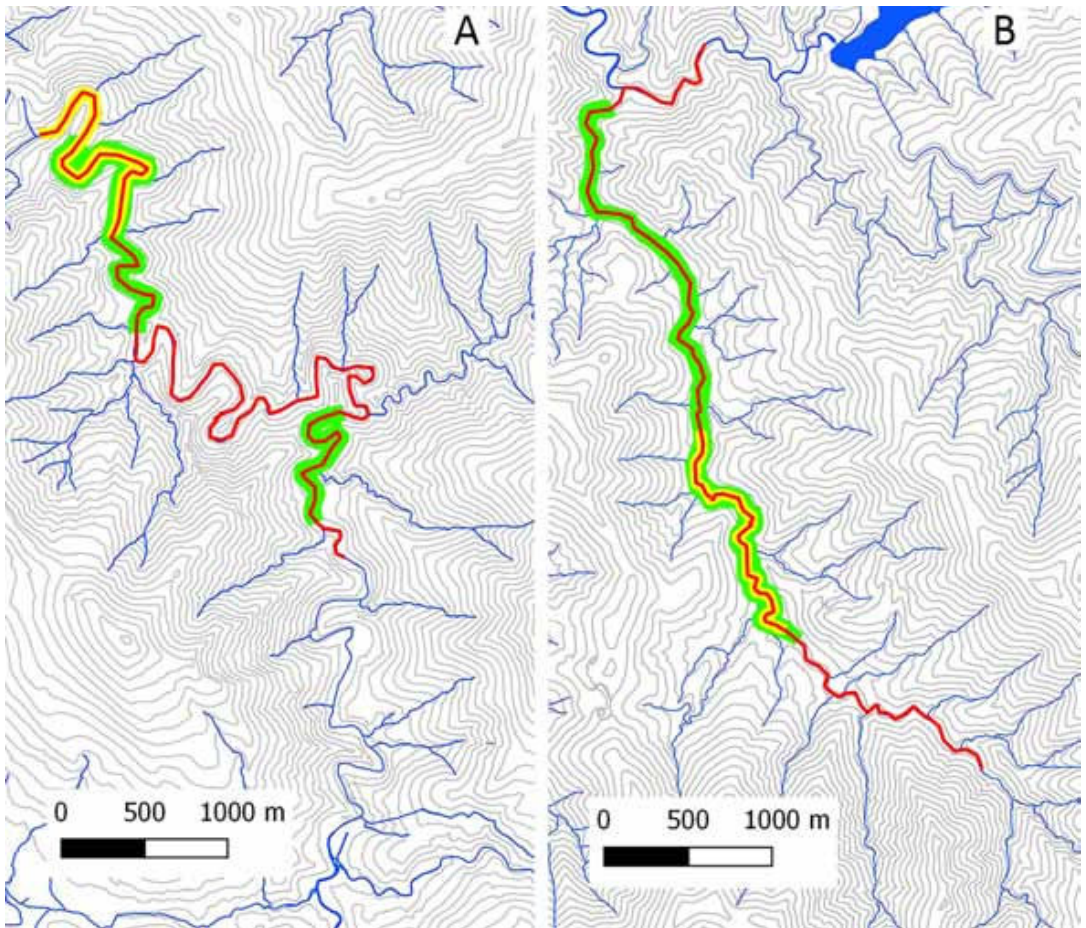


Figure 2. Study sites, in more detail: (A) Leitzarán and (B) Elama Streams. Information about the sections analysed in the active search for desman scats (red line), about the sections analysed in the first trial of the artificial shelters (yellow line) and about the sections analysed in the second trial of the artificial shelters (green line) was added in the figure.

In a preliminary sampling trial, in March 2016 we searched for desman scats in a 6 km segment of Elama Stream and an 8 km segment of Leitzarán Stream, following the standard active search procedure defined by Queiroz *et al.* (1998). In a situation of base flow and after 10 days without rain, we surveyed the streams inspecting with a hand torch all structures in the channel and on the banks where desmans could defecate (mainly rocks and logs). Each stream segment was examined in its entire length, without differentiating sections, for three consecutive days by 3 people, at an average standard speed of 200-300 m/h. During the surveys, the water level of both streams showed no fluctuations.

Next, we tested the efficiency of attracting desmans to home made artificial shelters or latrines. These artificial shelters were built with high-density ethylene-vinyl acetate (EVA) foam mats (ref. B517V1, mottez.com, 6 € each,

630X630X12 mm). Each mat was bent on top of a mound of boulders built in the middle of a shallow (10-30 cm deep) riffle, forming a “tent” that covered a dry platform over 0.25 m². The mat was fixed in place by means of rocks, piled as to offer a flat surface that could be easily accessed by desmans and would guarantee the persistence of droppings (Fig. 3, 4), and it was tied by a string to a nearby branch to prevent losing it in the event of a flood. The space between the rocks and the roof of the “tent” was not larger than 15 cm. The resulting structure thus simulated a small shelter or latrine, similar to those used by desmans to rest while they eat the food captured underwater (Niethammer, 1970). The structure was located in riffles, as these are the best places for desmans to capture food (Chapter 2; Esnaola *et al.*, 2018b; Richard, 1986).

To test their efficiency, we carried out a first sampling trial with artificial shelters in two smaller stream sub-sections of the same river stretches (1.8 km in Leitzaran and 1.4 km in Elama), where we did not find desman scats during the initial active search. We sampled the subsection of Leitzaran in April-June 2016, immediately after the first standard active search trial carried out in March, whilst the sampling in Elama was delayed until June 2016. We set 12 artificial shelters in Leitzaran Stream and 18 shelters in Elama (Fig. 2), their numbers and locations depending on the availability of riffles, with a mean distance of 160.9 m between them in Leitzaran (SD: 60.2 m, range 55-210 m) and 82.9 m in Elama (SD: 22.9 m, range: 55-135 m). The shelters in Leitzaran were kept for 92 days and checked every 10-15 days, whereas those in Elama were kept for 13 days and surveyed each 6-7 days. It is worth mentioning that sampling in Elama was suspended after 13 days because all the shelters got collapsed with scats. In both cases the streams registered base flow and little or no rainfall during the sampling time. On each visit we collected all the droppings found and preserved in individual vials in absolute ethanol, and all shelters were cleaned up to avoid finding the same scats later.

Later on, taking advantage of a subsequent trapping and radiotracking study, developed in September-October 2016 (Chapter 2; Esnaola *et al.*, 2018b), which provided precise information of the stretches occupied by the desman in both stream stretches, we carried out a new trial to better assess the effectiveness of sampling with artificial shelters. Thus, in October 2016, also with base flow and low rainfall, we set 16 shelters in each of the streams, in reaches where desman presence was confirmed by trapping and radiotracking (Chapter 2; Esnaola *et al.*, 2018b). This time we sampled a 3.5 km long section (two subsections of 1.0 km and 2.5 km) in Leitzaran, and a 4.0 km long section in Elama, and the shelters were again distributed on shallow riffles (Fig. 2). The mean distance between shelters was 233.3 m in Leitzaran (SD: 47.3 m; range 190-360 m) and 270.3 m in Elama (SD: 67.3 m; range: 170-410 m). All shelters were surveyed every 24 h for three consecutive days

and all scats were collected as described above. To minimise the loss of scats due to possible water level fluctuations, the shelters were surveyed early in the morning, just after the end of the desman nocturnal activity period. After each visit, all the shelters were cleaned up to be sure that scats found in a shelter were new at next visit.

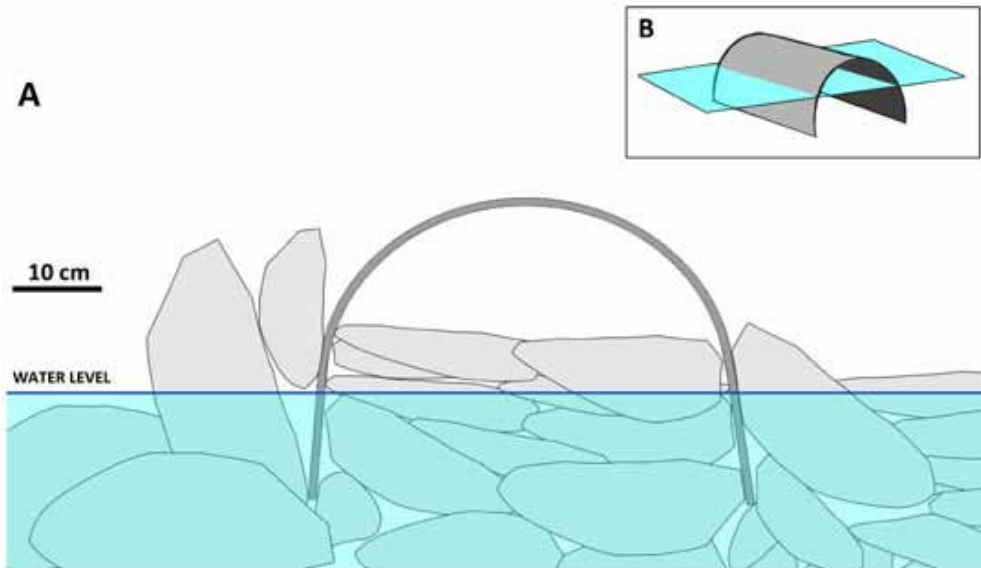


Figure 3. Scheme of a shelter.



Figure 4. Photograph of a shelter. Photo by Amaïur Esnaola.

The faeces were screened in the field, and only those with characteristics of desmans (musky smell, long, braided shape formed by the fusion of small spheres, length 15-30 mm) were preserved in individual vials in absolute ethanol.

Species identification by DNA metabarcoding

The source of the scats was identified by DNA metabarcoding of 15 random faeces collected in the first trial and almost all the faeces collected in the second one (N=150), as part of a molecular study of desman's diet (Chapter 4). DNA was extracted using the *Qiagen Powerfecal DNA kit* (Qiagen Iberia, S.L. Madrid), following the manufacturer guidelines. Subsequently, mitochondrial Cytochrome c oxidase subunit I (COI) gene was PCR amplified from extracts at the Analytical Services (SGIker) of the University of The Basque Country UPV/EHU, using the primer set described by Gillet *et al.* (2015). Samples were purified and a second reaction was performed to index each amplified product and attach Illumina adaptors using the *Illumina Nextera v2 kit*. Amplifications were performed with the *Quiagen Multiplex PCR Kit protocol*, using 12.5 mL Quiagen 2X (1 X final), 1.25 μ L forward primer (10 mM; 0.5 mM final), 1.25 μ L reverse primer (10 mM; 0.5 mM final), 8 mL H₂O and 2 mL DNA, in a final volume of 25 mL, with one activation step at 95 °C for 15 min followed by 40 cycles (denaturation at 94 °C for 30 s, annealing at 45 °C for 45 s, extension at 72 °C for 30 s) and final extension step at 72 °C for 10 min.

Once amplified, sequencing of PCR outputs was carried out in an *Illumina MiSeq NGS platform* (sequencing of 2x300 bp paired-end reads) with the *MiSeq® Reagent Kit v3 (600 cycle)*, following the manufacturer instructions. Sequencing was performed at the Analytical Services (SGIker) of the University of The Basque Country UPV/EHU.

Paired-end reads were merged using USEARCH (Edgar, 2010, 2013; Edgar and Flyvbjerg, 2015), demultiplexed by primers, adapter and primer sequences were removed, and reads were quality and length filtered using CUTADAPT (Martin, 2011). Then, singletons were removed and the remaining sequences were screened for chimeras using USEARCH. UPARSE algorithm (Edgar, 2013) was used to cluster sequences into Operational Taxonomic Units (OTUs) at a 97% similarity threshold (see Alberdi *et al.*, 2017). Finally, Genbank nt database was used to assign taxonomy to OTUs using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Species level assignments were performed when sequences matched with 100% similarity and 100% overlap, following Clare *et al.* (2013).

Results

The preliminary sampling based on the active search standard protocol (Queiroz *et al.*, 1998) yielded no desman faeces in the Elama Stream, and only two scats in the Leitzarar Stream (0.25 faeces/km). The desman shelters yielded a much higher number of faeces. On the first trial with artificial shelters (April-June) in sections with no previous confirmation of desman presence, over 50% of the shelters yielded faeces, and their production sustained over time (Tab. 1A). The deposition rate was higher for Elama than for Leitzarar Stream (24.5 vs 11.1 scats per km and survey, respectively). Actually, on the first trial, the visits to check the shelters were suspended in Elama after 13 days due to the intensive use of them by desmans. Visiting the shelters on a weekly basis, the stack of scats impeded individualizing samples. That situation was not observed in the Leitzarar.

Table 1. Numbers of scats collected in the study streams with artificial shelters. For each survey, it is shown the time elapsed since the shelters were set up (T, days), the number of shelters visited by desmans (P) and the number of faeces collected (N).

A) First trial. Shelters in the Leitzarar Stream were set up on 13th April 2016, those in Elama Stream on 11th June 2016.

Survey	Leitzarar (12 shelters)			Elama (18 shelters)		
	T	P	N	T	P	N
1	16	7	32	6	13	27
2	28	5	17	11	12	34
3	41	3	7	13	12	42
4	56	9	20			
5	68	4	15			
6	81	10	32			
7	92	8	17			
Total		10	140		13	103

B) Second trial. Sampling with surveys every 24 hours. The shelters in the Elama Stream were set up on 1st October 2016, those in the Leitzarar Stream on 30th October 2016.

	Leitzarar (16 shelters)			Elama (16 shelters)		
	T	P	N	T	P	N
1	5	15	15	1	11	38
2	6	15	15	2	14	42
3	11	24	24	3	12	32
Total		13	54		15	112

On the second trial with artificial shelters (October) in reaches where desman presence was confirmed beforehand, over 80% of the shelters were used by the 3rd night (Tab. 1B). As in the first trial, the deposition rate was higher for the Elama than for the Leitzaran Stream (10.6 vs 4.5 scats per km and survey, respectively).

In spring samples around 12667575 raw DNA sequences were obtained from faeces, which were reduced to about 11788479 after quality filtering. In autumn, instead, around 10297382 raw DNA sequences and about 8724646 filtered sequences were got. Bioinformatics' analyses and blasting showed that all the faecal samples contained abundant DNA sequences corresponding to the Pyrenean desman (100353 filtered reads per sample on average in spring and 10153 in autumn) with a 100% of similarity with the reference sequences. No sample was excluded because of low read numbers or bad sequence quality. No other potential source of faeces was identified.

Discussion

Population density of the Pyrenean desman ranges from 4 to 8 ind/km (Nores *et al.*, 1998), desman individuals prospecting daily their home ranges (Stone, 1987a). Although the rate at which they defecate is unknown, it is not unreasonable to estimate that tenths of scats can be produced per day and km, what, even with a large rate of loss, would yield hundreds of scats per km available, at least during dry, base flow periods. Nevertheless, these numbers contrast with the low yield collected during surveys, which ranges from 1.7-1.8 to 5.8 scats/km (Charbonnel *et al.*, 2015). The difficulty to find desmans' faeces in some streams occupied by the species produces false absences obtained through active search of scats (e.g. González-Esteban *et al.*, 2003), which are a matter of concern for administrations that carry out regional and national inventories since the 1980s. Recent studies (Charbonnel *et al.*, 2014) have worked to correct this problem.

The probability to detect desman faeces seems to be related to the composition and structure of stream channels (González-Esteban *et al.*, 2003a), being higher in channels with abundant emerging blocks accessible to the surveyor. The present study supports this hypothesis: the desman responds to an artificial increase in emergent structures, using them rapidly and continuously, and thus making it easier to detect it. Nevertheless, further research should be carried out to check the response of desmans to artificial latrines in streams with high availability of emergent structures or shelters.

On the other hand, it is difficult to explain the differences in yield of the artificial shelters among the studied streams, and our sampling design wasn't designed to do so. These might reflect either a higher population density in the Elama Stream (Chapter 2; Esnaola *et al.*, 2018b), or simply a higher relative increase in the surface of emergent structures per unit of surface area as a consequence of the building of artificial structures, what would trigger a stronger response by the desmans. In fact, as local deposition rates may be conditioned by many factors, researchers should keep from using them to infer any quantitative conclusion.

Whatever the case, the new sampling method presented in this paper greatly increases the detectability of desmans, providing an improved tool to test the species presence/absence with less risk of getting false absences. Additionally, it makes it easier to define the age of the faeces, thus improving the efficiency of methods that need abundant fresh material, such as genomics, population genetics, landscape genetics, dietary studies, or reproductive analyses (sex hormones). In particular, the need to find a method that allows the collection of fresh desman faeces has been highlighted by several authors (Gillet *et al.*, 2016; Hawlitschek *et al.*, 2018).

Moreover, this method is based on cheap, easy-to-use materials; building the shelters can be easily learnt in a single session, and two people can build 4-6 shelters per hour. Besides, even though this sampling procedure will always require two visits to the study area — to set the shelters first and to check them later —, it is noteworthy that checking for faeces in fixed spots is much easier and less timeconsuming than a full survey of all putative latrines along a stream stretch. Additionally, the high yield of fresh faeces favours in situ identification after basic training. Therefore, this method seems optimal for synchronic, regional-scale surveys, especially if they involve volunteers, as used for other mammals such as the water mole *Arvicola amphibius* in the UK (National Water Vole Monitoring Programme; <https://ptes.org/get-involved/surveys/countryside-2/nationalwater-vole-monitoring-programme/>).

Artificial shelters, thus, offer an important improvement over traditional desman scat surveys, and greatly enhance the possibility for future monitoring of the populations of this endangered species based on non-invasive sampling methods. Future work should define the protocol to use in these surveys (number of sampling units, effort, seasonality, etc.).

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