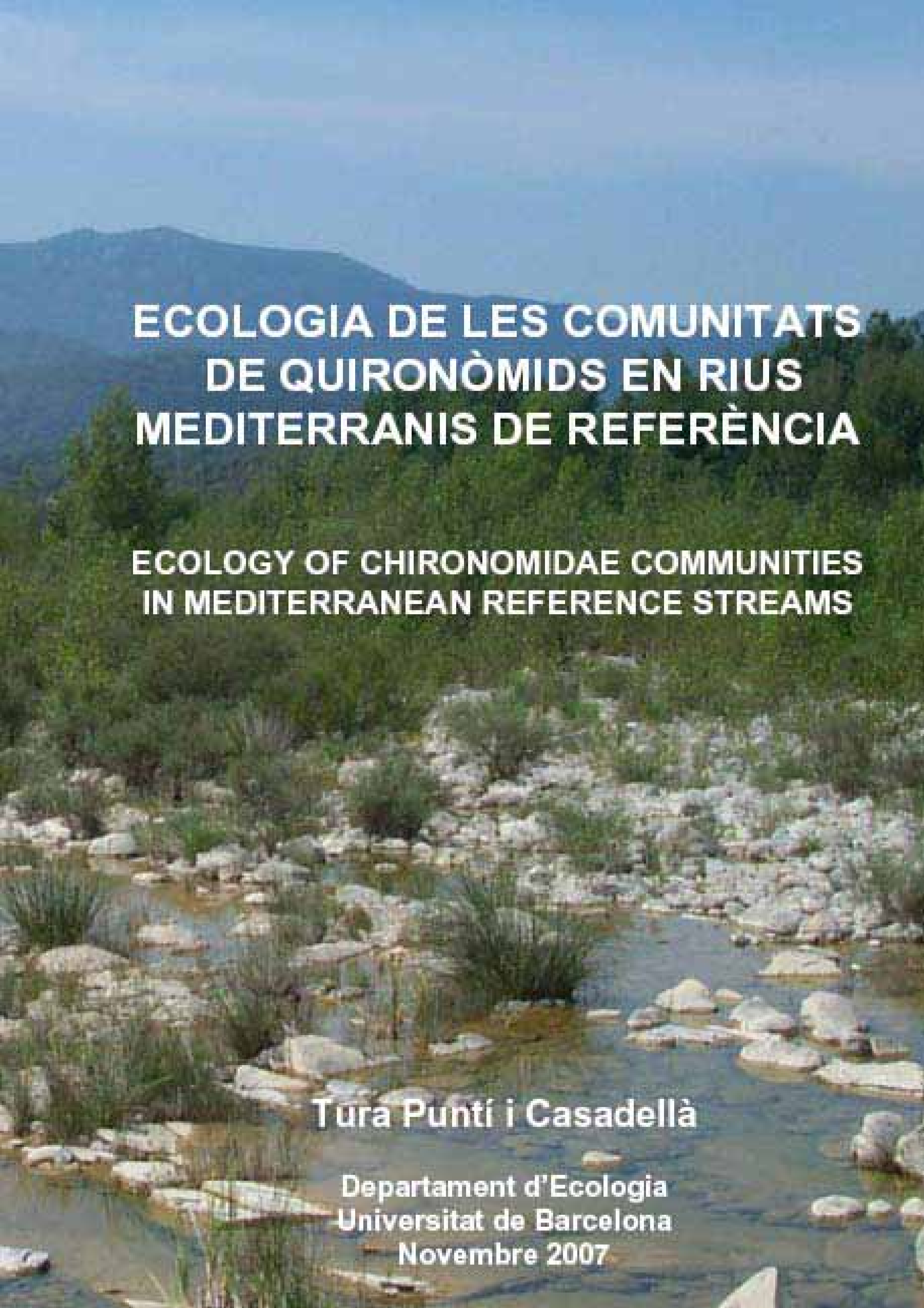

ECOLOGIA DE LES COMUNITATS DE QUIRONÒMIDS EN RIUS MEDITERRANIS DE REFERÈNCIA

ECOLOGY OF CHIRONOMIDAE COMMUNITIES IN MEDITERRANEAN REFERENCE STREAMS

Tura Puntí i Casadellà

Departament d'Ecologia
Universitat de Barcelona
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Memòria presentada per Tura Puntí i Casadellà per optar al títol de Doctor per la Universitat de Barcelona, sota la direcció dels doctors Narcís Prat i Fornells i Maria Rieradevall i Sant.

Tura Puntí i Casadellà

Barcelona, Novembre del 2007

El director de la tesi:	La directora de la tesi:
Dr. Narcís Prat i Fornells	**Dra. Maria Rieradevall i Sant**
Catedràtic d'Ecologia	Professora titular d'Ecologia
Facultat de Biologia (UB)	Facultat de Biologia (UB)

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Cuando salto por el acantilado

una abrumada espuma se formará,

burbujas de aire, de vida me llenarán.

La luz atraviesa mi corazón

dando suerte a todo tu interior.

Bañate conmigo y te mojaré,

vida tengo, vida soy.

Quiero esperarte,

pero fluyo y nada me detiene.

No me llenes de tus negreces,

que los malos sueños

no llegen a mi cauce.

Vive el agua

sueña ríos i torrentes

vacios de muerte,

llénalos de ti.

“Solilluna”

A la màgia de la vida

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Agraïments

Diuen que tot va començar a l'aigua no? Doncs a aquesta tesi si li hagués de posar un començament me n'aniria a una gota d'aigua... una gota d'aigua reivindicativa que cridava per aturar el transvasament de l'Ebre...

... quan jo em vestia de gota d'aigua és quan em vaig començar a interessar per l'estudi i la conservació dels rius, quan el moviment per una nova cultura de l'aigua es començava a fer sentir, i quan socialment hi va haver una gran explosió reivindicativa, on l'estat ecològic dels rius estava en boca de tothom (o quasi). En aquest moment és quan vaig conèixer a en Narcís, el que ha estat després un dels meus directors de tesi, i a qui des de sempre he admirat moltíssim per la seva gran capacitat de fer ciència i que mai ha deixat de creure en la transformació social donant un punt de vista més aplicat a l'ecologia... i està clar pel seu amor incondicional pels quironòmids que comparteix tant entusiasmadament amb la Maria! Si no hagués estat per aquesta passió compartida ben segur que no hagués fet mai una tesi de quironòmids! Però em vaig llançar, si! amb l'impuls de la gota d'aigua que volia conèixer més dels nostres petits i grans rius i més dels nostres grans i petits quironòmids. Gràcies Narcís per la teva capacitat resolutiva, pel teu entusiasme, per la teva visió holística, i gràcies Maria, pel teu ull clínic, per qüestionar lo inqüestionable i als dos per la oportunitat i confiança que m'heu donat d'endinsar-me en aquest món, i per fer-me creure que si, que els quiros es poden identificar, i amb alegria!

Una de les fonts d'inspiració principals d'aquesta tesi i a qui vull agrair tot el suport que m'ha donat durant tot aquest temps ha estat la Núria Bonada. Moltíssimes gràcies Núria per estar sempre aquí, sobretot darrera els mails entre les estades d'una o de l'altra, per tenir sempre una resposta i un cop de mà. Ha estat una gran sort coincidir amb tu i amb el teu somriure!

I una altra gran sort ha estat coincidir amb tots els ecobills... sense aquest grup de treball, tot hagués estat molt diferent. Gràcies als de la primera etapa (Caro, Rosa, Toni) els del mig (Mireia, Cesc, Blanca, Núria C., Rosa Andreu, Luisa) els d'ara (Iraima, Pau, Raul, Laura, Miguel, Mia, Esther, Cristian) a les que em vau ajudar a tallar caps (Cristina i Núria S.)... Us diria mil coses a cadascun de vosaltres, mil i més i mil gràcies, pels bons moments, per la gran qualitat humana, per entendre els heys heys, per les aixeres de campanyes, d'hores de lupes, de complicitat i de suport sempre, per trobar amb vosaltres amistat a més de companys de feina ... Per què he après molt al vostre costat, i les mil coses que us vull dir ja us les diré en persona!

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I... a tots els companys del Guadalmed 2, sense aquest projecte no hagués tingut la oportunitat de saber què és treballar coordinadament amb d’altres equips. Treballar conjuntament amb vosaltres ha estat una gran oportunitat per aprendre molt! Santi, Chary, Maria Luisa, Maruxa, Javier, Ana, Manolo, Biel, Jesús, Andrés i... gràcies especialment a l’altra becari, al Poquet: finalment ja ho hem aconseguit nen!

Durant tot aquest temps pel departament hi ha hagut moltes entrades i sortides, però sempre s’ ha respirat un ambient humà molt sa que hem viscut amb tots els becaris. Amb tots vosaltres he compartit sopars, consells, dinars a la paret amb el solet, reflexions als passadissos, i aquí hi sou tots els vells i els nous, amb tots he viscut el que tocava a cada moment! Gràcies Luciano, Biel, Dani, Sílvia, Oriol, Jaime, Xavi de P., Mary, Ainhoa, Carles, Izascun, Pere, Vicenç, Gonzalo, Gemma, Laia, Enric, Tània, Octavi, Esther, Eusebi, Júlio tots vaja!... i moltes i especials gràcies al Salva, perquè m’has donat l’empenta final en el moment que ho necessitava, visca les sads!

Fer aquesta tesi també m’ha donat la oportunitat de veure món! Gràcies al “ministerio” he pogut sortir a explorar més rius mediterranis. La primera parada va ser Austràlia. Allà vaig treballar amb un altra enamorat dels “non-biting midges”, el Don Edward, i un altra gran científic l’Andrew Storey “Thank you Andrew and Don for your help, your extreme attention and hospitality, I will never forget my experience at Western Australia ...” i gràcies a les meves dues germanes australianes a la Eleanor i a la Miky, i a totes les llumetes que em vaig trobar allà, va ser una gran aventura viure l’esperit aussie.

La segona parada va ser al mediterrani xileno, allà el nostre guia va ser el Ricardo Figueroa. “Gracias Ricardo por hacerme ver que las cosas se pueden salir de la libreta, por tu creatividad y tus ganas y tu querer de los ríos chilenos”. Gracies també a tota la gent de l’EULA, per totes les facilitats que ens vau donar, Xirino, Alexis, Fernando, Alberto, Paola, Marta i especialment a la Marie Claire... allà ens vau fer sentir com a casa! A Epuyén també per començar a somiar amb les llunes.

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Mirant a Sant Francesc, Olot

Un vespre del 2007

Introducció

INTRODUCCIÓ

Context general i marc teòric

Aquesta tesi s'ha desenvolupat en un moment en el que els rius pateixen fortes pressions antròpiques, i en un context on es pretén implementar una normativa (la *Directiva Marc de l'Aigua*) que vol millorar l'estat de salut dels rius d'Europa amb l'objectiu d'assolir el bon estat ecològic de les seves aigües ens els propers anys. La biodiversitat dels ecosistemes aquàtics està disminuint ràpidament en els últims temps (Allan, 1995), com han demostrat nombrosos estudis d'avaluació de la qualitat biològica dels rius (per exemple: Barbour *et al.,* 1999). En el cas dels rius mediterranis de la Península Ibèrica, els treballs desenvolupats arrel del projecte GUADALMED (Jáimez & Cuéllar, 2004; Bonada *et al.,* 2004a), mostren que hi ha molts trams de rius mostrejats que es troben en un estat inferior al bon estat ecològic. Un prerequisit fonamental perquè es puguin implementar programes de gestió adequats és estudiar quines són les condicions de referència per tal de conèixer els objectius de qualitat a assolir. Però per tal d'avaluar l'estat ecològic dels nostres rius necessitem un bon i millor coneixement de base de la taxonomia, distribució i autoecologia de les espècies, ja que encara hi ha moltes llacunes d'informació en determinats grups biològics. El present treball aborda l'ecologia de les comunitats d'un dels grups de macroinvertebrats bentònics més diversos i abundants dels nostres rius però alhora més oblidats: els quironòmids*,* una família de dípters que s'ha utilitzat àmpliament en estudis de monitoratge com indicadors de la pol·lució orgànica amb diferents aproximacions, per exemple utilitzant les exúvies pupals (Rieradevall & Prat, 1986; Ruse, 2002).

A la figura 1 s'il·lustra de manera sintètica el marc teòric del nostre treball. S'hi indiquen els principals processos que actuen com a filtres de la biodiversitat global a diferents escales espacials, i com aquests determinen la composició de les comunitats de quironòmids en rius mediterranis de referència. Així doncs, en primer lloc hi haurà una selecció d'espècies per factors històrics i climàtics que determinaran la composició del *pool* d'espècies regional. Després els filtres ambientals i biòtics seran els d'escala més petita (regió, conca o subconca, tram). Com a resultat final del filtratge i la selecció d'espècies s'obtindrà un subgrup de les espècies provinents del *pool* regional que són les que poden coexistir en una comunitat (Poff, 1997). La comunitat local resultant tindrà unes característiques estructurals (riquesa d'espècies, patrons d'abundàncies) i funcionals (distribució de grups tròfics) determinades. En aquesta tesi s'aborden les característiques estructurals de les comunitats de quironòmids.

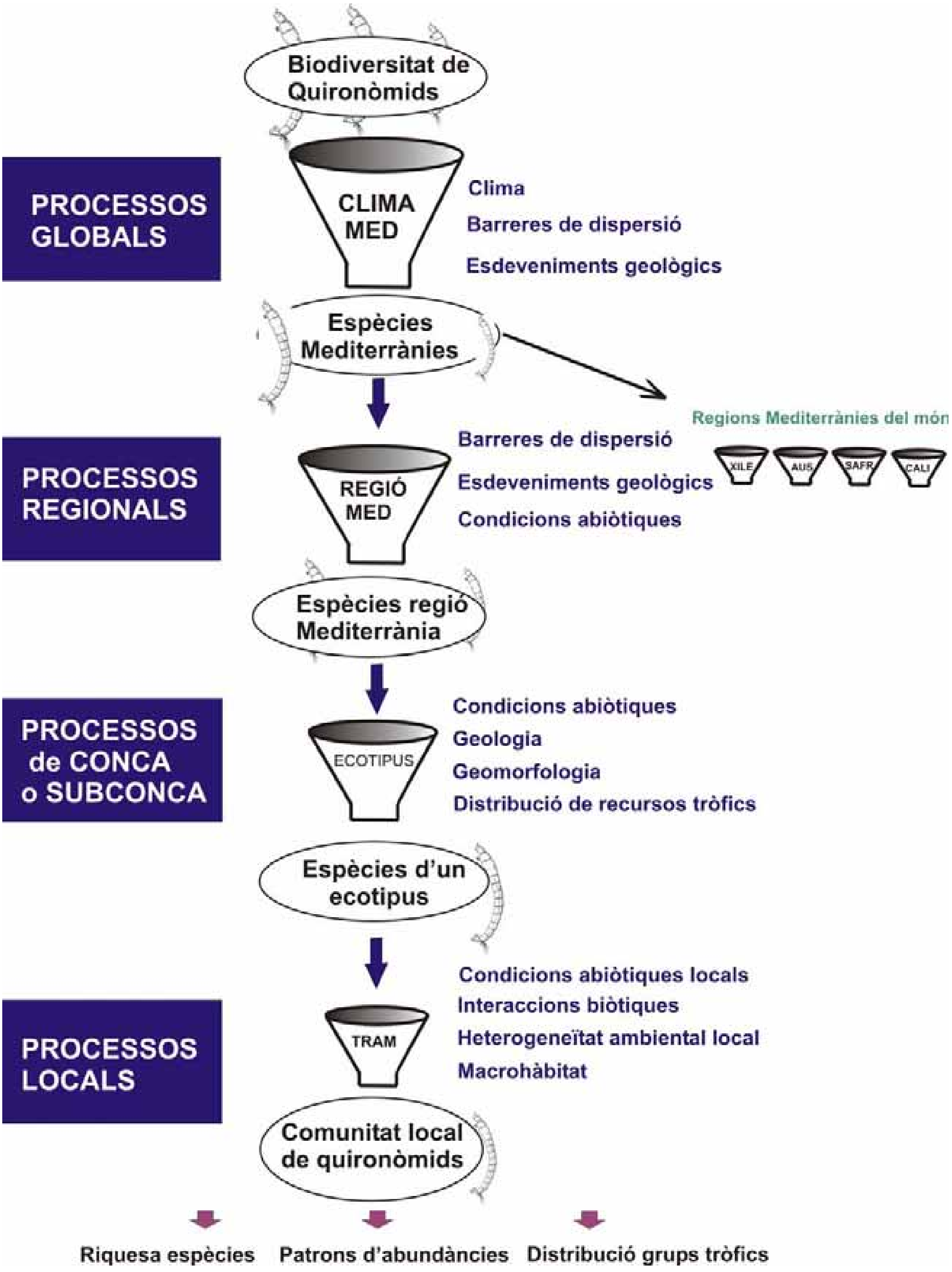


Figura 1. Procesos a diferents escales espacials que determinen la composició de les comunitats de quironòmids en cada tram de referència.

Els rius mediterranis

Aquesta és una tesi centrada en l'estudi de rius situats en zones de clima mediterrani, tot seguint una línia de treball que fa molts anys que duu a terme el grup de recerca en el que estem integrats (grup F.E.M., Departament d'Ecologia, Universitat de Barcelona). Les cinc regions del planeta que comparteixen un patró climàtic típicament mediterrani estan ubicades entre 32-40º Nord i Sud de latitud (Aschmann, 1973) (figura 2). Els cursos d'aigua situats en aquestes regions caracteritzades per tenir clima mediterrani s'anomenen rius mediterranis, els quals estan afectats per una forta estacionalitat en el règim de precipitació i temperatures, amb estius calorosos i secs i hiverns freds i humits (Di Castri, 1973; Gasith & Resh, 1999). A més a més, la temperatura i la pluja pot variar molt d'un any a l'altre, fet que implica una elevada heterogeneïtat ambiental (Mount, 1995). Com a conseqüència de les variacions anuals i interanuals en el règim de cabals, els rius d'aquestes zones estan sotmesos freqüentment a avingudes i sequeres (Martín Vide & Olcina, 2001). En funció de la intensitat i la freqüència d'aquestes perturbacions naturals, aquests rius poden presentar règims hidrològics permanents, intermitents o efímers. Els organismes que habiten en aquests sistemes presenten com a conseqüència adaptacions del seu cicle de vida, que els hi fan tenir trets específics (*species traits*) particulars, sent atributs comuns d'aquesta biota tant la resistència com la resiliència a l'efecte dels canvis ambientals, per exemple a la sequera (Meyer & Meyer, 2000; Lake, 2003). D'altra banda, les regions influenciades pel clima mediterrani es consideren un punt calent de biodiversitat mundial (Myers *et al*., 2000), sobretot pel que fa a les comunitats terrestres (per exemple: vegetació o artròpodes terrestres). Hem de destacar però que hi ha una manca de coneixement important pel que fa a la biodiversitat dels ecosistemes aquàtics mediterranis (Álvarez-Cobelas *et al*., 2005).

A més a més, davant dels futurs escenaris del canvi climàtic global, s'espera que en les àrees de clima mediterrani hi hauran pluges estacionalment més irregulars, temperatures més elevades (Bolle, 2003), i una tendència a l'increment de la intensitat i freqüència de sequeres (Arnell, 1999). També es preveu un increment de la proporció de rius amb característiques mediterrànies en àrees que actualment són temperades (Bonada *et al*., 2007a). Així doncs, és per tots aquests motius que l'estudi de la biodiversitat i els patrons ecològics que tenen lloc en els rius mediterranis, són de gran interès.

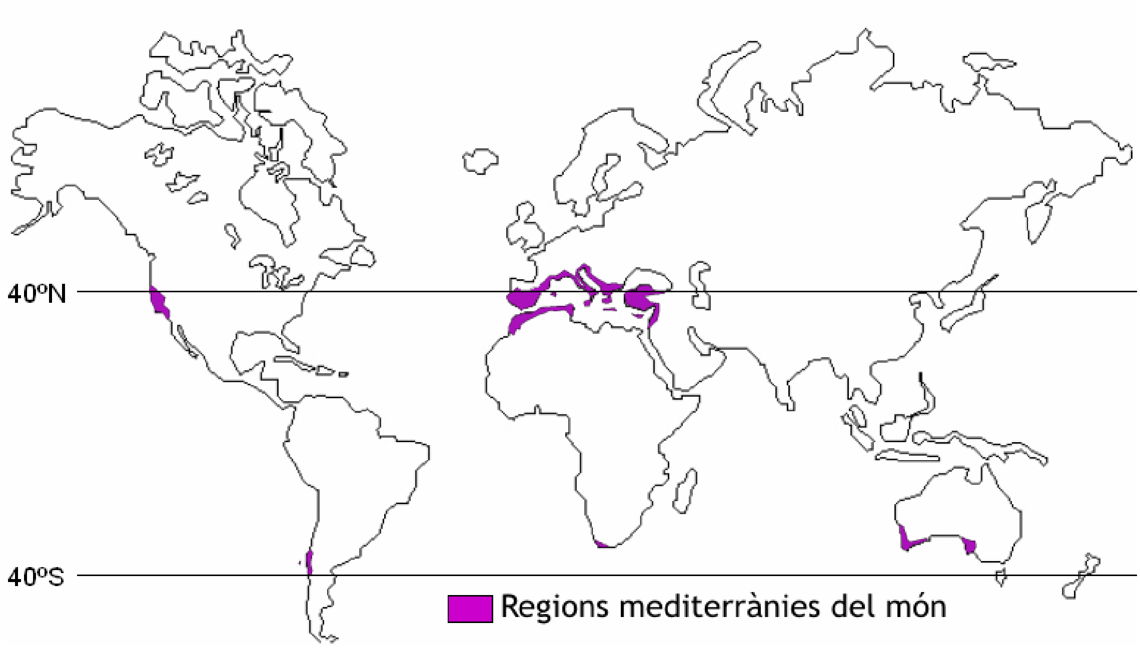


Figura 2. Mapa de la distribució de les regions de clima mediterrani en el món.

Condicions de referència i el projecte GUADALMED

La degradació dels ecosistemes fluvials és patent a causa de multitud d'actuacions humanes com ara la regulació dels cabals, l'eutrofització i l'alteració de l'estructura de l'hàbitat, la destrucció de la vegetació de ribera pel pas d'infraestructures, la manca de cabals mínims circulants per la derivació d'aquests a l'agricultura o explotacions hidroelèctriques, etc., tal i com sumaritzen Allan & Flecker (1993). Totes aquestes pertorbacions han modificat les condicions ambientals d'aquests ecosistemes reduint la capacitat que tenen per acollir una comunitat biològica diversificada. Particularment, en els rius mediterranis aquestes perturbacions antròpiques han estat molt importants sobretot a les parts mitges i baixes de les conques fluvials, especialment a causa de l'elevada densitat de població i del model intensiu de l'ús de l'aigua (Prat, 1984; Aguiar *et al*., 2002).

La Directiva Marc de l'Aigua (DMA) (European Comision, 2000) estableix com a mesura del grau de conservació o degradació d'un ecosistema aquàtic el concepte d'estat ecològic, el qual es determina per la qualitat biològica dels diferents elements (fitobentos, macròfits, macroinvertebrats i peixos), juntament amb d'altres elements de qualitat hidromorfològics i fisicoquímics (Wallin *et al.,* 2003). El grau d'alteració de l'estat ecològic es mesura segons la desviació dels paràmetres ambientals i biològics respecte als valors que considerem de referència. Durant els últims anys s'han desenvolupat molts estudis per tal d'avaluar l'estat ecològic dels nostres rius seguint les directrius de la DMA, ja que el principal objectiu d'aquesta directiva europea és obtenir el bon estat ecològic de totes les masses d'aigua l'any 2015 (Irvine, 2004). Concretament, des del 1998 el projecte GUADALMED ha estat estudiant l'estat ecològic dels rius mediterranis de la Península Ibèrica segons les directrius de la DMA i centrant-se amb l'estudi de les comunitats de macroinvertebrats. Entre d'altres en la primera fase del projecte (1998-2001) es va elaborar un protocol de mostreig estandarditzat (Jáimez-Cuéllar *et al*., 2004) utilitzant indicadors biològics i hidromorfològics, per tal de fer comparables les dades obtingudes per tots els equips de treball. Amb l'aplicació d'aquest protocol es van estudiar les comunitats de macroivertebrats de zones perturbades i d'altres de referència, per tal d'avaluar l'estat de salut dels rius mediterranis (Vivas *et al.,* 2004).

Aquesta tesi s'emmarca en la segona fase del projecte GUADALMED (2002-2005), el qual s'ha centrat en estudiar exclusivament les condicions de referencia dels rius mediterranis de la Península Ibèrica. Donada la manca de coneixement que hi ha de les comunitats de macroinvertebrats d'aquests rius poc alterats, estudiar quines són les característiques de les condicions de referència és imprescindible per tal de fer una bona diagnosi de l'estat ecològic. Per tant tots els estudis realitzats en la segona fase del projecte s'han fet en condicions de referència (o poc alterades) i conseqüenment els llocs amb les comunitats degradades per les perturbacions d'origen humà no es tracten en aquesta tesi.

Previ a la definició de les condicions de referència, és necessària l'elaboració d'una tipologia dels rius, ja que la gestió dels ecosistemes aquàtics requereix una classificació de la variabilitat natural i de les seves comunitats biològiques (Reynoldson, 1997; Verdonschot, 2006). Dues aproximacions diferents són les que s'han utilitzat més freqüentment per elaborar les tipologies fluvials. En una aproximació *bottom-up*, es parteix de les comunitats biològiques per agrupar els rius (per exemple: Heino *et al*., 2003). En canvi en una classificació *top-down* les tipologies de rius es defineixen a través de variables ambientals, basant-se en el coneixement previ del territori (per exemple: Munné & Prat, 2004). Posteriorment les regions o els tipus fluvials que s'obtenen *a priori* s'han de validar amb les comunitats biològiques (Soininen *et al*., 2004; Ferréol *et al*., 2005). Aquesta classificació *top-down* està basada en la idea que el territori està diferenciat en regions que es caracteritzen per un clima, topografia, geologia i vegetació particulars, entre d'altres. Per tant s'assumeix que dins d'una regió o tipus fluvial, les condicions han de ser relativament homogènies i que trobarem unes comunitats biològiques semblants (Omernik & Bailey, 1997).

La proposta que fa la Directiva Marc de l'Aigua per a la classificació de les masses d'aigua d'Europa segueix una aproximació *top-down*. Concretament, per a l'elaboració de les tipologies de rius es proposen dos sistemes: el sistema A, el qual diferencia les masses d'aigua tenint en compte únicament tres descriptors ambientals dins d'unes determinades ecoregions (l'altitud, la mida de conca i la geologia), i el sistema B, que proposa uns factors obligatoris (altitud, latitud, longitud, geologia i mida de conca) juntament amb d'altres factors opcionals, com per exemple variables hidrològiques o morfològiques.

A la primera part del projecte GUADALMED es va presentar una tipologia preliminar per la regió mediterrània de la península Ibèrica utilitzant tant el sistema A com el B (Bonada *et al*., 2004a). Però per tal de millorar els resultats obtinguts, en la segona fase del projecte es van incloure nous punts de mostreig i noves variables ambientals, utilitzant exclusivament el sistema B.

Un cop tipificades les diferents masses d'aigua, s'hi hauran d'assignar els estats de referència corresponents (Stoddard, 2005). Per aquesta tasca s'hauran de seleccionar els trams de rius dins de cada regió que presentin un estat de conservació i naturalitat elevats, i una alteració antropogènica gairebé inexistent. La selecció de les estacions de referència s'ha realitzat freqüentment utilitzant una metodologia *a priori* (Reynoldson *et al*., 1997; Stoddard, 2005), i també aquesta és la metodologia utilitzada en el projecte GUADALMED (Bonada *et al*., 2004b; Sánchez *et al.,* 2005; Sánchez *et al.,* submitted). Aquest mètode de selecció consisteix en elaborar un llistat de criteris que inclogui les perturbacions i pressions derivades de les activitats humanes que poden afectar l'estat ecològic de la zona d'estudi. Després de dur a terme la selecció *a priori* dels punts de mostreig, s'haurà de fer una validació per tal de refinar la selecció dels punts de referència (Nijboer *et al.,* 2004). En general, segons aquest sistema un punt de mostreig serà estrictament de referència quan compleixi tots els criteris, mentre que si hi ha algun criteri (un o dos) que no compleix, es considerarà un punt mínimament perturbat. En aquests casos, i per algunes tipologies de rius, per exemple en trams baixos on és pràcticament impossible trobar estrictament punts de referència, haurem de considerar els punts menys alterats o amb el màxim potencial ecològic utilitzant els termes de la DMA (Chaves *et al.,* 2006).

En la segona fase del projecte GUADALMED es va establir una xarxa d'estacions de referència de 162 punts, localitzats en 34 conques d'estudi de la Península Ibèrica. La selecció d'aquestes estacions es va fer en base als criteris resultat de la primera fase del projecte (Bonada *et al*., 2004b). En canvi, la validació d'aquestes estacions de referència s'ha fet tenint en compte els criteris de selecció d'estacions de referència en rius mediterranis elaborats per Sánchez-Montoya *et al*. (2005) (veure taula 1). Les conclusions finals de com s'han de seleccionar les condicions de referència estan recollides en el treball:

Sánchez-Montoya, M.M; Vidal-Abarca M. R.; Puntí T.; Poquet J. M.; Prat N.; Rieradevall M.; Alba-Tercedor J.; Zamora-Muñoz C.; Robles S.; Álvarez M.; Toro M. and Suárez M. L. Defining criteria to select reference sites in Mediterranean streams (submitted). *Hydrobiologia.*

Aquest treball, del qual som també coautors, és imprescindible ja que sense ell no haguéssim fet la validació adequada de les estacions de referència mostrejades en aquesta tesi, i forma part de la tesi de M.M. Sánchez-Montoya.

Elements	**Bloc**	**Criteris**
Biològics	**Zona de ribera**	1. Cobertura i composició d'espècies adequada al tipus
		2. Absència de plantacions i/o cultius
		3. Absència d'àrees impermeables a la plana d'inundació (carreteres asfaltades, grans construccions…)
	Espècies introduïdes	4. Absència d'impacte sever sobre la biota autòctona per espècies introduïdes
Fisicoquímics	**Contaminació puntual**	5. Sense evidències d'abocaments urbans
		6. Sense evidències d'abocaments industrials
		7. Absència de canals de retorn procedents de rec
	Contaminació difusa	8. Percentatge total d'agricultura <30% (inclosos secà i regadiu)
		9. Percentatge total d'àrea impermeable < 5 % (incloses zona urbana, industrial, infrastructures i àrees de recreació)
		10. Absència d'impacte sever per pastoreig
		11. Usos naturals de la conca > 70%
Hidromorfològics	**Morfologia fluvial**	12.Composició del substrat adequada al tipus
		13. Absència de canalització (marges i fons no fixats)
		14. Absència d'estructures transversals que provoquen retenció de sediments
		15. Absència d'activitats extractives de graves i sorres
	Condicions hidrològiques i regulació	16. Absència de derivacions d'aigua pel rec o d'altres finalitats.
		17. Absència d'embassaments o preses que modifiquen el cabal natural del riu
		18. Absència de transvasaments entre conques

Taula 1. Criteris de referència per la selecció de les estacions de mostreig (Sánchez-Montoya *et al*., 2005).

Un altra aspecte que s'ha considerat en el projecte GUADALMED és que per fer la diagnosi de l'estat ecològic es poden utilitzar diferents sistemes, entre ells sistemes multivariants que permetin predir la comunitat que hauria d'estar present en un determinat tipus de riu en condicions de referència i comparar-la amb la comunitat actual. El sistema predictiu està basat en comparar la composició de les comunitats de macroinvertebrats observada i esperada, on la composició esperada s'obté a partir de la relació entre la classificació biològica de les estacions de referència i les variables ambientals estudiades (Wright *et al*., 1984). Per tant per dur a terme una bona aplicació dels mètodes predictius, és imprescindible un bon coneixement de les comunitats biològiques en condicions de referència.

Un altra resultat del projecte GUADALMED ha estat l'elaboració d'un mètode predictiu per les regió mediterrània de la Península Ibèrica (MedPacs) i s'està treballant en una publicació que està en fase de finalització i de la qual també som coautors, i que forma part de la tesi de J.M. Poquet.

Poquet J.M., Alba-Tercedor J., Puntí T., Sánchez-Montoya M.M., Robles S., Álvarez M., Zamora-Muñoz C., Sáinz-Cantero C.E., Vidal-Abarca M.R., Suárez M.L., Toro M., Pujante A.M., Rieradevall M. and Prat. N. A MEDiterranean Prediction and Classification System (MEDPACS) for aquatic macroinvertebrate communities as a tool to assess the ecological status of Mediterranean rivers.

Els quironòmids

Una part molt important de la biodiversitat dels rius mediterranis està formada pels macroinvertebrats bentònics (Alba-Tercedor *et al*., 1992). Aquests organismes tenen un paper molt important tant des del punt de vista funcional com estructural dels ecosistemes fluvials (Allan, 1995), i s'han utilitzat àmpliament en el monitoratge dels rius (Rosenberg, 1993). Per tant la informació sobre l'estructura de les comunitats de macroinvertebrats és important, no només des del punt de vista de conservació de la biodiversitat sinó també des del punt de vista de gestió i restauració dels ecosistemes fluvials. Els estudis que es fan en ecologia de les comunitats s'haurien de fer a nivell específic (McCreadie & Adler, 1998), ja que això ens donarà una informació més precisa de la resposta de les comunitats, davant de per exemple els factors ambientals (Lenat & Resh, 2001). La resolució taxonòmica dependrà però de l'objectiu de l'estudi plantejat inicialment. En els rius mediterranis de la Península Ibèrica podem dir que hi ha un bon coneixement de la majoria de grups de macroinvertebrats, ja que s'hi han realitzat tesis doctorals d'efemeròpters i plecòpters (Puig, 1983), simúlids (González, 1990) i tricòpters (Bonada, 2003), entre d'altres. En aquesta tesi, hem aprofundit en l'estudi dels quironòmids, els quals són una família de dípters nematòcers àmpliament diversificada, molt abundants, ubicus i de gran importància en els sistemes aquàtics (Pinder, 1986; Cranston, 1995) i coneguts per la seva tolerància a la contaminació i la seva dificultat taxonòmica a l'hora de classificar-los a nivell de gènere o espècie. Pràcticament no existeix una massa d'aigua, no importa altitud o latitud, en la qual no hi trobem alguna larva de quironòmid. S'estimen unes 10000 espècies a tot el món, encara que aquesta dada pot ser molt més gran ja que hi ha moltes regions poc estudiades (Armitage, 1995).

Malgrat l'atenció que aquesta família ha rebut per part dels especialistes (Cranston, 1995), els quironòmids han estat sovint ignorats en la majoria d'estudis ecològics, a causa principalment de la seva dificultat en la identificació taxonòmica a nivell específic, sobretot treballant amb larves, bàsicament per les limitacions de les claus taxonòmiques i per la laboriosa tasca del muntatge dels individus per a la seva observació. És per aquests motius que la majoria dels estudis d'ecologia aquàtica que treballen amb les comunitats de macroinvertebrats, han deixat de banda els quironòmids, identificant-los com a molt a nivell de subfamília. Tot i això, els quironòmids són un grup d'organismes que s'han utilitzat àmpliament en estudis d'avaluació de la qualitat biològica en rius mediterranis (Prat *et al.,* 1983; Rieradevall & Prat, 1986). Malgrat tot, manca molta informació pel que fa a l'ecologia d'aquest grup en condicions de referència, ja que normalment sempre s'han estudiat aquestes comunitats comparant condicions impactades amb d'altres ben conservades (per exemple: Calle-Martínez & Casas, 2006).

El seu cicle vital comprèn quatre estadis de duració desigual: ou, larva, pupa i adult, desenvolupant-se els tres primers en tot tipus de medis aquàtics (Figura 3). Es solen distingir quatre estadis larvaris, els quals són caracteritzables a través de les mesures de la càpsula cefàlica (Schmid, 1993). L'alimentació de les larves de quironòmids és molt variable, hi ha grups amb una alimentació característica, com poden ser els Tanypodinae que majoritàriament són depredadors, però la majoria són detritívors alimentant-se d'una barreja de partícules de matèria orgànica amb organismes petits i amb proporció variable de material fresc (Berg, 1995). La duració dels estadis de pupa i adult és molt curta en comparació amb la fase larvària, podent anar des d'unes hores a varis dies. Un cop la pupa és madura i rep els estímuls adequats, se'n va cap a la superfície de l'aigua i té lloc l'eclosió de l'adult, que com a conseqüència desprén l'exúvia pupal. La majoria dels adults no s'alimenten ja que les seves peces bucals solen estar molt atrofiades. La còpula té lloc generalment a l'aire quan una femella s'apropa a l'eixam de mascles. Després de la còpula la femella tria el lloc on dipositar els ous, algunes femelles moren immediatament, mentre que d'altres poden viure encara unes hores. També trobem gran diversitat pel que fa al número de generacions anuals que poden presentar els quironòmids, i per tant trobarem taxons que seran univoltins, bivoltins o multivoltins depenent dels casos (Pinder, 1986; Armitage *et al.*, 1995; Prat & Rieradevall, 1995).

La identificació de larves a nivell específic és més difícil si la comparem per exemple amb la de les exúvies pupals, per a les que existeix una clau única que permet identificar la majoria d'espècies de la regió paleàrtica (Langton, 1991). Per això no és estrany que la majoria de treballs sobre quironòmids ho siguin utilitzant les exúvies (Rieradevall & Prat, 1986; Cobo & González, 1991; Casas & Vílchez-Quero, 1993). Però malgrat la major dificultat en la identificació taxonòmica, la utilització de les larves comporta una sèrie d'avantatges en comparació amb les exúvies. Entre d'altres, amb les larves podem relacionar directament les espècies col·lectades amb els hàbitats mostrejats, evitem maximitzar els mostreig d'espècies rares o d'altres que vinguin de tributaris i que per tant no siguin representatives de la localitat d'estudi i les abundàncies que obtenim són menys dependents dels períodes d'emergència com ho són amb les exúvies. A més a més, des del punt de vista funcional, les larves són l'element més important ja que són les que viuen en els rius i les que defineixen la biodiversitat d'aquest. És per aquests motius que en aquesta tesi hem treballat amb les comunitats de quironòmids centrant-nos en l'estadi larvari, de forma similar com ho feia Prat *et al.* (1983) al Llobregat.

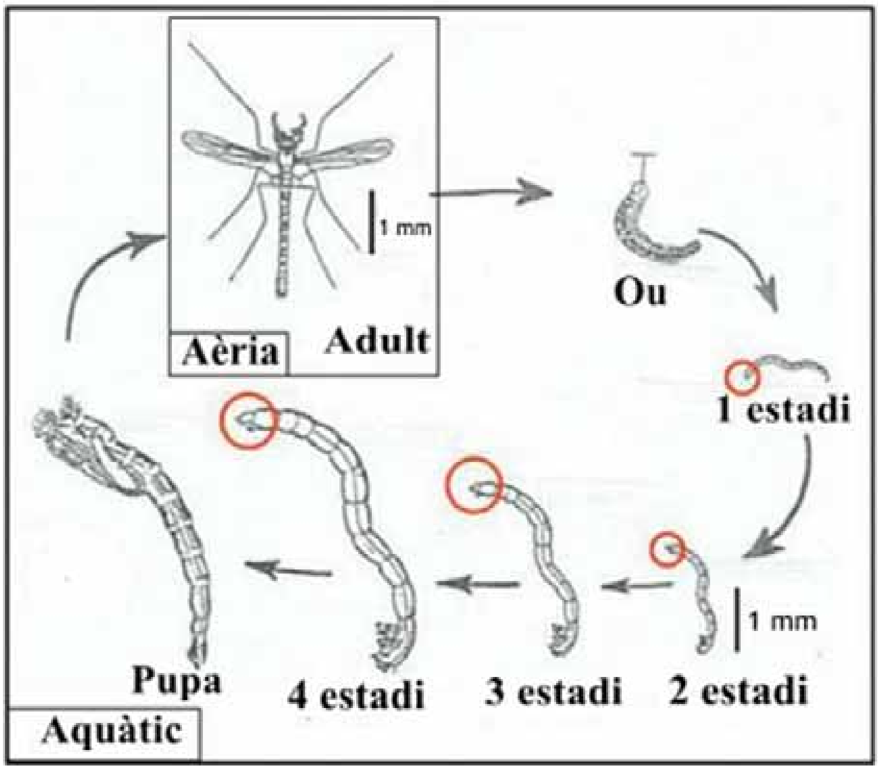


Figura 3. El cicle biològic dels quironòmids.

OBJECTIUS I ESTRUCTURA DE LA TESI

L'objectiu principal d'aquesta tesi ha estat contribuir al coneixement de l'ecologia de les comunitats de quironòmids, en el context dels rius mediterranis en condicions de referència i abordant també qüestions aplicades d'acord amb les directrius que es desprenen de la DMA.

Aquest treball l'hem dividit en quatre capítols, cadascun dels quals és un article independent, dos d'ells **(Capítol I i II)** ja estan publicats en revistes indexades *, i els altres dos estan en forma de manuscrit a l'espera de rebre una resposta per part de les revistes **(Capítol III i IV).**

La tesi es centra en l'estudi de la variabilitat espacial de les comunitats de quironòmids **(Capítols II, III i IV)**, mentre que la temporal s'ha tractat menys extensivament **(Capítol II).** Paral·lelament també s'ha estudiat la variabilitat espacial de tota la comunitat de macroinvertebrats a una resolució taxonòmica menor (a nivell de família) **(Capítol I).**

En el **capítol I** es defineixen els ecotipus fluvials de la regió mediterrània d'estudi de la Península Ibèrica. Aquesta regionalització es fa utilitzant una classificació *top-down* basada en paràmetres ambientals. A més a més, es testa la validesa dels ecotipus obtinguts *a priori,* amb les comunitats de macroinvertebrats per tal de valorar la utilitat d'aquesta classificació com a base per a futurs programes de gestió. Les diferències entre classificacions *a priori top-down* i les comunitats de macroinvertebrats és un tema que ja ha estat abordat per altres autors però no per la regió mediterrània exclusivament (per exemple: Verdonschot & Nijboer, 2004). A més a més la concordança entre tipologies i l'estructura de la comunitat d'un grup taxonòmic concret no ha estat analitzat especialment en el cas dels quironòmids. És per això, que en el **capítol II** s'aborda aquesta qüestió estudiant la correspondència entre les comunitats de quironòmids i les tipologies de rius obtingudes en el **capítol I.**

La nostra àrea d'estudi ha estat principalment la regió mediterrània de la Península Ibèrica, però també s'ha fet una comparació intercontinental, a una escala espacial més gran **(capítol IV)**. S'ha cregut convenient treballar a diferents escales espacials ja que l'escala d'observació d'un sistema és molt important alhora de determinar els patrons i processos ecològics que hi tenen lloc (Tonn *et al.*, 1990; Poff, 1997).

Un dels altres objectius centrals d'aquesta tesi és analitzar quins són els factors ambientals que estructuren les comunitats de quironòmids en rius mediterranis, i com responen a diferents escales espacials (**capítol II i III**). En molts estudis s'ha demostrat que tant els factors regionals com els locals són importants a l'hora d'explicar les variacions en l'estructura de les comunitats biològiques (Vinson & Hawkins 1998; Sandin & Johnson, 2000; Chaves *et al.,* 2005), i en aquests capítols s'ha abordat aquesta qüestió pel que fa a les comunitats de quironòmids. A més a més, en aquests dos capítols es realitza una classificació de les comunitats biològiques *bottom-up*, que pot ser utilitzada per futurs programes de conservació i gestió dels rius.

A banda de la variabilitat espacial, s'ha estudiat la variabilitat temporal d'aquests sistemes, ja que és un dels principals factors que influeixen en l'estructura de les comunitats de macroinvertebrats (Resh & Rosenberg, 1989) i també en els quironòmids de rius mediterranis (Langton & Casas, 1999). És per això que en el **capítol II** s'ha estudiat tant l'escala espacial com la temporal en la regió mediterrània de Catalunya.

El **capítol III** es centra exclusivament en una escala espacial més extensa que el capítol anterior (regió mediterrània de la Península Ibèrica), amb l'objectiu principal de determinar els òptims i toleràncies de les espècies de quironòmids pels gradients ambientals de la zona d'estudi. Abordar aquesta última qüestió és especialment interessant, ja que no es disposa de molta informació dels requeriments ecològics dels quironòmids en rius mediterranis de referència.

Finalment, el **capítol IV** fa una anàlisi comparativa de l'estructura de la comunitats de quironòmids en tres regions mediterrànies del món. Aquest treball segueix les hipòtesis de Bonada *et al.* (in press), que va fer una comparació intercontinental semblant però utilitzant tota la comunitat de macroinvertebrats. Concretament s'estudia com afecten els factors regionals, locals i històrics sobre diferents aspectes de l'estructura de les comunitats de quironòmids: la composició taxonòmica i els patrons de riquesa i abundància. La comparació entre les dades de les diferents regions mediterrànies, és possible perquè en tots els mostrejos de la tesi, s'ha utilitzat el mateix protocol de mostreig (Jáimez-Cuéllar *et al.*, 2004).

Donat que al llarg dels capítols s'usa abastament i repetida diferents anàlisis estadístiques de les dades amb mètodes multivariants, hem cregut oportú elaborar un recull dels mètodes aplicats en aquesta tesis (taula 2), per tal de mostrar els mètodes utilitzats i facilitar la interpretació dels acrònims dels resums.

Anàlisis	**Tècnica**	**Programa**	**Capítol**
Anàlisi de similaritats en dos dimensions de les comunitats de macroinvertebrats/quironòmids	Ordenació no mètrica de proximitats (NMDS)	PRIMER 6.0	I, II
Anàlisi jeràrquic d'agrupació de les comunitats de macroinvertebrats	Mètode d'agrupació jeràrquic (Cluster Group Average)	PRIMER 6.0	I
Anàlisi jeràrquic d'agrupació de les comunitats de quironòmids	Mètode d'agrupació jeràrquic (Cluster B flexible)	PC-ORD 4.20	IV
Càlcul dels òptims i toleràncies	Regressió ponderada (WA)	C2 1.3	III
Classificació de rius	Mètode d'agrupació no jeràrquic (K-means)	SPSS 10.0	I
Classificació de rius-comunitats de quironòmids	Mètode d'agrupació no jeràrquic (K-means)	SPSS 10.0	II, III
Estimació dels paràmetres de les distribucions d'abundàncies d'espècies	Estimació del màxim versemblant	Programa de S.Pueyo	IV
Identificació de taxons característics per grups preestablerts	Mètode del valor indicador (IndVal)	PC-ORD 4.20	I, II, III, IV
Partició de la variança de la composició de la comunitat de quironòmids	Anàlisi Parcial de Redundància (pRDA)	CANOCO 4.5	III
Relació entre quironòmids i paràmetres ambientals	Anàlisi Canònic de Correspondències (CCA)	CANOCO 4.5	II
Relació entre quironòmids i paràmetres ambientals	Anàlisi de Redundància (RDA)	CANOCO 4.5	III
Representació de distribució d'abundàncies d'espècies	Log-histograma	Programa de S.Pueyo	IV
Riquesa taxonòmica local	Anàlisi de rarefacció	PRIMER 6.0	IV
Riquesa taxonòmica regional	Anàlisi de doble rarefacció	Programa de S.Pueyo	IV
Selecció de variables discriminants	Minimització per passos de la Lambda de Wilks	SPSS 10.0	I, II
Testar diferències entre grups *a priori* per les comunitats de macroinvertebrats/quironòmids	Anàlisi de similaritat ANOSIM de un factor	PRIMER 6.0	I, IV
Testar diferències entre grups *a priori* per les comunitats de quironòmids utilitzant dos factors	Anàlisi ANOSIM de dos factors creuats	PRIMER 6.0	II
Testar diferències entre grups *a priori* per les comunitats de quironòmids utilitzant dos factors	Anàlisi ANOSIM de dos factors niats	PRIMER 6.0	IV
Testar diferències no paramètriques entre grups pre-establerts	Test de Kruskal-Wallis	JMP 6.0	II, IV
Testar diferències paramètriques entre grups pre-establerts	Anàlis de la variança (ANOVA)	JMP 6.0	II
Testar normalitat de sèries de dades	Test de Shapiro-Wilk	JMP 6.0	II, III, IV
Variació del gradient ambiental	Anàlisi de Components Principals (PCA)	STATISTICA/PRIMER 6.0	I, IV

Taula 2. Anàlisis de dades aplicades en aquesta tesis amb indicació del capítol on s’ha utilitzat.

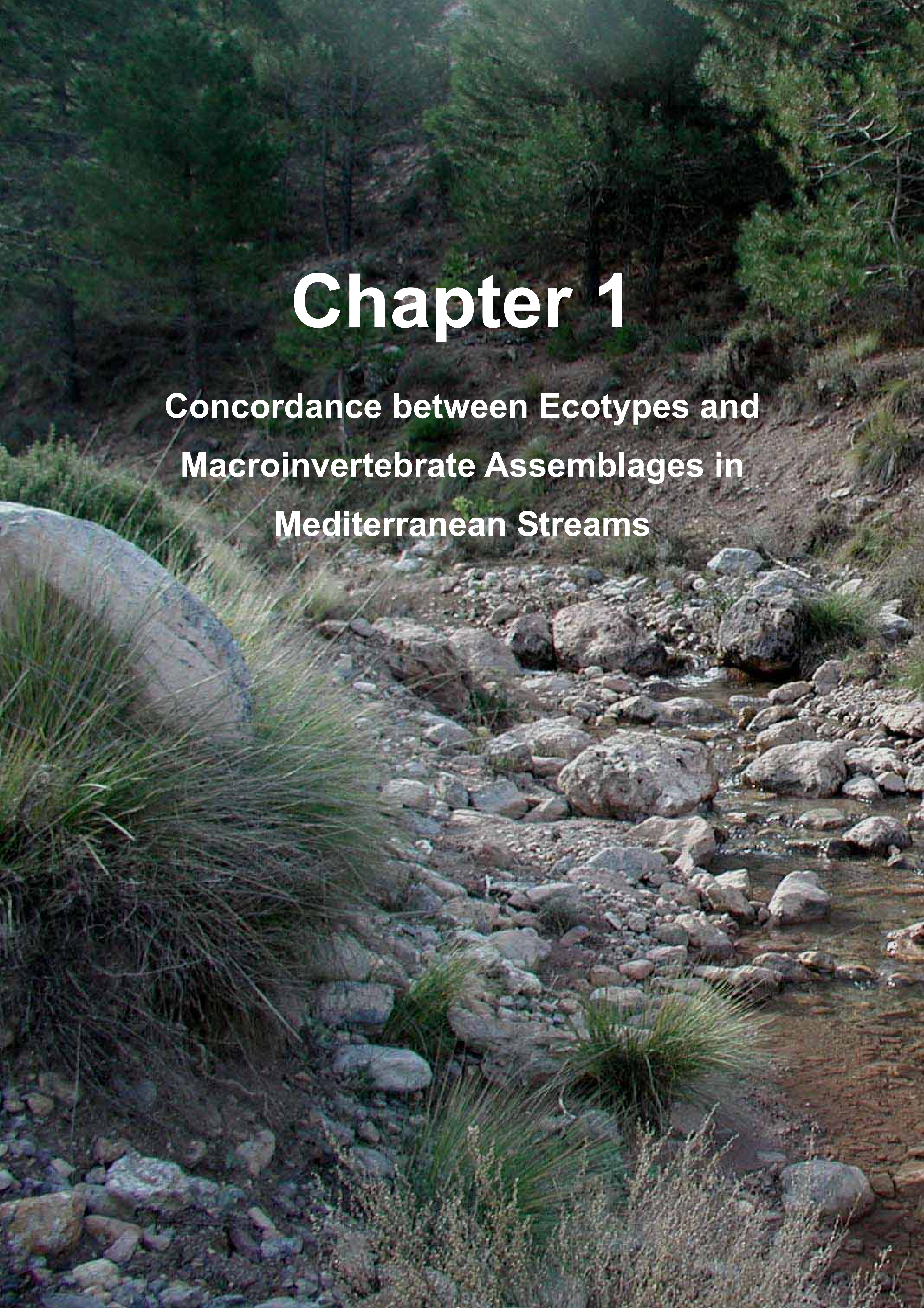
Llistat d' articles publicats o enviats per la seva publicació

* **Capítol I.** Sánchez-Montoya M.M., Puntí T., Suárez M.L., Vidal-Abarca M.R., Rieradevall M., Poquet J.M., Zamora-Muñoz C., Robles S., Álvarez M., Alba-Tercedor J., Toro M., Pujante A.M., Munné T. & Prat N. (2007) Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. *Freshwater Biology*, 52, 22-40.

* **Capítol II**. Puntí T., Rieradevall M. & Prat N. (2007) Chironomidae assemblages in reference conditions from Mediterranean streams: seasonality, environmental factors and ecotypes. *Fundamental and applied limnology*, 170, 149-165.

* **Capítol III**. Puntí T., Rieradevall M. & Prat N. (submitted) Optima and tolerances of chironomidae in mediterranean streams in reference conditions.

* **Capítol IV**. Puntí T., Rieradevall M., Pueyo S., Edward D.H.D., Storey A., Figueroa R. & Prat N. (submitted) Chironomid community structure in streams of three mediterranean climate regions: taxonomical composition and patterns of richness and abundances.



Chapter 1

Concordance between Ecotypes and Macroinvertebrate Assemblages in Mediterranean Streams

Concordança entre ecotipus i comunitats de macroinvertebrats en rius mediterranis

Resum

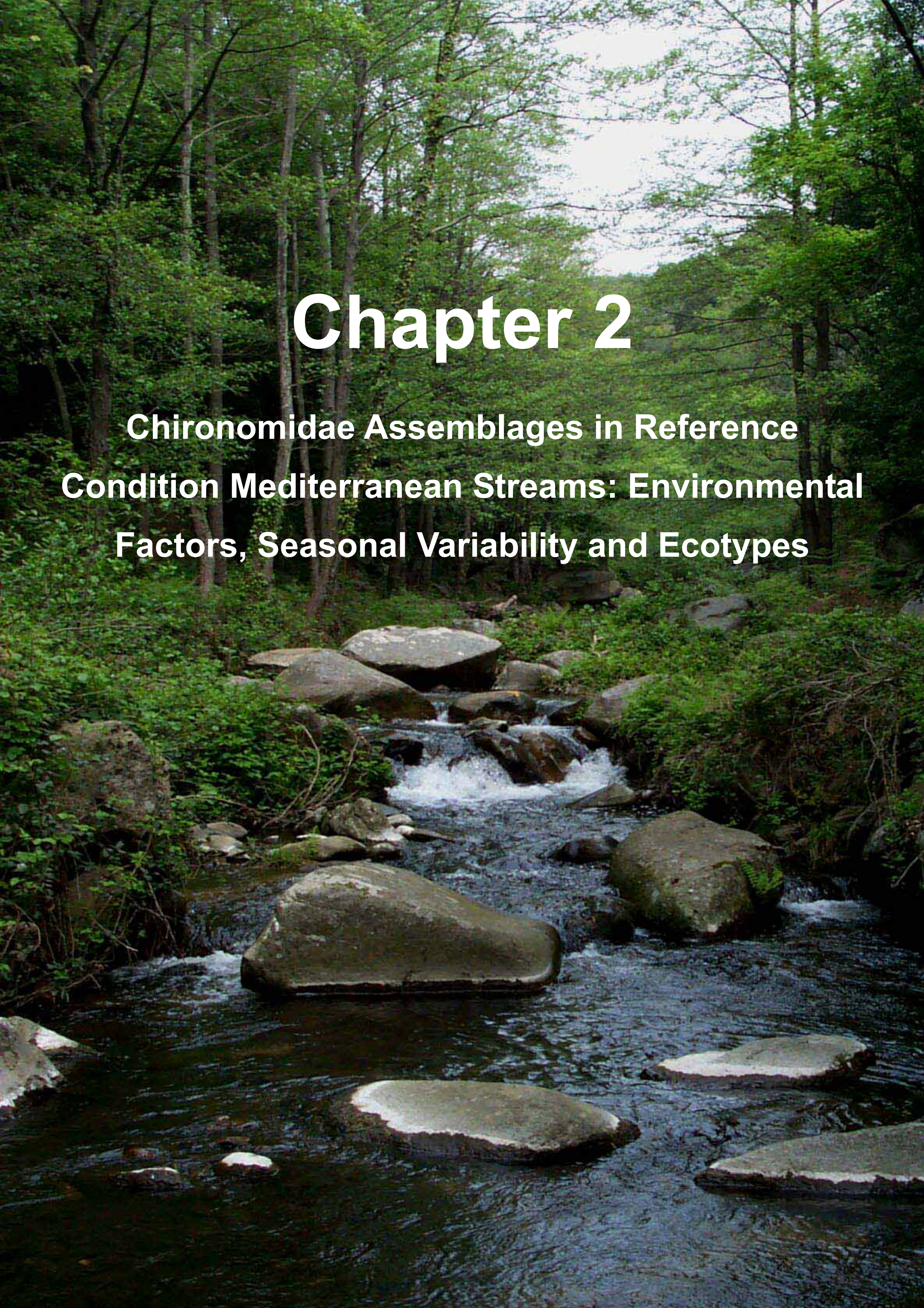
Segons les directrius de la Directiva Marc de l'Aigua, l'avaluació de l'estat ecològic dels rius s'ha de fer identificant les condicions de referència específiques per cadascun dels ecotipus fluvials assignats. En aquest treball s'han utilitzat dues aproximacions per tal d'establir una tipologia pels rius mediterranis, la *bottom-up* que utilitza la composició de les comunitats biològiques i la *top-down* basada en els valors dels paràmetres ambientals. S'han utilitzat els macroinvertebrats bentònics ja que són un component important de la biota aquàtica i un dels grups més utilitzats en la seva gestió. La classificació utilitzant les variables ambientals *top-down* es fa utilitzant els 162 punts de referència mostrejats en la segona fase del projecte GUADALMED. Com a resultat d'aquesta regionalització s'obtenen cinc ecotipus: (1) rius temporals, (2) trams mitjos de rius evaporítics-calcàris, (3) capçaleres silíciques i rius d'elevada altitud, (4) capçaleres calcàries de mitjana a elevada altitud i (5) trams mitjos-baixos dels rius. La classificació de les comunitats de macroinvertebrats *bottom up* es fa únicament amb els punts estrictament de referència (105), (no es valida per tant l'ecotipus 5, ja que aquest grup no presenta cap punt de referència). L'anàlisi de similaritats (ANOSIM) ens mostra que la composició de les comunitats de macroinvertebrats són diferents en tres dels quatre ecotipus testats anteriorment. Així, les capçaleres silíciques (3) es diferencien clarament dels altres tres ecotipus, mentre que els rius evaporítics (2) i les capçaleres calcàries (4) no mostren diferències clares pel que fa a les comunitats de macroinvertebrats. Els rius temporals (1) representen l'ecotipus més heterogeni de les comunitats de macroinvertebrats, a causa de la gran variabilitat d'aquests rius especialment pel que fa a la salinitat i la hidrologia, i es diferencien clarament de la resta. Per tant, els rius temporals i les capçaleres silíciques presenten unes comunitats biològiques clarament diferenciades, en canvi els ecotipus 2 i 4 tenen unes comunitats biològiques menys diferents. Aquests resultats hauran de ser considerats quan s'estableixin les classes de qualitat per la classificació de l'estat ecològic dels rius mediterranis. A més a més els rius temporals requeriran una atenció especial a causa de la gran diversitat de comunitats biològiques que inclouen.

Abstract

According to the guidelines of the European Water Framework Directive, assessment of the ecological quality of streams and rivers should be based on ecotype-specific reference conditions. Here we assess two approaches for establishing a typology for Mediterranean streams: a top-down approach using environmental variables and bottom-up approach using macroinvertebrate assemblages. Classification of 162 sites using environmental variables resulted in five ecotypes: (1) temporary streams, (2) evaporite calcareous streams at medium altitude, (3) siliceous headwater streams at high altitude, (4) calcareous headwater streams at medium to high altitude and (5) large watercourses. Macroinvertebrate communities of minimally disturbed sites (n = 105), grouped using UPGMA on Bray-Curtis similarities, were used to validate four of the five ecotypes obtained using environmental variables; ecotype 5, large watercourses, was not included since this group had no reference sites. Analysis of similarities (ANOSIM) showed that macroinvertebrate assemblage composition differed among three of the four ecotypes, resulting in differences between the bottom-up and top-down classification approaches. Siliceous streams were clearly different from the other three ecotypes, evaporite and calcareous ecotypes did not show large differences in macroinvertebrate assemblages and temporary streams formed a very heterogeneous group due to large variability in salinity and hydrology. This study showed that stream classification schemes based on environmental variables need to be validated using biological variables. Furthermore, our findings indicate that special attention should be given to the classification of temporary streams.

Keywords: Water Framework Directive (WFD), Mediterranean streams, GUADALMED project, typology, benthic macroinvertebrates.

Sánchez-Montoya M.M., Puntí T., Suárez M.L., Vidal-Abarca M.R., Rieradevall M., Poquet J.M., Zamora-Muñoz C., Robles S., Álvarez M., Alba-Tercedor J., Toro M., Pujante A.M., Munné T. & Prat N. (2007) Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. *Freshwater Biology*, 52, 22-40.



Chapter 2

Chironomidae Assemblages in Reference Condition Mediterranean Streams: Environmental Factors, Seasonal Variability and Ecotypes

Comunitats de quironòmids en rius mediterranis de referència: factors ambientals, variabilitat estacional i ecotipus

Resum

La Directiva Marc de l'Aigua (DMA) requereix la necessitat de contribuir en la millora del coneixement dels macroinvertebrats aquàtics, especialment en grups poc estudiats com és el cas dels quironòmids, essent especialment necessari pels rius mediterranis del sud d'europa, que és on hi ha una manca de coneixement important. En aquest treball s'estudia la distribució espacial i temporal dels quironòmids en 31 punts de mostreig de Catalunya durant dues estacions de l'any: primavera i estiu, i com a resultat s'identifiquen un total de 127 taxons que pertanyen a 67 gèneres de larves de quironòmids. L'anàlisi canònica de correspondències ens indica que la variació de l'estructura de les comunitats és explicada principalment per la temperatura, l'altitud i la geologia, juntament amb factors hidrològics tals com el cabal i la duració del període sec, que tenen un paper molt important en els rius mediterranis. Després de dur a terme una classificació basada exclusivament en les comunitats biològiques, s'obtenen quatre grups de rius, amb els corresponents tàxons indicadors associats. Un dels grups està format quasi exclusivament per mostres d'estiu, fet que ens indica que les diferències estacionals són un dels factors més importants alhora de determinar la composició d'aquestes comunitats. Paral·lelament s'estudia la concordança entre les comunitats biològiques de quironòmids i tres ecotipus (obtinguts segons la metodologia proposada per la DMA). Els resultats del NMDS ens mostren que hi ha una superposició important entre les comunitats de quironòmids i els ecotipus, malgrat les comunitats que pertanyen a l'ecotipus "capçaleres silíciques i rius d'elevada altitud", es separen clarament de la resta d'ecotipus estudiats. Els nostres resultats suggereixen que una classificació *top-down* utilitzant els ecotipus, no necessàriament implica comunitats diferents de quironòmids. Per tant, la classificació basada amb les comunitats biològiques pot ser més apropiada que la classificació ambiental. A més a més, s'evidencia que l'heterogeneitat temporal és un factor molt important d'aquests rius mediterranis, ja que com a resultat de la classificació biològica, hi ha un rius que tenen clarament comunitats de quironòmids diferents a la primavera i a l'estiu, mentre que en d'altres la variació temporal no és tan important.

Abstract

Chironomidae spatial and temporal distributions were investigated over two seasons at 31 reference sites in eight Mediterranean river basins in NE Spain. A total of 126 taxa included in 67 genera of chironomid larvae were identified. Canonical correspondence analysis indicated that variation in the assemblage structure of chironomids was primarily explained by temperature, altitude and geology, together with hydrological factors such as discharge and dry period, which play an important role in structuring communities in Mediterranean streams. A final classification based solely on Chironomidae reference assemblages produced four biological groups, and the corresponding indicator taxa were identified. One group is almost exclusively formed by summer samples, showing that seasonal differences are one of the most important driving forces behind differences in Chironomidae assemblage composition in Mediterranean streams. Furthermore we tested the agreement between Chironomidae assemblages and three river ecotypes (WFD approach) obtained after conducting an environmental classification in the Mediterranean region of Spain. Non-metric multidimensional scaling showed a large overlap of chironomid assemblages among ecotypes, although communities belonging to the ecotype “Siliceous headwaters and high altitude streams” proved to be the most different from the other ecotypes studied. Our results suggest that a top-down classification of streams (using ecotypes) does not necessary imply exclusive assemblages of chironomids. Thus, classification based on biological data may be more appropriate than environmental classification with subsequent testing using biological data. These findings have important implications for the application of the WFD in Mediterranean streams.

Keywords: Chironomidae, stream typology, seasonality, Mediterranean climate, reference sites, multivariate approach, Water Framework Directive (WFD).

Introduction

Chironomidae comprise one of the most abundant and species-rich families of freshwater insects in fluvial systems and they are widely distributed in all types of aquatic environments (Pinder, 1986), as well as being at the same time commonly used for bioassessment purposes (e.g. Rosenberg, 1993; Edward *et al.,* 2000). They have also been used for river classification (Wilson, 1977; Laville & Vinçon, 1991) and several studies have found shifts in chironomid assemblages along the river continuum (Prat *et al.,* 1983; Ward & Williams, 1986; Lindegaard & Brodersen, 1995). These studies have demonstrated that several factors, such as substrate type, current regime, water temperature and food availability, strongly affect the composition of chironomids related to longitudinal river zonation. Moreover, Chironomidae assemblages have been used effectively to classify fluvial ecosystems according to environmental characteristics (Lindegaard, 1995; Calle-Martínez & Casas, 2006). Thus, chironomids respond to a variety of environmental factors that influence their richness (Coffman, 1989), and spatial or temporal distribution (Schmid, 1992; Bazzanti *et al.,* 1996). We hypothesized those chironomid assemblages would present distribution patterns that are influenced by both local and global factors, as is the case of macroinvertebrates at the family level (Sandin, 2003).

Several previous studies have established that temporal variability is one of the main factors influencing Chironomidae assemblage structure in stream systems (Langton & Casas, 1999; Rossaro *et al.,* 2006), which is especially important in streams in a Mediterranean-type climate due to their great temporal heterogeneity characterized by a strong seasonality of rainfall and air temperature (Gasith & Resh, 1999). Accordingly, seasonal differences were expected in the Mediterranean streams studied.

Taxonomy is a difficult issue in studying chironomids. Thus, several authors had described chironomid communities in streams in Mediterranean-type climates using mainly pupal exuvia, due to sample processing facilities and the easier identification of these diptera at species level as compared with larvae (Rieradevall & Prat, 1986). To our knowledge no study of larval chironomid assemblages has been carried out exclusively in reference conditions in Mediterranean streams, and there are very few reports for other stream types (e.g. Ward & Williams, 1986).

The European Water Framework Directive (WFD) (European Comission, 2000) emphasizes the need to define an appropriate system of classification of streams in a given region, establishing different river types (or ecotypes). For each ecotype, reference conditions should be established in order to compare the stream’s present biological conditions with the undisturbed ones. This is a central issue in the current biological assessment promoted by the WFD in all European countries. The process of defining river ecotypes has now been completed in many European countries using a range of methodologies. In the Mediterranean region of Spain, several studies have used environmental parameters to establish stream ecotypes and select reference sites according to WFD specifications (Bonada *et al.,* 2004a; Munné & Prat, 2004; Sánchez-Montoya *et al*., 2007). In general, we should expect comparable biological assemblages at sites of the same ecotype because environmental features influence stream biota (Richards *et al.,* 1996). Although some research has evaluated the environmental classification of rivers using macroinvertebrates (e.g. in Europe, Verdonschot & Nijboer, 2004; Sánchez-Montoya *et al*., 2007), there have been few studies using exclusively Chironomidae assemblages (Calle-Martínez & Casas, 2006), and to our knowledge, none using larvae, despite their great importance in terms of abundance, species richness and functional ecology in fluvial systems (Coffman, 1995).

In this paper we report data on chironomids from several Mediterranean streams in Catalonia (NE of Spain), the aim being to examine their taxonomic composition and community structure in minimally disturbed sites. The specific objectives of this study are: (i) to classify Mediterranean streams using chironomids and determine the most important factors that characterize these communities; (ii) to analyse seasonal variation in larval Chironomidae assemblage composition between spring and summer and its relevance for biological characterization; and (iii) to study the correspondence between Chironomidae assemblages and ecotypes defined by Sánchez-Montoya *et al*. (2007) using a top-down approach.

Methods

Study area

Eight basins along the Mediterranean coast in Catalonia (NE Spain) were sampled (Figure 1). Streams and rivers flowing across this area are subjected to a Mediterranean climate, characterized by high seasonality with hot dry summers and cool wet winters. A wide range of conditions was covered, from small streams at higher altitudes to large streams in middle reaches, as well as several intermittent and temporary streams that dry out in summer or karstic streams that present nearly constant flow throughout the year. Calcareous and sedimentary rocks predominate in this region, although some siliceous areas are present in the Pyrenees (north of the area) and Montseny (central in the area) ranges. The vegetation of the basins studied mainly comprised sclerophyllous and evergreen shrubs, although in some areas deciduous and coniferous forest were present. A detailed description of the studied basins can be found in Prat *et al.* (2000) and Munné & Prat (2004).

Sampling sites

Sites were selected according to 18 criteria used to establish reference conditions in Mediterranean streams (Sánchez-Montoya *et al.,* 2005). Mediterranean Reference Criteria (MRC), want to reflect the particular characteristics of Mediterranean streams and the most frequent impacts present here. In particular, stream reaches were classified according to their watershed disturbances and habitat characteristics, considering land use in the catchments, alteration of riparian zone, instream channel naturalness, importance of river regulation by dams, point-source of pollution and presence of invasive species among others. However, as can be expected after many years of human interference, in middle reaches of streams it was impossible to find strict reference sites due to several pressures. Both point and diffuse sources of pollution and alterations in river morphology and hydrology are present at these sites. In these cases the least disturbed sites in the available streams of the region were considered. Finally, a total of 31 sampling sites were visited on two occasions: spring and summer of 2003 (Figure 1). Later, each site was classified into one of the five ecotypes defined according to the Mediterranean streams typology (Sánchez-Montoya *et al.,* 2007), and as established in the GUADALMED 2 project: ecotype 1 “Temporary streams”; ecotype 2 “Evaporite calcareous medium altitude streams”; ecotype 3 “Siliceous headwaters high altitude streams”; ecotype 4 “Calcareous headwaters medium and high altitude streams”; and ecotype 5 “Large watercourses”. Few of the selected sites belonged to ecotypes 1 and 5, and for this reason concordance among ecotypes and biological assemblages was tested exclusively with the other three ecotypes.

Sampling procedure and biological data

Macroinvertebrate samples were collected from all available habitats with a kick net of 250 µm mesh size, following the GUADALMED protocol (Jáimez-Cuéllar *et al.*, 2004). Samples were examined in the field, and successive samples were then taken until no more macroinvertebrate families were found by the observer. They were preserved in formalin 10% and sorted in the laboratory. All chironomid larvae were sorted, counted and identified to the maximum possible taxonomical level for each sampling site. First, larvae were grouped by their similar morphological appearance (size, colour, setae presence) under a stereomicroscope, and all (if few individuals) or a part (many individuals) were mounted according to Pinder (1983). Individuals were then cleared in 10% potassium hydroxide at 85 ºC, rinsed in distilled water, and dehydrated in ethanol (70% and 96%). The head capsule of individuals was also removed from the body and they were mounted under separate cover slips in Euparal.

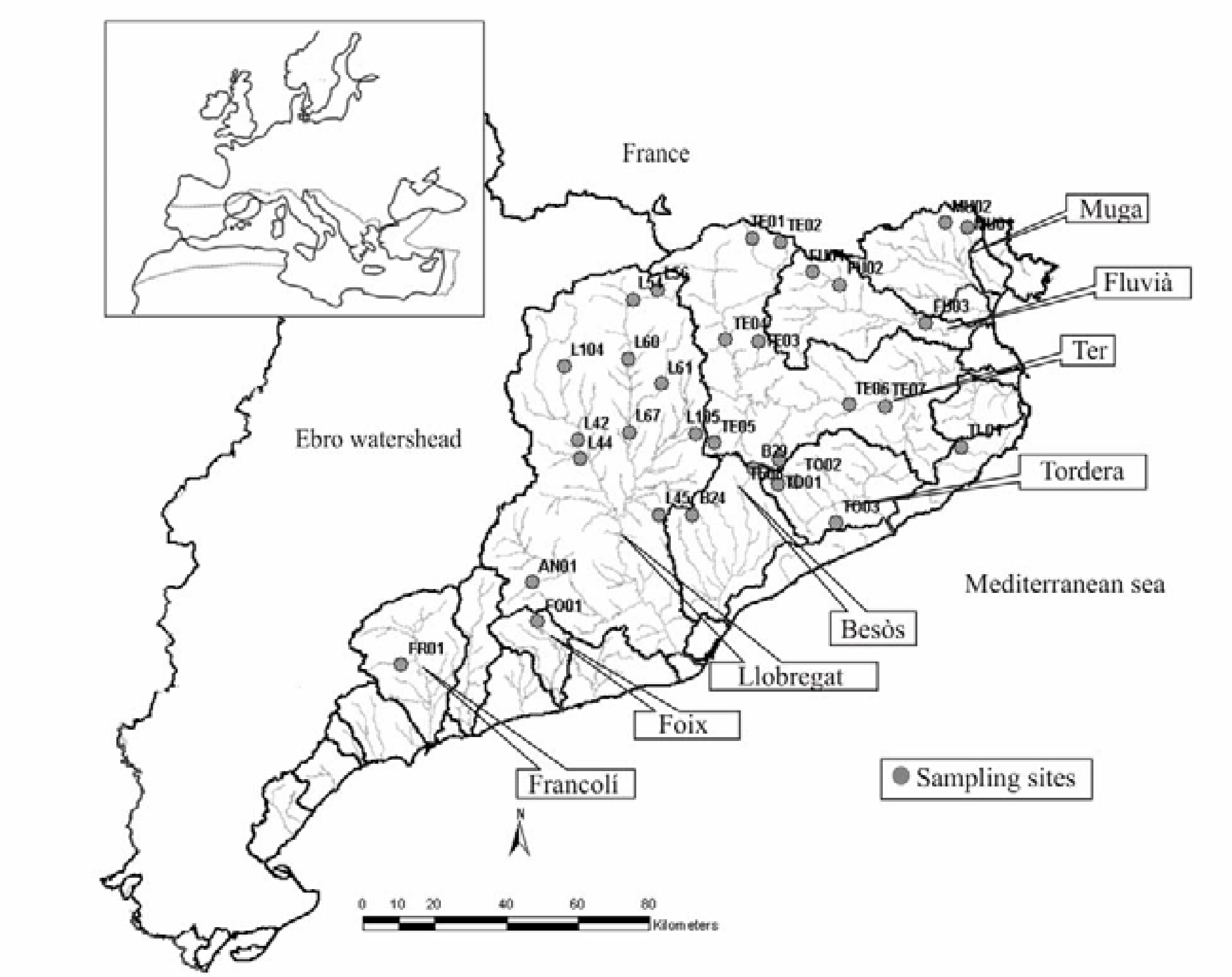


Figure 1. Reference sites (31) located in several Mediterranean basins sampled in NE Spain. Dotted line of the European map represents the Köppen (1923) Med-climate boundary.

Several taxonomical keys were used to match chironomid larvae to genus, species group or species whenever possible. Orthocladiinae and Podonominae taxa were identified using the approach of Wiederholm (1983) and Schmid (1993). For Tanypodinae we followed Rieradevall & Brooks (2001) and for Chironominae Wiederholm (1983) and Nocentini (1985). For specific genera we used available keys such as those for *Corynoneura* (Rieradevall, unpubl. data) and *Diamesa* (Ferrarese & Rossaro, 1981), among many others. In the case of *Micropsectra* and *Tanytarsus* the species were identified according to material (larvae and associated mature pupae) from Spain and Portugal (collection of M. Rieradevall). In some cases we could not identify the chironomid species or species group with certainty because of the small size of many individuals (second or third larval instars), as is the case of some groups that are difficult to separate at the larval stage (for example, *Orthocladius-Cricotopus).* Consequently, different taxonomical levels were mixed in the "species matrix".

After identification, relative abundance of Chironomidae (percentage of each taxon per sampling site) was calculated. The total abundances in the sample were also recorded for each taxon: one from 1-3 individuals, two from 4-10, three from 11-100 and four from more than 100 individuals. At the end we thus had five data sets combining the factors we wished to compare: taxonomic resolution (only genus or "the best available", species in many cases) and biological data (presence-absence, relative abundance or rank of abundance).

Environmental data

A total of 35 environmental variables were measured or calculated for each site (Appendix 1). Conductivity, pH, temperature, dissolved oxygen and discharge were measured *in situ* with portable meters. Discharge was estimated from depth, width and water velocity measurements. Additionally, one litre of water was collected and taken to the laboratory for suspended solids, alkalinity and nutrient analysis. Analyses of ammonia, nitrites and nitrates were then conducted with spectrophotometers according to standard procedures (APHA, 1992). Variables related to riparian characteristics (Munné *et al.,* 2003) and diversity of habitat (Pardo *et al.,* 2004) were also measured, along with others such as the basin geology, altitude, stream order, distance from the origin, dry period percentage and basin area, calculated using Geographical Information Systems (GIS).

Data analysis

In order to establish the main links between environmental variables and Chironomidae assemblage patterns, a canonical correspondence analysis (CCA) was performed. First, a detrended correspondence analysis (DCA) (Hill & Gauch, 1980) was conducted to test if a model with unimodal (CCA) or linear (RDA) response curve should be used in ordination analysis. Results of the DCA showed that gradient length was 2.98 for axis one to 2.46 for axis four; thus, both RDA and CCA may give correct results (Jongman *et al.,* 1996). As little difference in the percentage of total variance explained was detected (RDA: 42% and CCA: 38.2%) we considered it more appropriate to perform a CCA analysis, as unimodal responses are more characteristic of biological data (Ter Braak, 1987). Furthermore, to determine the proportion of chironomid distribution explained by the measured environmental variables, an indirect correspondence analysis (CA) was conducted. The biological matrix used for both CA and CCA analysis was the relative abundances of species data fourth-root transformed, and the option down weighting of rare species was invoked. All ordination analyses were performed using CANOCO for Windows v. 4.5 (Ter Braak & Ŝmilauer, 1998).

A total of 35 environmental variables measured initially were tested for autocorrelation using the non–parametric Pearson correlation coefficient. Percentage of siliceous surface, alkalinity, stream order and distance from origin were removed from further analysis, because they were highly correlated ($r > 0.8$) with other variables (percentage of calcareous surface, conductivity and area). In addition, environmental variables that followed a non-normal distribution (after a Shapiro-Wilk test) and presented high skewness values were log-transformed prior to inclusion in the ordination analysis (Appendix 1). Jump v6 (JMP, 2005) programme was used to perform these analyses. Finally, environmental variables were individually tested to determine their significance using forward selection, as suggested by Økland & Eilertsen (1994). In forward selection the Monte Carlo permutation test in CANOCO was run with 999 unrestricted permutations. The remaining 15 explanatory variables were significant ($p\text{-value} < 0.05$) with inflation factors <20, and all of them were used for the CCA analysis.

To cluster samples into similar groups and identify the Chironomidae assemblage characteristic of each group, a k-means cluster was applied with values of the first two canonical axes obtained by CCA. Finally, four predefined groups were used because they showed major ecological relevance and were easily interpretable, in comparison to tests performed with groups 3, 5 and 6. Moreover, a stepwise discriminant analysis using the Wilk's lambda method was used to select the most significant environmental variables that defined each K-means group obtained in a hierarchical way (Ferrán-Aranaz, 2001). Both analyses (K-means and discriminant) were performed with the SPSS programme (SPSS Inc., 1999).

We also used the indicator value method (IndVal) (Dufrêne & Legendre, 1997) to identify species discriminating between k-means groups. This method identifies indicator taxa that best characterize groups of sites, and each taxon is associated with an indicator value (IV value) and a p-value obtained by Monte Carlo permutations (9999 runs). The indicator value varies between 0 and 100, attaining its maximum value when all individuals of a species occur in all sites of a given group and never in other groups. The PCORD programme (McCune & Mefford, 1999) was used to perform this analysis.

In order to analyze seasonal changes among sampling sites of each k-means group, Euclidean distances in the space of X_1 and X_2 axis of CCA were measured from spring to summer for each sampling site. The values obtained give us an idea of whether Chironomidae assemblages from spring and summer are different among each k-means group.

Differences of composition (percentages of subfamilies) and larval abundance between season and three ecotypes were assessed using a Kruskal-Wallis non-parametric ANOVA test (Chi-square statistic), because data did not follow a normal distribution using a Shapiro-Wilk test. In the case of taxon richness the same differences were tested with an analysis of variance (ANOVA, F statistic), because these variables did follow a normal distribution and showed homogeneity of variance (Levene's test). The Jump v6 (JMP, 2005) package was used to perform these analyses.

To examine patterns of variation in Chironomidae assemblage structure among sites we performed ANOSIM (Clarke & Warwick, 1994) using Bray-Curtis similarities. Thus, a two-way crossed analysis with ecotype as the level factor was performed, considering different biological matrices to analyze the influence of taxonomic resolution over Chironomidae assemblage composition. Each test in ANOSIM produces an R statistic which compares the differences among samples within a group (ecotypes or seasons in our case) with the similarities among samples between groups. R will assume values near 1 when similarities between samples within groups are higher than those between samples from different groups, and values near -1 in the opposite case. Values close to 0 are indicative of no differences among groups. When ANOSIM results were significant, ANOSIM pair-wise comparisons among different groups were calculated to distinguish between possible contrasting effects.

We then performed a non-metric multidimensional scaling "NMDS" (Kruskal & Wish, 1978) to visualize spatial patterns of community structure. NMDS is an ordination method based on ranked distances and is highly suitable for our data because it performs well with data that are non-normally distributed or which contain numerous zero values (McCune & Mefford, 1999). The stress value was recorded as a measure of the ordination effectiveness on preserving the similarity ranks. Relative abundance of species matrix was used to perform this analysis, because it was this data set that showed higher values of R in the ANOSIM results. Fourth-root transformation was conducted over biological matrix. These analyses were run removing a total of 25 very rare taxa from the data set (occurrence at <2% of sites), because they usually obscure general patterns in classification analysis (Gauch, 1982). Furthermore, samples with fewer than 60 individuals or fewer than 10 taxa were not included in the multivariate analysis because they were considered outliers after running preliminary tests. The PRIMER v6 package (Clarke & Warwick, 1994) was used to perform ANOSIM and NMDS.

Results

Chironomidae and environmental factors

A total of 13402 Chironomidae larvae from 31 sites were sorted and identified in our study (4758 prepared), with 126 taxa corresponding to 67 genera being obtained from the two seasons. A new genus for the Iberian Peninsula (*Saetheria*, Jackson) was reported, according to the updated check list of Chironomidae in the Iberian Peninsula (Cobo *et al.,* 2002; Soriano & Cobo, 2006). Appendix 2 presents the complete list of Chironomidae taxa recorded in the study area. The total explained variance in the data matrix was 38.2%, including the 15 environmental variables selected (Appendix 1) after running forward selection in CCA analysis. Canonical axes from CA and CCA analyses represent a low percentage of chironomid variability, with 10.3% in the first CA axis and 8.1% in the first CCA axis (Table 1). However, the results indicate that a high percentage of all chironomid variability shown on the CA axis was explained by environmental variables (78.83% for the first axis, 79.58% for the second, 73.92% for the third and 62.77% for the fourth). This suggests that the measured variables are among those responsible for the differences in assemblages. Although the Monte Carlo permutation test indicates that all canonical axes were significant with the set of variables used, only the first two canonical axes were used because they include the maximum variability expressed by the environmental variables, and all variables that were significant on axes 3 and 4 were also significant on axes 1 and 2 (Table 1). The first CCA axis (8.12% of the variation explained) was negatively correlated with basin area, temperature, calcareous surface and conductivity, while it was positively correlated mainly with altitude (Figure 2.1). Sampling sites situated to the right of the first axis were characterized by higher altitudes, and cold waters of low mineralization (Figure 2.2). On the left, the sampling sites are those with higher temperatures, conductivities and basin area. Thus, axis 1 showed a gradient of decreasing altitudes and increasing water mineralization. The second axis (5.03 % of the variation explained) was negatively correlated with discharge and basin area, and positively correlated with percentage of dry period (Figure 2.1). In the negative part of this axis, we found sampling sites corresponding to middle reaches of permanent streams, mainly from the Llobregat basin, while in the positive part intermittent streams (with low absolute discharge) and most of the summer samples were found (Figure 2.2). This axis could be interpreted as a hydrological environmental gradient, with increasing dry period and decreasing discharge and basin area.

	Axis 1	Axis 2	Axis 3	Axis 4
CA				
Eigenvalues	0.384	0.235	0.207	0.185
% of variance accumulated by Chironomidae taxa	10.3	16.6	22.2	27.2
CCA				
Eigenvalues	0.302	0.187	0.153	0.116
Species-environment correlations	0.912	0.919	0.906	0.885
% of variance accumulated by Chironomidae taxa	8.1	13.2	17.3	20.4
Environmental variables				
Basin area	-0.449**	-0.403**	0.065	0.260
Conductivity	-0.282*	0.253	0.396**	-0.079
Discharge	-0.271*	-0.312*	-0.056	-0.170
Altitude	0.511**	-0.128	-0.383**	-0.433**
Dry period	0.321*	0.658**	0.154	0.151
Calcareos surface	-0.492**	0.246	0.289*	-0.309*
Channel width	-0.421**	-0.397**	0.045	-0.049
Riparian structure	0.120	0.383**	-0.013	-0.343*
Channel shape	-0.346*	-0.302*	-0.152	0.141
Oxygen	-0.016	-0.506**	0.405**	-0.057
pH	-0.358**	-0.299*	0.320*	-0.195
Temperature	-0.567**	0.286*	-0.417**	0.177
Pebbles and gravels	0.306*	-0.359**	-0.114	0.369**
Flow and depth regimes	0.231	-0.389**	-0.445**	-0.076
Heterogeneity elements	0.285*	0.306*	-0.477**	-0.060
Monte Carlo test (999 permutations)	**F**	**p-value**		
Significance of first canonical axis	3.363	0.0010		
Significance of all canonical axis	1.566	0.0010		

Table 1. Eigenvalues and percentage of variance explained by Chironomidae from CA and CCA analysis. The Pearson correlations (r) between environmental variables and the four canonical axes from CCA are given** p-value<0.01, *p-value<0.05. Results from the Monte Carlo test checking for axis significance in CCA are also presented.

Consequently, the four groups of sites distinguished by Figure 2.2 are: Headwater – higher altitude streams, mainly siliceous (Group A: bottom right of Figure 2.2); middle streams – permanent regime (Group B: bottom left of Figure 2.2); headwater- middle altitude streams, mainly spring samples (Group C: top right of Figure 2.2); and calcareous middle altitude streams, summer samples (Group D: top left of Figure 2.2).

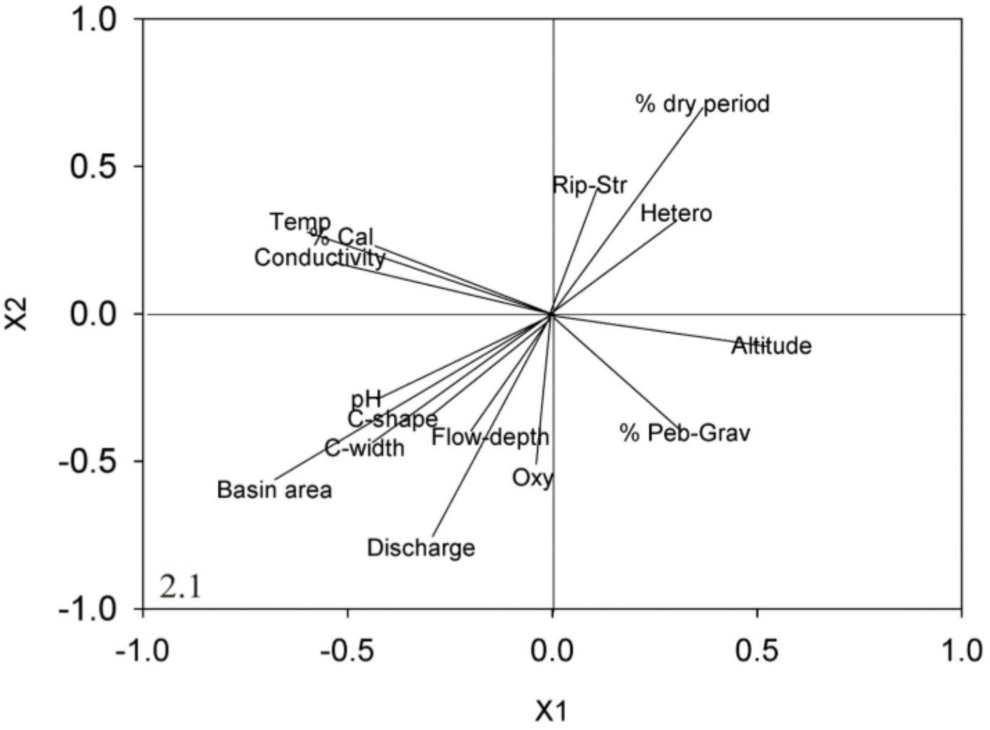


Figure 2.1. Canonical correspondence analysis (CCA) plots representing first (X1) and second (X2) axis. Position of environmental variables that best explain the variance among taxa included in the analysis.

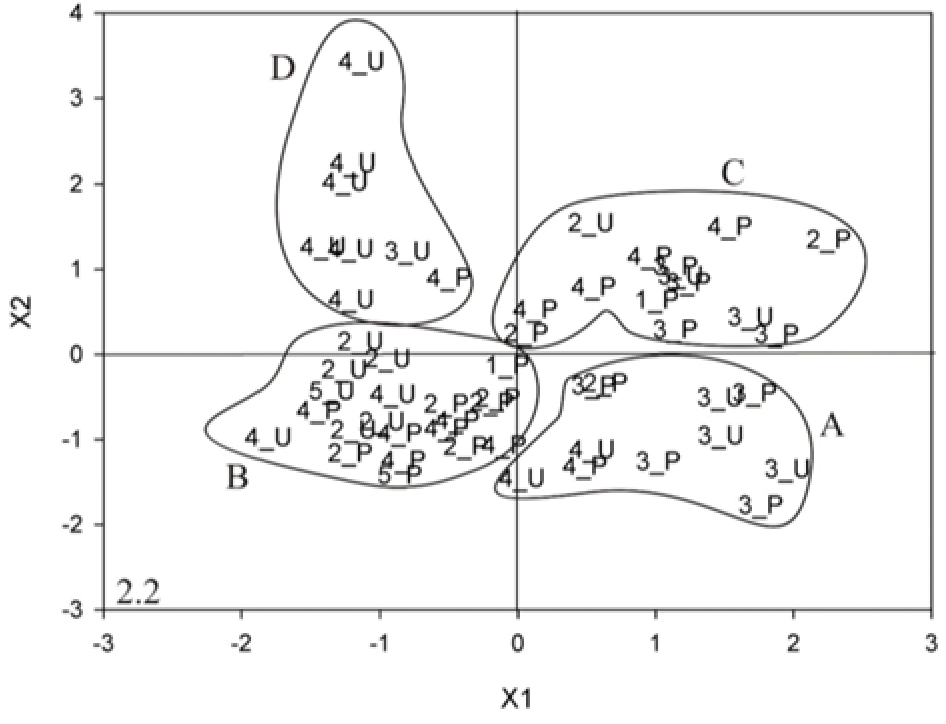


Figure 2.2. Canonical correspondence analysis (CCA) plots representing first (X1) and second (X2) axis. Distribution of sampling sites labelled by the corresponding ecotype and season sampled (P: spring; U: summer). The four groups obtained with the K-means analysis are enclosed in the same area.

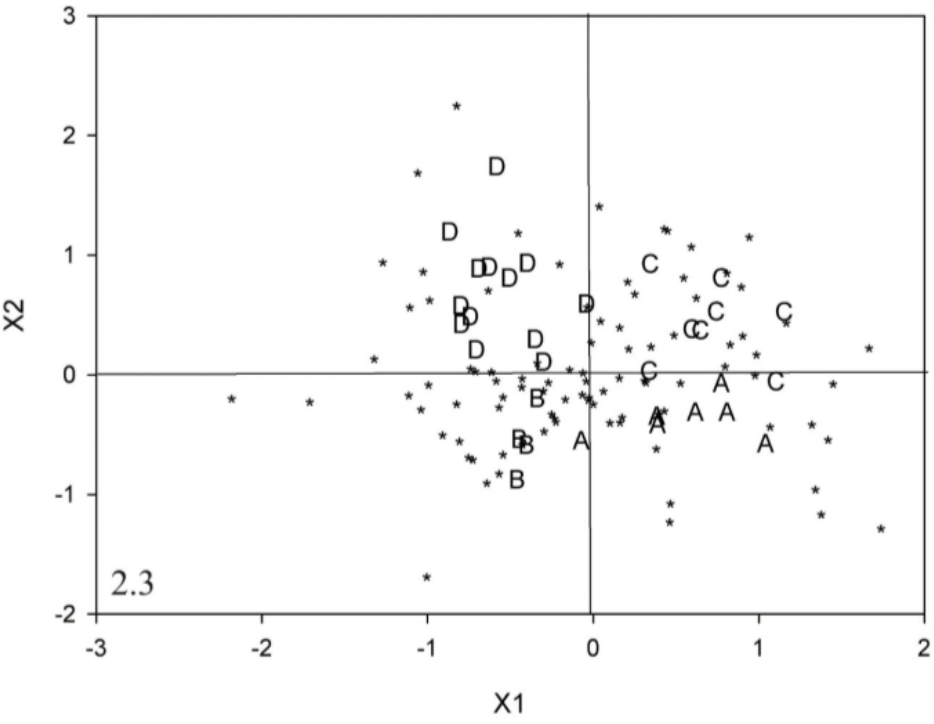


Figure 2.3. Canonical correspondence analysis (CCA) plots representing first (X1) and second (X2) axis. Distribution of taxa. Only indicator taxa (IndVal results) are labelled according to the k-means group.

Percentage of dry period, altitude, percentage of pebbles and gravel, and temperature were the variables that differentiated between the four k-means groups obtained after applying a discriminant analysis. Thus, different kinds of variables discriminate the four groups obtained, which differed mainly according to hydrological, geological and habitat characteristics.

The IndVal results (Table 2) reveal several taxa with high indicator values (IV value> 23) (Figure 2.3). Many taxa are characteristic of group A (headwater-higher altitude streams, mainly siliceous): *Tvetenia bavarica-calvescens, Tvetenia discoloripes* (Goetghebuer, 1940)*, Micropsectra sp.4, Thienemanniella partita* (Schlee, 1968), *Thienemanniella vittata* (Edwards, 1924) *and Micropsectra sp.5.* On the other hand, group B (middle stream reaches) has few indicator taxa, it being mainly dominated by the complex *Orthocladius-Cricotopus, Potthastia gaedii group* and *Cricotopus (Isocladius)*. Group C has characteristic taxa such as *Brillia bifida* (Meigen, 1830)*, Trissopelopia* spp.*, Zavrelimyia* spp.*, Parametriocnemus stylatus* (Kieffer, 1924) and *Stempellinella* spp.*,* which are headwater middle altitude streams, with spring samples being dominant in this case. Many chironomids are characteristic of group D, which are also mainly calcareous middle altitude streams, but in this case composed mostly of summer samples. Indicator taxa present in this group are mainly Tanytarsini and Tanypodinae, for instance, *Ablabesmyia longystila* (Fittkau, 1962), *Rheocricotopus chalybeatus* (Edwards, 1929) and *Rheotanytarsus* spp.

Group A	**IV**	**p-value**	**Group B**	**IV**	**p-value**
Tvetenia bavarica-calvescens	59.0	0.001	*Orthocladius - Cricotopus*	60.8	0.001
Tvetenia discoloripes	56.2	0.001	*Potthastia gr. gaedii*	58.6	0.003
Micropsectra sp.4	54.7	0.002	*Cricotopus (Isocladius)*	27.6	0.020
Thienemanniella partita	52.8	0.001	*Paratanytarsus* spp.	23.7	0.049
Thienemanniella vittata	50.7	0.003			
Micropsectra sp.5	38.0	0.005			
Brillia longifurca	23.5	0.050			
Group C	**IV**	**p-value**	**Group D**	**IV**	**p-value**
Brilia bifida	61.7	0.001	*Ablabesmyia longistyla*	80.9	0.001
Trissopelopia spp.	60.6	0.001	*Rheocricotous chalybeatus*	60.7	0.002
Zavrelimyia spp.	47.7	0.005	*Rheotanytarsus* spp.	56.7	0.003
Parametriocnemus stylatus	47.1	0.033	*Microtendipes pedellus*	56.6	0.002
Stempellinella spp.	42.8	0.003	*Corynoneura coronata*	50.7	0.002
Rheocricotopus fuscipes	38.4	0.027	*Procladius* spp.	46.6	0.002
Paratrissocladius excerptus	32.8	0.034	*Paramerina* spp.	40.01	0.008
Corynoneura lobata	30.8	0.036	*Phaenopsectra* spp.	33.8	0.028
			Paracricotopus niger	33	0.031
			Paratendipes spp.	28.6	0.019
			Tanytarsus gr. chinyensis	28.3	0.018
			Stempellinella spp.	25	0.027
			Corynoneura sp. A	24.5	0.015

Table 2. Results of the IndVal method for each group obtained from K-means analysis. Only taxa with an indicator value (IV) over 20 and a p-value <0.05 are shown.

Seasonal changes and chironomid assemblages

Euclidean distances calculated between spring and summer samples for each sampling site in the CCA space give a good picture of the seasonal change of individual sites. The distances measured in Figure 2.2 are shown in Table 3, which also indicates whether each sampling site belongs to the same K-means group in spring and summer, or if a seasonal change is detected. In several sites it was not possible to calculate Euclidean distance because only one sample was available (some sites from groups B and C were totally dry in summer or the number of larvae was very low). Mean Euclidean distances from sites without a seasonal change in group assemblage (most A and B sites) (0.73 ± 0.43) were lower than distances between sites that changed from one group to another (normally from any group to group D) (2.19 ± 0.58).

Sampling site	dE(sp. su)	k group spring	k group summer
B24	3.132	C	D
FU1	2.678	B	D
L45	2.411	C	D
MU2	2.075	A	D
FR1	1.901	A	B
FO1	1.736	C	C
AN1	1.614	B	D
TE3	1.519	C	D
L105	1.355	D	D
TE6	1.106	B	B
TE4	1.012	B	B
L61	0.962	B	B
L104	0.912	B	B
L60	0.824	B	A
L67	0.672	B	B
TO1	0.635	C	C
L54	0.559	B	B
TE2	0.545	A	A
TE1	0.471	A	A
TO2	0.279	C	C
L42	0.273	B	B
TE8	0.254	A	A
L56	0.201	A	A
FU2		C	dry
MU1		B	dry
TL1		C	dry
L44		B	(l)
TE5		C	(l)
TO3		C	(l)
B29		C	(l)
FU3		B	(l)

Table 3. Euclidean distance calculated from the position of spring and summer samples of each sampling site in Figure 2. Sampling sites are ordered according to Euclidean distance. The faunistic group from the K-means cluster is indicated for spring and summer for each site. Dried sites (dry) or those with a low number of Chironomidae larvae (l) are also shown at the end of the table.

In general, our data show that few temporal changes in chironomid composition occur in groups A and B. Most sites belonging to these groups present a permanent regime, and lower Euclidean distances between spring and summer assemblages were found. As indicated previously, group D mainly comprises (except one site) summer samples and the majority of sites from this group were in group C in spring (the sites from headwaters of calcareous and evaporitic areas, mainly small streams); Euclidean distances between these groups of samples were greater than those of other sites.

Chironomid composition also differed over time at the subfamily level. Orthocladiinae was the dominant subfamily in both seasons, with 68 % and 48 % for spring and summer, respectively (Table 4). Diamesinae were more abundant in spring than in summer (4.73 - 1.11%), while Chironomini (5.23 - 12.81%), Tanytarsini (12.88 - 19.66%) and Tanypodinae (8.95-18.12%) increased in summer. Thus, significant differences were detected between spring and summer for all subfamilies except Tanytarsini. As Podonominae and Prodiamesinae showed low representation in both seasons, they were not considered in the analysis. In general, in both seasons the abundant taxa were also the most frequent ones: *Orthocladius-Cricotopus*, *Tvetenia bavarica-calvescens* and *Rheotanytarsus* spp. Several taxa such as *P. stylatus*, *Conchapelopia* spp.*,* *Thienemannimyia* spp. and *B. bifida* were frequent in most sampling sites in both seasons, although they were not very abundant. In spring, twenty exclusive taxa were found, such as several taxa of *Diamesa* spp., which were generally cold-adapted and inhabit flowing water, and most larvae of *Metriocnemus* spp. and *Paraboreochlus* spp. that were found typically in mosses. In contrast, eleven taxa were exclusive to the summer season: for instance, Chironomini taxa associated with lentic habitats, such as *Cryptochironomus* spp. or *Paratendipes* spp. The total number of taxa captured was 115 in spring and 107 in summer. No differences were detected as regards richness (total number of taxa F= 1.61, p= 0.2; and total number of genera F= 0.15, p= 0.6) or abundance (Chi= 0.16, p= 0.68) from spring and summer assemblages.

		Season				**Ecotype**	
Subfamily	**n**	**Spring (Mean ± SD)**	**Summer (Mean ± SD)**	**Chi-square**	**p-value**	**Chi-square**	**p-value**
Podonominae	1	0.04 ± 0.2	0	0.74	0.38	2.57	0.27
Tanypodinae	12	8.9 ± 8.6	18.1 ± 13.7	7.11	0.007**	3.5	0.17
Diamesinae	6	4.7 ± 7.2	1.1 ± 2.7	6.94	0.008**	1.87	0.39
Prodiamesinae	1	0.2 ± 0.8	0.2 ± 0.6	2.75	0.09	0.8	0.67
Orthocladiinae	72	68.2 ± 16.2	48 ± 22.7	9.42	0.002**	4.84	0.08
Tr. Chironomini	22	5.2 ± 6.9	12.8 ± 12.3	8.38	0.003**	0.69	0.70
Tr. Tanytarsini	19	12.9 ± 13	19.6 ± 14	2.96	0.08	0.89	0.64

Table 4. Mean and standard deviation (SD) of subfamily percentage from all sampling sites for the two periods studied and results of Kruskal-Wallis test (**p < 0.01) for the two seasons and five ecotypes analysed. Total number of taxa for each subfamily considering spring and summer together (n) is presented.

Importance of ecotypes

No differences between defined ecotypes were found (Table 4) at the subfamily level, and the same results were obtained when richness and abundances were tested (for total number of taxa F= 1.3, p= 0.2; total number of genera F= 1.5, p= 0.2; and abundances Chi= 1.9, p= 0.3). Differences among the three ecotypes tested were studied using ANOSIM. There were significant overall differences between the three ecotypes studied in all five combinations of taxonomical levels and biological data considered (Table 5). However, these differences were small, with low R-values (<0.3) indicating an important overlap between chironomid assemblages with the established ecotypes. The most significant differences considering pair-wise comparisons were found between ecotypes 3 and 4, with higher values of R (0.4). In the case of ecotypes 2 and 3, values of R were lower (0.3) and significant differences were found only for the species matrix, relative abundances data. No differences were detected between ecotypes 2 and 4, which shared many chironomid taxa, as shown by the values of R near to zero. Relative abundance data provided higher values of R than rank of abundances or binary data. Taxonomic resolution influenced ANOSIM results, increasing the R values when species instead of genera were used (Table 5).

As the ANOSIM analyses were significant for some ecotypes, a non-metric multidimensional scaling (NMDS) was performed to determine the difference between them (Figure 3). The NMDS plot reflected similar trends to those obtained in ANOSIM tests, with a stress value of 0.24. However, except for most of the sites of ecotype 3 (clustered on the right-hand side of the graph) no other clear pattern emerged. Thus, ecotype 3 (“Siliceous headwaters high altitude streams”) presented the most exclusive chironomid assemblages among the ecotypes analyzed. Greater differences were present between ecotypes 3 and 2, while sites belonging to ecotype 4 overlapped with sites of ecotype 2. Samples belonging to ecotype 3 also showed some overlap with the other two, for instance, the summer assemblage of sampling site MU2 “ecotype 3” was more related to assemblages of ecotype 2 (in the left part of the figure 3). Otherwise, mixed with siliceous headwaters we found some samples belonging to ecotypes 4 (Te5-spring) and 2 (FO1-spring) that present assemblages similar to ecotype 3.

		Global ANOSIM	**Pair - wise comparations between ecotypes**		
		Ecotype	**2 & 3**	**2 & 4**	**3 & 4**
Taxonomical level	Biological data	Global R	R	R	R
Species	Presence/absence	0.24**	0.311	0.05	0.375**
	Relative abundances	0.284**	0.395*	0.025	0.464**
	Rank abundances	0.259**	0.33	0.05	0.41**
Genus	Presence/absence	0.201*	0.173	0.011	0.288*
	Relative abundances	0.261**	0.315	0.071	0.402**

Table 5. Two-way ANOSIM results for global and pair-wise comparisons among ecotypes studied using two taxonomical levels (species and genus) and three sorts of biological data (presence/absence, relative abundances and rank of abundances). R values are shown at different p values (*< 0.05; **< 0.01).

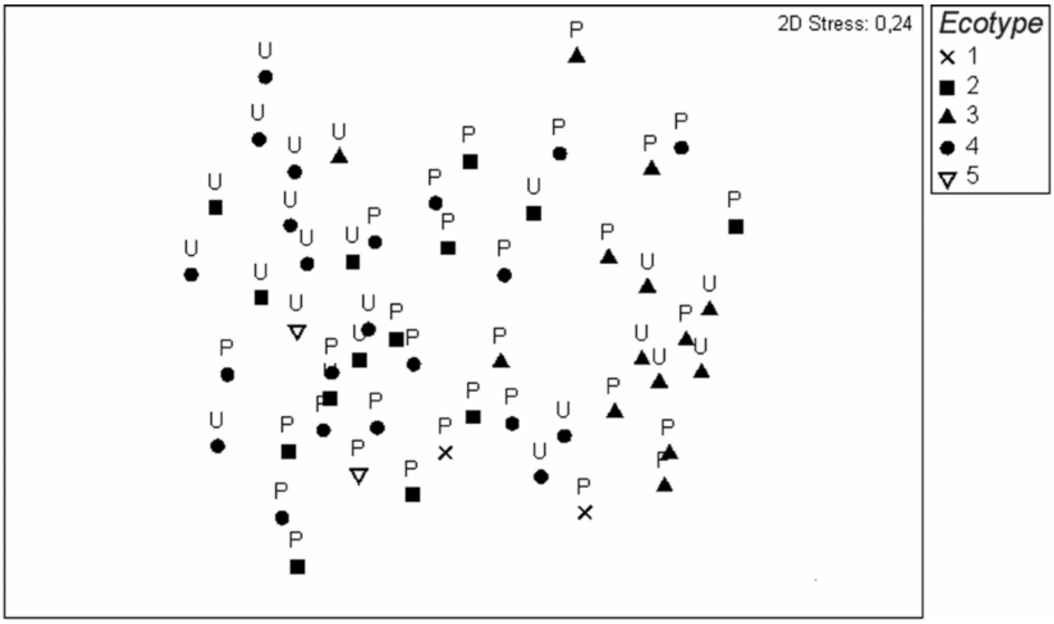


Figure 3. Non-metric multidimensional scaling (NMDS) plot for sampling sites studied and related to defined ecotypes using a top-down approach and seasons (P: spring; U: summer). Stress values are included (2S).

Discussion

To date, and due to the difficulties surrounding chironomid larvae taxonomy, few studies have been conducted in streams using chironomid species data, and at most they have identified genera (Prat *et al.,* 1983). In this study the regional pool of taxa in reference conditions was around 24% of the species recorded in Spain (Cobo *et al.,* 2002; Soriano & Cobo, 2006). The number of taxa found is noteworthy considering that the region sampled represents only a small part of the Mediterranean region of Spain (approximately 5.83%, see: Figure 1). In fact, in order to properly understand the ecology of these communities, species identification is important, as species belonging to the same genus may show different responses to environmental variables.

The present study provides baseline information about the environmental variables affecting distribution patterns of Chironomidae assemblages in Mediterranean streams in reference conditions. It is clear that a combined understanding of both local and large scale factors is needed to assess the importance of factors structuring macroinvertebrate communities (Sandin, 2003; Chaves *et al.,* 2005), together with other factors not considered here such as food (Pinder, 1983) and species interactions (Tokeshi, 1995). In particular, the key factors that determine the distribution of Chironomidae in Mediterranean steams are altitude and river size, which are highly related to temperature, flow and substratum composition (Coffman, 1989; Casas & Vílchez-Quero, 1993; Lindegaard & Brodersen, 1995). However, in our study one of the most important factors that influences the composition of Chironomidae assemblages, is temporal heterogeneity derived from changes in flow regime (Langton & Casas, 1999) similarly to what has been reported for other macroinvertebrates (Bonada *et al.,* 2006). The hydrological regime is known to be one of the main constraints on biotic communities in most Mediterranean regions (Gasith & Resh, 1999) and specifically in temporary systems mainly due to the effect of drought (Lake, 2003). Not surprisingly, influences of hydrological factors on the Mediterranean streams sampled, explained by dry period and discharge are remarkable.

Overall, we were able to differentiate among four groups of chironomids in relation to the influence of environmental variables. For instance, headwater sites (group A) are associated with a Chironomidae community explained by the highest altitudes and lowest temperatures, while sites from group B present a differentiated assemblage from middle altitude/permanent regime sites; however, both groups of sites present few seasonal differences between spring and summer. This is not the case of the small and middle altitude stream sites from groups C and D, where seasonal differences reflect particular assemblages during spring (group C) and summer (group D). For instance, group D was almost totally composed of summer samples, with a dominance of taxa belonging to Chironominae and Tanypodinae, which are typical of lentic habitats and warmer waters.

Seasonal changes in the composition of Chironomidae communities in Mediterranean streams have been previously considered by several authors (Prat *et al.,* 1983; Rieradevall & Prat, 1986; Langton & Casas, 1999). In our case, these changes may be caused by differences in temperature affecting particularly the cold adapted species, and this is consistent with the seasonal succession common in temporary waters (Bazzanti *et al.,* 1996; Williams, 1996). Flow reduction produces an important change in the condition of the substratum and in the thermal regime, and this can favour the colonization of either more cosmopolitan species or species that prefer more lentic environments; therefore, this determines the composition and structure of communities inhabiting temporary waters (Casas & Vílchez-Quero, 1989; Rüegg & Robinson, 2004). Thus, seasonal variation should be one of the most important factors to consider in bioassessment, at least in Mediterranean streams, and this aspect can easily be studied using chironomid assemblages because many species change from permanent flowing conditions to pools remaining in intermittent streams. Furthermore, with future scenarios of global climate change, expecting an increase of the proportion of streams with Mediterranean characteristics (Bonada *et al.,* 2007a), a better understanding of these systems and their biota becomes a basic requirement for water management (Álvarez-Cobelas *et al.,* 2005).

We were also interested in testing the correspondence between a top-down stream classification for the Spanish Mediterranean region (Sánchez-Montoya *et al.,* 2007) and chironomids communities. Correspondence between ecotypes and chironomid assemblages was generally weak, with the exception of ecotype 3 (high altitude streams in siliceous bedrock), and the river ecotypes studied did not have a chironomid community as specific and differentiated as was hypothesized. Obviously, increasing the environmental scope of the study, by including more samples of ecotypes 1 and 5, may have revealed different patterns from those observed here. Several recent studies have examined differences between environmental classifications and stream macroinvertebrate assemblage structure (Hawkins & Vinson, 2000; Waite *et al.,* 2000). These studies have mostly shown that landscape classifications have weak correlations with biological assemblages. In European streams, distributions of macroinvertebrates are well distinguished using ecoregions (large scale) but in terms of stream types, this resolution is insufficient to separate communities (Verdonschot & Nijboer, 2004). When different ecotypes present similar biological communities (as in the case of ecotypes 2 and 4 here) the reference condition will be the same, and it would not be necessary to maintain the ecotypes separately for biomonitoring purposes. For this reason, the fact that the correspondence of ecotypes with biological communities is not as clear as expected should not be overlooked. Considering that our results are similar to those found when the comparison is made using all the macroinvertebrate data at the family level (Sánchez-Montoya *et al.,* 2007), we suggest that a classification based on biological data is better than an environmental top-down classification.

In conclusion, the assessment of communities of Chironomidae is a useful tool to classify stream communities, especially in terms of revealing temporal flow changes. It would be interesting to compare these results with other taxonomical levels because controversy remains as to which taxonomic resolution (family or genus/species of macroinvertebrates) produces the most robust results when assessing environmental classifications (Hawkins *et al.,* 2000).

Acknowledgements

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Appendix 1. Variables measured grouped on three spatial scales. Variables used in the CCA analysis are marked with an asterisk (p-value <0.05 and Pearson correlation r<0.8).

Scale	Variable	Code	Transf	Description
Basin	Basin area*	Basin area	Log	Basin area drained in each site (km ^{2})
	% Siliceous surface	%Sil		Percentage of siliceous materials in basin from each site
	% Calcareos surface*	%Cal		Percentage of calcareous materials in basin from each site
	% Evaporitic surface	%Eva		Percentage of evaporitic materials in basin from each site
Reach	Oxygen*	Oxy		Concentration of oxygen (mg L $^{-1}$)
	Conductivity *	Conductivity	Log	Water conductivity (µS cm^{-1})
	pH*	pH		Water pH
	Temperature*	Temp		Temperature (ºC)
	Suspended solids	SS	Log	Concentration of suspended solids (mg L $^{-1}$)
	Alkalinity	Alcal		Alkalinity (meq L $^{-1}$)
	Nitrates	NO3-	Log	Concentration of N-NO3$^-$ (mg L $^{-1}$)
	Nitrites	NO2-	Log	Concentration of N-NO2$^-$ (mg L $^{-1}$)
	Amonium	NH4+	Log	Concentration of NH4$^+$ (mg L $^{-1}$)
	Channel width*	C-width		Channel width: up to 1m (1); from 1 to 10 m (2); over 10 m (3)
	Channel depth	C-depth		Channel depth: up to 0.1m (1); from 0.1 to 0.5 m (2); over 0.5 m (3)
	Riparian Cover	Rip-Cove		Proportion of riparian area covered by trees and shrubs
	Riparian Structure*	Rip-Stru		Proportion of the riparian vegetation composed of trees and shrubs separately
	Riparian Quality	Rip-Qual		Absence of introduced species, garbage, and other human impact on riparian vegetation
	Riparian Naturality	Rip-Nat		Human impact altering channel form
	Channel shape*	Chan-shape		Channel shape according to the QBR field sheet (Munné *et al.*, 2003)
	Discharge*	Discharge	Log	Water discharge (l/s)
	Altitude*	Altitude		Altitude of each site (meters a.s.l.)
	Stream order	Str-Order		Stream order (strahler method)
	Distance from the origin	Dist-Ori	Log	Distance from the origin (meters)
	Dry period*	% dry period		Percentage of months with discharge equal to zero
Bedform	Embeddedness	Embed		Percentage of embeddedness in riffles or sedimentation in pools
	Riffles vs.pools	R/L		Frequency of riffles in sampling reach: distance between riffles/stream width
	% Boulders and stones	%Bou-Stones		Percentage of boulders and stones
	% Pebbles and gravels*	%Peb-Grav		Percentage of pebbels and gravels
	% Sand	%Sand		Percentage of sand or silt
	% Clay	%Clay		Percentage of clay
	Flow and depth regimes*	Flow-depth		Number of classes present in sampling reach: slow-depth, slow-shallow, fast-depth and fast-shallow
	Shade	Shade		A score running from not shaded to completely shaded
	Heterogeneity elements*	Hetero		Percentage of leaf litter, presence of wood and branches, tree roots and natural dams
	Instream vegetation	Inst-veg		Types and abundance of different instream vegetation formations: % of plocon, pecton and macrophytes

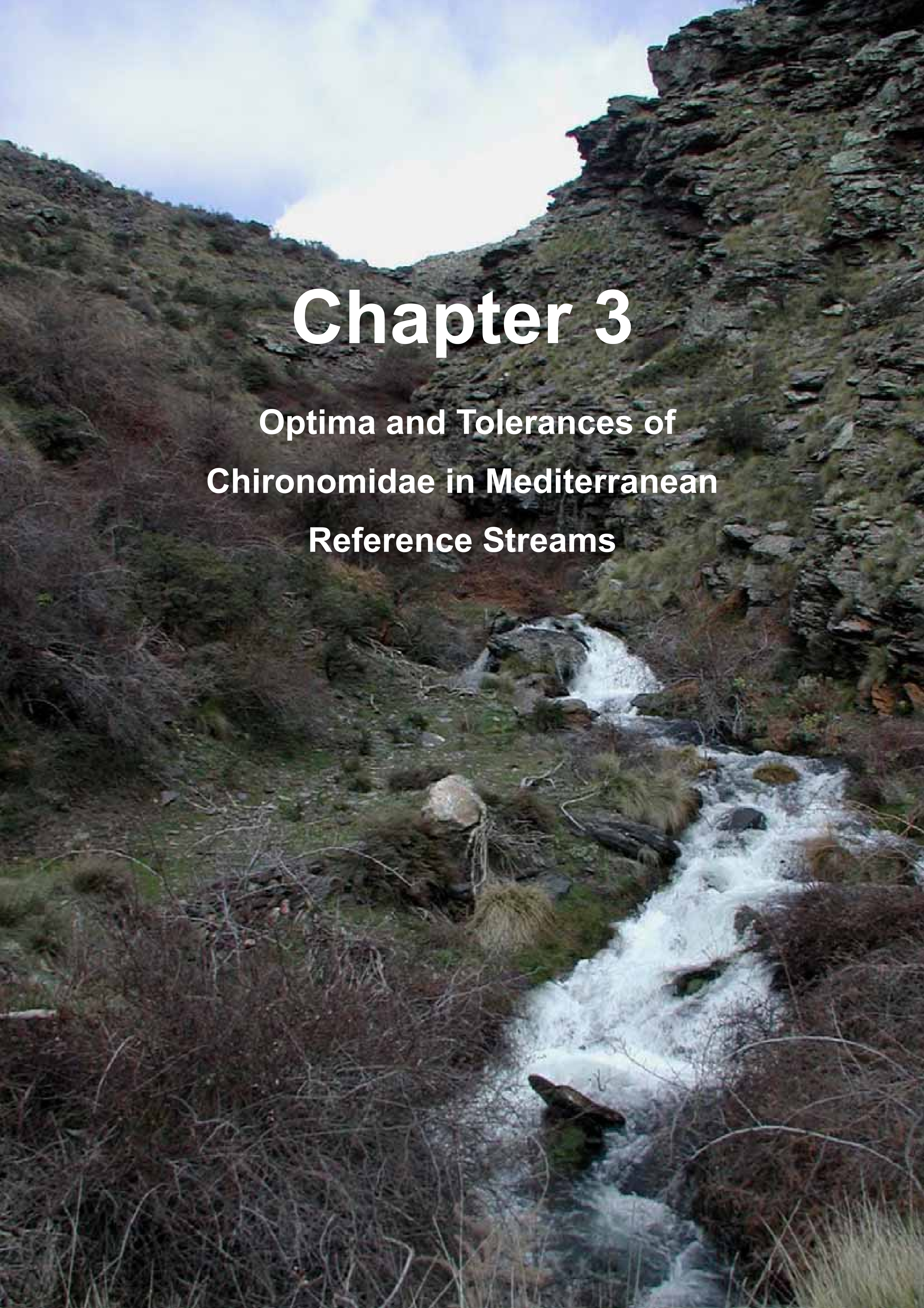
Appendix 2. List of chironomid taxa collected in basins studied, along with their abundance (number of larvae collected) and frequency of taxa (% of taxa present at each sampling site) recorded over two seasons: spring and summer.

	Spring		**Summer**	
Taxa	Abundance (n)	Frequency (%)	Abundance (n)	Frequency (%)
SUBFAMILY PODONOMINAE				
Paraboreochlus minutissimus (Strobl, 1984)	2	3	0	0
SUBFAMILY TANYPODINAE				
Ablabesmyia longistyla (Fittkau, 1962)	21	17	158	48
Conchapelopia (Fittkau, 1957)	110	48	137	52
Krenopelopia (Fittkau, 1962)	2	7	0	0
Larsia (Fittkau, 1962)	26	14	10	4
Macropelopia (Thienemann, 1916)	27	21	117	36
Nilotanypus dubius (Meigen, 1804)	18	21	6	12
Paramerina (Fittkau, 1962)	24	21	79	28
Procladius (Skuse, 1889)	20	10	58	20
Rheopelopia (Fittkau, 1962)	77	52	88	32
Thienemannimyia (Fittkau, 1957)	104	45	185	44
Trissopelopia (Kieffer, 1923)	65	31	102	20
Zavrelimyia (Fittkau, 1962)	109	38	85	16
SUBFAMILY DIAMESINAE				
Boreoheptagyia monticola (Serra-Tosio, 1964)	0	0	1	4
Diamesa hamaticornis (Kieffer, 1924)	51	24	0	0
Diamesa sp.A sensu Schmid'93	13	10	0	0
Diamesa zernyi-thienemanni group	249	38	13	4
Potthastia gaedii group (Meigen*)*	103	38	39	20
Potthastia longimana (Kieffer, 1922)	1	3	1	4
SUBFAMILY PRODIAMESINAE				
Prodiamesa olivacea (Meigen, 1818)	30	14	17	28
SUBFAMILY ORTHOCLADIINAE				
Brillia longifurca (Kieffer, 1921)	5	10	6	12
Brillia bifida (Meigen, 1830)	164	45	86	24
Cardiocladius (Kieffer, 1912)	49	10	125	12
Corynoneura arctica (Kieffer, 1923)	0	0	6	4
Corynoneura coronata (Edwards, 1924)	46	14	36	24
Corynoneura indet.	13	10	46	24
Corynoneura lacustris (Edwards, 1924)	0	0	13	4
Corynoneura lobata (Edwards, 1924)	67	31	97	20
Corynoneura scutellata group	122	31	35	24
Corynoneura sp.A sensu Schmid'93	0	0	15	12
Cricotopus (Cricotopus) trifascia (Edwards, 1929)	9	7	7	12

	Spring		**Summer**	
Taxa	Abundance (n)	Frequency (%)	Abundance (n)	Frequency (%)
Cricotopus (Isocladius)	50	24	3	8
Cricotopus (Isocladius) sylvestris group	7	3	19	12
Epoicocladius flavens (Malloch, 1915)	4	10	27	12
Eukiefferiella brevicalcar (Kieffer, 1915)	94	38	2	8
Eukiefferiella cf.lobifera sensu Schmid'93	17	7	14	8
Eukiefferiella claripennis (Lundbeck, 1898)	16	10	0	0
Eukiefferiella clypeata (Kieffer, 1923)	0	0	16	20
Eukiefferiella coerulescens (Kieffer, 1926)	10	21	0	0
Eukiefferiella devonica (Edwards, 1929)	44	24	32	12
Eukiefferiella fuldensis (Lehmann, 1972)	4	7	1	4
Eukiefferiella gracei (Edwards, 1929)	154	21	10	12
Eukiefferiella ilkleyensis (Edwards, 1929)	99	45	31	16
Eukiefferiella indet.	1	3	0	0
Eukiefferiella minor-fittkaui	63	45	51	24
Eukiefferiella tirolensis (Goetghebuer, 1938)	26	10	0	0
Heleniella ornaticollis (Edwards, 1929)	4	7	7	4
Heleniella sp.1	1	3	0	0
Heterotrissocladius marcidus (Walker, 1856)	13	7	11	16
Limnophyes (Eaton, 1875)	4	10	4	12
Metriocnemus fuscipes group (Meigen)	1	3	0	0
Metriocnemus indet.	2	7	0	0
Metriocnemus eurynotus group (Holmgren, 1883)	13	6	0	0
Nanocladius (Nanocladius) bicolor (Zetterstedt, 1838)	1	3	0	0
Nanocladius (Nanocladius) rectinervis (Kieffer, 1911)	5	7	5	8
Orthocladiinae unknown	33	31	4	16
Orthocladius (Euorthocladius) indet.	14	14	2	4
Orthocladius (Euorthocladius) rivulorum (Kieffer, 1909)	51	34	0	0
Orthocladius/Cricotopus	1540	86	361	76
Paracladius conversus (Walker, 1856)	5	7	1	4
Paracricotopus niger (Kieffer, 1913)	99	28	79	40
Parakiefferiella cf. coronata sensu Schimd'93	10	7	0	0
Parakiefferiella cf. gracillima sensu Schimd'93	7	7	0	0
Parakiefferiella indet.	0	0	1	4
Parametriocnemus stylatus (Kieffer, 1924)	150	72	133	72
Paraphaenocladius pseudirritus (Strenzke, 1950*)*	1	3	0	0
Paratrichocladius (Santos Abreu, 1918)	212	48	51	16
Paratrissocladius excerptus (Walker, 1856)	31	28	97	36
Parorthocladius (Thienemann, 1935)	0	0	4	4
Psectrocladius (Allopsectrocladius) obvius (Walker, 1856)	0	0	5	4
Psectrocladius (Allopsectrocladius) platypus (Edwards, 1929)	0	0	20	4

	Spring		Summer	
Taxa	Abundance (n)	Frequency (%)	Abundance (n)	Frequency (%)
Pseudosmittia holsata (Thienemann & Strenzke, 1940)	1	3	0	0
Rheocricotopus (Psilocricotopus) chalybeatus (Edwards, 1929)	168	34	390	56
Rheocricotopus (Rheocricotopus) effusus (Walker, 1856)	33	17	0	0
Rheocricotopus (Rheocricotopus) fuscipes (Kieffer, 1909*)*	511	52	0	0
Rheocricotopus indet.	10	3	0	0
Smittia (Holmgren, 1869)	7	7	0	0
Stilocladius montanus (Rossaro, 1979)	0	0	2	4
Symposiocladius lignicola (Kieffer & Potthast, 1915)	1	3	0	0
Synorthocladius semivirens (Kieffer, 1909)	21	38	89	32
Thienemannia (Kieffer, 1909)	2	7	0	0
Thienemanniella acuticornis (Kieffer, 1912)	1	3	4	4
Thienemanniella clavicornis (Kieffer, 1911)	59	21	5	12
Thienemanniella indet.	41	7	1	4
Thienemanniella majuscula (Edwards, 1924)	3	3	6	4
Thienemanniella partita (Schlee, 1968)	148	24	90	24
Thienemanniella sp.1	4	7	4	4
Thienemanniella vittata (Edwards, 1924)	160	45	108	12
Tvetenia bavarica-calvescens	657	90	520	72
Tvetenia discoloripes (Goetghebuer, 1940)	194	45	232	36
SUBFAMILY CHIRONOMINAE				
TRIBE CHIRONOMINI				
Chironomus (Meigen, 1803)	5	3	17	20
Cryptochironomus (Kieffer, 1918)	3	3	14	8
Dicrotendipes notatus (Meigen, 1818)	0	0	2	4
Harnischia (Kieffer, 1921)	2	3	0	0
Microtendipes pedellus group (Pinder, 1976)	23	24	152	48
Microtendipes rydalensis group (Pinder, 1976)	3	7	28	8
Nilothauma brayi (Goetghebuer, 1921)	0	0	1	4
Paracladopelma (Harnisch, 1923)	0	0	18	8
Paratendipes (Kieffer, 1911)	8	7	71	20
Phaenopsectra (Kieffer, 1921)	26	24	38	28
Polypedilum (Polypedilum) albicorne (Meigen, 1838*)*	2	3	1	4
Polypedilum (Polypedilum) cf. cultellatum	87	21	16	20
Polypedilum (Polypedilum) laetum group_sp1	109	28	207	48
Polypedilum (Polypedilum) laetum group_sp2	87	7	0	0
Polypedilum (Polypedilum) nubeculosum group	85	24	63	20
Polypedilum(Polypedilum) pedestre (Meigen, 1830)	5	7	0	0
Saetheria (Jackson)	2	1	1	1
SUBFAMILY CHIRONOMINAE	2	3	1	4

	Spring		Summer	
Taxa	Abundance (n)	Frequency (%)	Abundance (n)	Frequency (%)
TRIBE TANYTARSINI				
Cladotanytarsus (Kieffer, 1921)	12	10	8	8
Micropsectra sp.1	152	17	80	32
Micropsectra sp.2	74	34	38	16
Micropsectra sp.3	1	3	9	8
Micropsectra sp.4	46	28	136	40
Micropsectra sp.5	29	14	34	12
Micropsectra sp.6	2	3	13	8
Neozavrelia (Goetghebuer, 1941)	21	14	21	24
Paratanytarsus (Thienemann & Bause, 1913)	27	21	16	8
Rheotanytarsus (Thienemann & Bause, 1913)	329	55	231	52
Stempellina bausei group (Kieffer)	16	3	5	4
Stempellina indet.	0	0	4	8
Stempellinella (Brundin, 1947)	89	28	26	16
Tanytarsus chinyensis group	5	3	39	20
Tanytarsus sp.1	31	17	70	28
Tanytarsus sp.2	39	21	10	8
Tanytarsus sp.3	82	34	108	32
Tanytarsus sp.4	21	10	11	4
Virgatanytarsus (Pinder, 1982)	41	28	106	36



Chapter 3

Optima and Tolerances of Chironomidae in Mediterranean Reference Streams

Òptims i toleràncies dels quironòmids en rius mediterranis de referència

Resum

Aquest capítol pretén contribuir al coneixement de la distribució i l'ecologia de les comunitats de quironòmids en rius mediterrànis, determinant primer els principals gradients ambientals i aportant nova informació dels òptims i toleràncies de les espècies als paràmetres característics d'aquests gradients. El resultats d'aquest estudi provenen d'un mostreig extensiu realitzat a la regió Mediterrània de la Península Ibèrica en condicions de referència (63 estacions de mostreig en 22 conques d'estudi). Després d'estudiar les respostes ecològiques de les comunitats de quironòmids actuant a diferents escales espacials s'observa que la proporció de variança explicada pels factors locals (23.3%) és superior que la explicada pels factors geogràfics (8.5%) i regionals (8%). S'obtenen tres grups de rius tenint en compte els principals gradients ambientals: 1) rius de capçaleres silíciques, 2) rius petits de mitjana altitud i 3) rius mitjans calcaris, definint per cadascun dels grups les corresponents espècies característiques. Els òptims i la tolerància de les espècies de quirònomids han estat establerts per les següents variables: altitud, percentatge de substrat silícic, àrea de conca, pH, temperatura i cabal. Després d'estudiar els requeriments ecològics de les espècies, s'observen diferents respostes per espècies que pertanyen al mateix gènere. Per exemple alguns gèneres com *Diamesa* els trobem restringits en àrees de capçalera, mentre que d'altres com *Eukiefferiella* presenten unes preferències ecològiques més àmplies amb òptims i toleràncies diferents per espècies congenèriques. Les nostres dades accentuen la importància de la identificació taxonòmica per tal d'aprofundir en els patrons de distribució i els requeriments ecològics de les espècies de quironòmids en rius mediterranis. A més a més, davant l'actual escenari del canvi climàtic global, conèixer els requeriments de les espècies pot ser una eina molt útil alhora de predir respostes de les espècies cap a variables que poden canviar en el futur, com poden ser la temperatura i la hidrologia.

Abstract

A total of 141 chironomid taxa were recorded from 63 minimally disturbed near-pristine sites in 22 catchments of the Iberian Mediterranean coast. First, we used a partial redundancy analysis (pRDA) to study Chironomidae community responses to a number of environmental factors acting at several spatial scales. The proportion of variation explained by local factors was higher (23.3%) than that explained by geographical (8.5%) or regional factors (8%). Furthermore, drainage area, longitude, pH, percentage of siliceous rocks in the drainage basin and altitude were identified as the best predictors of Chironomidae assemblages. Using a k-means cluster analysis, we classified sites into three major groups on the basis of the Chironomidae fauna present. These groups were explained mainly by longitudinal zonation and geographical position and were defined as: (1) Siliceous headwater streams; (2) Mid-altitude streams with small catchment areas; and (3) Medium-sized calcareous streams. Distinct species assemblages with associated indicator taxa were established for each stream category using IndVal analysis. Moreover, species responses to the key environmental variables identified previously were determined and optima and tolerances were established by weighted average regression. Distinct ecological requirements were observed between genus and between species of the same genus. For instance, some genera were restricted to headwater systems (e.g. *Diamesa*) while others (e.g. *Eukiefferiella*) had wider ecological preferences but with distinct distributions among congeneric species. In the present scenario of climatic change, the response of species to alterations in environmental variables (e.g. temperature and hydrology) can be predicted on the basis of the specific ecological requirements of species (e.g. optima and tolerances).

Key words: Chironomidae assemblages, spatial variation, environmental gradient, partitioning variance, optima and tolerances, autoecology.

Introduction

One of the main focal points of community ecology is the identification of factors that determine composition patterns in stream communities and the study of how these factors influence diversity and abundance of such communities (Allan, 1995). Aquatic macroinvertebrate communities respond to multiple environmental gradients, many of which are scale-related (Levin, 1992; Vinson & Hawkins, 1998) and consequently communities are shaped not only by local scale processes but also by constraints on a wider scale, like geologic and climatic factors (Menge & Olson, 1990; Poff, 1997; Heino *et al*., 2002). Recently, several studies have examined the relationships between freshwater communities and environmental variables measured at distinct spatial scales (Johnson *et al*., 2007; Mykra *et al*., 2007). Although the relative importance given to factors that affect the community structure of benthic macroinvertebrates differs among studies, most report that local scale factors exert the most influence (e.g. Death & Joy, 2004; Sandin & Johnson, 2004).

Moreover, biological responses to environmental factors can be studied using an auto-ecological approach at population level (Tokeshi, 1999). For instance, estimation of optima and tolerances for each species is an excellent way to obtain auto-ecological information on relevant environmental conditions. Accordingly, ecological characterization requires species identification, as species of the same genus may show distinct responses to environmental factors (Rossaro *et al*., 2006).

Chironomidae are the most broadly distributed, species-rich and frequently the most abundant family of benthic macroinvertebrates in freshwater systems (Pinder, 1986; Coffman, 1995) and they comprise a heterogeneous group of species with variable responses to environmental gradients (Lindegaard, 1995; Lencioni & Rossaro, 2005; Helson *et al*., 2006). Ecological information on chironomids is still fragmentary, especially when examining larvae because species identification is time-consuming and requires sound taxonomic expertise. However, Chironomidae are widely used for bioassessment purposes as indicators of trophic conditions in lakes (Brundin, 1974; Saether, 1979; Real *et al.,* 2000) and organic pollution in running waters (Prat & Ward, 1994; Orendt, 1999; Ruse, 2002). This family is also used in paleolimnology studies for environmental reconstruction (Walker, 2001). Moreover, several studies identifying spatial community patterns and the most significant environmental factors contributing to Chironomidae assemblages have been performed in Europe (e.g. Lindeegard & Brodersen, 1995; Lencioni & Rossaro, 2005). However, fewer have addressed the Mediterranean area (González *et al*., 1985). These studies consisted of relatively short spatial gradients (including one or two catchments), established using mostly pupal exuviae and not exclusively working in reference conditions. Moreover, there is little detailed auto-ecological information on Chironomidae species in Mediterranean streams and to our knowledge only a few studies report the specific ecological requirements of Chironomidae taxa in near-pristine watercourses (but see: Calle-Martínez & Casas, 2006).

According to the Water Framework Directive (European Commission, 2000), a prerequisite for effective management of water systems is information on the state of freshwater biodiversity in near- pristine (reference) ecosystems. Thus, here we analyzed the ecological requirements of the most frequent chironomid species after determining the most important environmental gradient present in Mediterranean catchments of the Iberian Peninsula in reference conditions (or in the least disturbed sites in mid and lower sections of the catchments).

We performed a large scale examination of Chironomidae assemblages, including a range of stream types with distinct geology, morphology and physicochemical features (Sánchez-Montoya *et al.,* 2007). These characteristics should affect the composition of these assemblages, and despite the large dispersion capacity of Chironomidae (Armitage, 1995), we hypothesized that distinct Chironomidae communities would be present in the area.

Thus, the specific aims of the study were to: i) assess the contribution of environmental variables at distinct spatial scales (geographical, regional and local) to structuring Chironomidae assemblages; ii) identify the environmental variables most strongly related to assemblage structure; iii) determine the assemblage groups in Mediterranean reference streams and their representative indicator species; and iv) define the optima and tolerances of Chironomidae taxa for the relevant ecological variables responsible for assemblage composition.

Methods

Study area

The study area (Figure 1) covered approximately 78560 Km^2 of the Iberian Mediterranean coast, and included large watersheds (e.g. Júcar with 18136 Km^2) and very small ones (e.g. Chillar with 54 Km^2). A thermal, pluviometric and altitudinal gradient is present from north to south and from mountains to the coast (west to east usually). The annual range of temperatures is between -2ºC and 42ºC and annual precipitation from 280 mm to 1000 mm, with strong storms that often cause flooding during spring and autumn (MIMAM, 2000). Because of the Mediterranean climate, with hot dry summers and cool wet winters (Di Castri, 1973), rivers show high seasonality, with an annual and interannual variability in discharge regimes and frequent and predictable periods of flooding and drying (Gasith & Resh, 1999). Limestone and other sedimentary rocks are dominant along this coast, although some siliceous areas are present in the ranges of Sierra Nevada (south) and Montseny and Pyrenees (north). Sclerophylous and evergreen trees and shrubs are dominant, although in some areas deciduous forests are found. A total of 63 sites belonging to 22 river catchments were sampled during spring of 2003 (Figure 1). The sites sampled in our study cover an altitudinal (12 to 1940 meters) and latitudinal range (from the Muga stream in the north-east to the Guadiaro in the south of Spain) (Appendix 1). An extensive description of the catchments studied can be found in Robles *et al*. (2004).

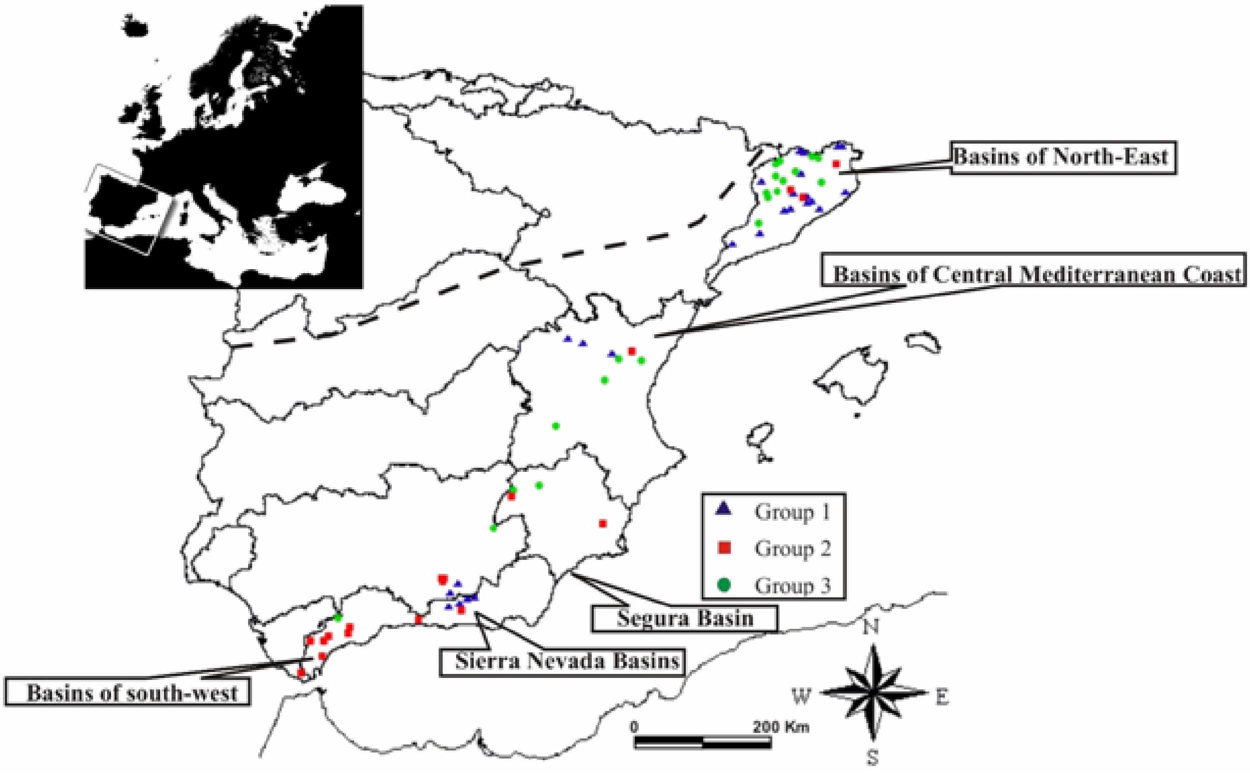


Figure 1. Location of 63 reference sites sampled along the Mediterranean coast of the Iberian Peninsula. The boundary of the Mediterranean climate according to Köppen (1931) is included (dashed line) as well as symbols of three groups of samples based on multivariate analysis (see text for explanation).

Only minimally disturbed sites were selected to ensure that they represent near-pristine conditions (as is the case in most of the headwaters) or the least disturbed sites in mid-reaches. Our site network covered very small streams at high altitude to mid-reaches of several medium-sized watercourses as there are no undisturbed large streams in the area. Sites were selected on the basis of the 18 criteria used to establish reference conditions in Mediterranean streams described in Sánchez-Montoya *et al.* (2005). Following this approach, a reference site is identified in function of several features related to: the catchment (e.g. no canalization or water derivations, natural uses in catchments >70%), the site (e.g. natural riparian vegetation appropriate to the type, absence of pointed and diffuse sources of pollution) and in-stream characteristics (e.g. no transversal structures (dams), no sand and gravel extraction activity).

As streams present in Mediterranean zones have a high probability of drying up during part of the year (usually in summer), only samples taken in spring were considered in this study, thereby ensuring that water flows and biological assemblages were comparable, because when the river is reduced to pools the community may differ both for macroinvertebrates (Bonada *et al.*, 2006) and Chironomidae (Puntí *et al.*, 2007).

Environmental descriptors

The environmental data set (41 variables) was divided into three groups of variables: geographical (site coordinates); regional (e.g. catchment, land use) and local (e.g. water chemistry) (Appendix 2).

The spatial variables were calculated by including all terms of a cubic trend surface regression (i.e. x, y, x^2, xy, y^2, x^3, x^2y, xy^2 and y^3) with x (latitude) and y (longitude) and using a similar approach to that described by Borcard *et al.* (1992). The use of this spatial component in the analysis allows the inclusion of large scale spatial structures in the data set (Meot *et al.*, 1998). The spatial component explains patterns in the species data not shared by any of the environmental data measured and represents an indirect synthetic descriptor of a number of biological process or environmental factors not measured explicitly in the study (see: Magalhaes *et al.,* 2002; Johnson *et al.,* 2007).

In the regional variables group, geological characteristics and drainage area were calculated using a digital terrain model (DTM 30 x 30 m) (*Centro Geográfico del Ejército, Ministerio de Defensa*, Spain, 2005) and Arc/Info software (Version 9.0, 2005), whereas the classification of catchment land cover was obtained from the CORINE LAND COVER (2000).

Local variables included riparian characteristics (see: Munné *et al.,* 2003) and bedform variables referred to habitat condition (see: Pardo *et al.,* 2004). Water chemistry was measured *in situ* with standard portable equipment (e.g. conductivity, pH, temperature oxygen and discharge), and additionally, water samples were analyzed in the laboratory for alkalinity, chlorides and sulphates following standard procedures described in APHA (1992). Other local environmental variables such as altitude, stream order and percentage of dry period were derived from Geographical Information Systems (GIS).

Biological sampling

Benthic macroinvertebrates were sampled using a multi-habitat sampling procedure with a kick net (mesh size between 250-400 µm) following the protocol established in the GUADALMED project (Jáimez-Cuéllar *et al*., 2004; Sánchez-Montoya *et al.,* 2007). Samples were preserved in the field using a 10% formalin solution and sorted in the laboratory. All chironomid material collected for this study was larvae, which were sorted, counted and mounted on slides for identification with high power magnification and at the maximum possible taxonomical level. Larvae were first grouped by their similar morphological appearance (shape of the capsule, colour, body setae, size) under a stereomicroscope, and all (when fewer than ten individuals of each morphological type) or part (when more than 10 individuals of each type) were mounted on slides following Pinder (1983). A total of 12409 larvae were examined (4347 mounted).

We used identification keys and species description selected from European literature, including: Ferrarese & Rossaro, 1981; Wiederholm, 1983; Nocentini, 1985; Schmid, 1993; Rieradevall & Brooks, 2001. In addition and for some genera (e.g. *Corynoneura*, *Micropsectra* and *Tanytarsus*), the authors' own experience in the identification of larvae and their reference collections were used. In some cases we could not identify the chironomid species because of the small size of many individuals (second and third instars) and the difficulty in differentiation some groups (e.g. *Orthocladius-Cricotopus)* at the larval stage, therefore in the final biological matrix a number of taxonomical levels were mixed. Abbreviations of species names follow a standardized coding system developed by Schnell *et al.* (1999). Finally, relative abundance of Chironomidae (percentage of each taxon per sampling site) was calculated and used for multivariate analysis.

Data analysis

Detrended correspondence analysis (DCA) (Hill & Gauch, 1980) of species relative abundances was performed to assess the degree of species turnover across ecological gradients, and to determine the gradient length of the biological dataset. The gradient lengths of the first two axes were 3 and 2.7 standard deviation (SD) units respectively, indicating that either a linear and unimodal species response model should perform reasonably well (Lepŝ & Ŝmilauer, 2003). We considered that methods based on a lineal response model were best suited to our data (variance explained of redundancy analysis (RDA) 64% and canonical correspondence analysis (CCA): 59%), and therefore we used a RDA to examine the relationship between Chironomidae assemblages and the explanatory variables. RDA is a constrained form of the linear ordination method of principal components analysis (Legendre & Legendre, 1998). All analyses were run on transformed Chironomidae abundance data (fourth root). When necessary, environmental variables were $\log_{10}$ or square-root arcsin transformed in order to approximate normally distributed random errors (Appendix 2). There is no consensus whether rare taxa should be removed from the dataset using multivariate analysis (Marchant, 1999; Cao *et al*., 2001). In our case, to prevent a disproportionate effect of Chironomidae taxa with low occurrence on the results (Gaugh, 1982), taxa occurring in at least 2 samples and with a relative proportion of 2% or more in at least one sample were included in the multivariate analysis. All ordinations were run with the CANOCO programme, version 4.5 (Ter Braak & Ŝmilauer, 1998).

To estimate the fraction of variance in community composition explained by the three sets of explanatory variables (geographical, regional and local), direct gradient analysis known as partial constrained ordination (pRDA) was performed. Partial constrained ordinations allow examination of relationships between desired environmental variables and biological variables by removing the effects of known factors of no interest. It is possible to use the same variable both as a covariable and as an environmental variable in different parts of the same analysis. In variation partitioning, covariables are useful for distinguishing the relative contributions of groups of variables to explaining species composition (Legendre & Legendre, 1998).

Firstly, constrained ordinations were run to determine the environmental variables that were significant ($p<0.05$). Only significant variables were considered as the environmental variables used in the pRDA. Variables included in the three groups (geographical, regional and local) as well as the individual effects explained by each variable (lambda 1 or marginal effects) are shown in Appendix 2. Using these data, series of pRDAs were run for Chironomidae assemblages following Borcard *et al.* (1992) and Liu (1997). pRDAs were carried out in the following steps: (i) running one RDA using species data and all 3 groups of environmental variables as explanatory variables and no covariables, to explain the total amount of variation explained (TVE) by the 3 environmental groups; (ii) running pRDA using one of the 3 environmental variable groups as explanatory variables, and the remaining two groups together as covariables, to obtain pure effects for each group of variables; (iii) calculating the variation shared by several combinations between groups of variables: interaction effects; and (iv) calculating the unexplained proportion of variation (1-TVE).

In addition, RDA using forward selection was run to detect main environmental variables that could best explain the variability of dataset analyzed. Bonferroni-adjusted forward selection was used to reduce redundancy between variables and the significance of each remaining variable was tested with MonteCarlo permutation (9999 permutations $p<0.05$). The significance level was set to α/n for each variable tested to compensate the number of statistical tests (Legendre

& Legendre, 1998). Environmental variables were chosen only when their addition did not cause any variation inflation factor higher than 20. Pearson correlations between the first four canonical axes and environmental variables were analyzed in order to interpret the meaning of these axes and their significance.

To obtain groups of Chironomidae assemblages, samples were clustered using their projections onto the first two axes of the ordination results with a K-means method in the SPSS programme (SPSS, 1999). We applied the indicator value method (IndVal) (Dufrêne & Legendre, 1997) to determine the most representative Chironomidae taxa among the groups of K-means obtained. IndVal is based on the comparison of relative abundances and relative frequencies of taxa in distinct *a priori* site groups. Each taxon obtained is associated with an indicator value (IV value) that varies between 0 and 100, and a p-value obtained by Monte Carlo permutations (9999 runs). The PC-ORD programme (McCune & Mefford, 1999) was used to perform this analysis.

Finally, to calculate the optima and tolerances of several species of chironomids using independent environmental variables, a Weighted Average (WA) regression was performed with the C2 programme, version 1.3 (Juggins, 2003). This analysis estimates the optimum of an environmental variable for each species using the average of the values of the variable in sites where taxa are present, weighted by species' relative abundances. The WA assumes that each taxon has a Gaussian response to an environmental variable, where the species optimum (the mode) and the tolerance (the standard deviation from the optimum) can be calculated (Birks *et al.*, 1990). WA regression has been widely applied in paleolimnology to infer environmental conditions using optima and tolerances of Chironomidae species (Brodersen & Anderson, 2002; Porinchu *et al.,* 2002).

Results

Relative importance of geographical, regional and local variables

A total of 141 taxa of Chironomidae included in 73 genus were identified in the 63 sites sampled (Appendix 3). However, only 117 taxa with relative abundances >2% were included in multivariate analyses. The percentage of variance for each group of explanatory variables is shown in Table 1. The results of the Variance Partition analysis indicated that the total variation explained by all the groups (TVE) is 48.3%. Of this explained variation, the pure effect of local variables accounted for 23.3%, whereas pure geographical and regional effects accounted for 8.5% and 8% respectively. Thus, local scale variables explained substantially more of the among-site variance in community composition. The total shared variance of the three explanatory groups accounted for 4.1%, where as the joint effect of regional and local (RL) factors accounted for 3.1% and the geographical and local (GL) factors 1.5%. A negative value (-0.2%) was obtained between geographical and regional variables, indicating that the variance explained by the GR term was substantially lower than the unique variance explained by G and R. The pure or unique effects of the three variable groups accounted for 82.4% and combinations of variable groups (interaction terms) the remaining 17.59% of the total explained variability.

Variation explained by factors	Code	Variance (%)
Pure effect geographical	G	8.5
Pure effect regional	R	8
Pure effect local	L	23.3
Shared effect geographical and regional	GR	-0.2
Shared effect geographical and local	GL	1.5
Shared effect regional and local	RL	3.1
Shared effect geographical regional and local	GRL	4.1
Total explained	TVE	48.3
Unexplained	UX	51.7
Total variance	TV	100

Table 1. Percentage of variance explained (pure and shared effect) for each group of variables.

Best predictors of Chironomidae assemblages

The first four axes of the RDA explained 19.2% of the total variation of the 117 Chironomidae taxa in the 63 sites. Five environmental variables were included in the model after applying forward selection corrected with Bonferroni: catchment area was the first variable selected (explaining 6.64% of the total variance), followed by longitude (3.42%), pH (3.42%), altitude (2.91%) and percentage of siliceous rocks in the catchment (2.81%). Table 2 gives the results of RDA using only the five forward selected variables. These results showed the combination of geographical, regional and local factors could best explain the variation in among-site differences in Chironomidae assemblages, even though in the analysis of partition of variance, local variables represented the highest percentage of variance explained.

	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**
Eigenvalue	0.08	0.043	0.029	0.024
Species-environment correlations	0.828	0.781	0.836	0.806
Cumulative % variance of species data	8	12.3	15.2	17.6
Cumulative % variance of species-environment relation	41.8	64	79.1	91.8
Correlations with first four axes				
pH	0.596**	0.061	-0.323*	-0.131
Altitude	-0.546**	-0.267*	0.304*	0.243
Catchment Area	0.481**	-0.252*	0.063	0.095
% of siliceous geology	-0.500**	-0.044	-0.361**	0.507**
Longitude	-0.075	-0.513**	-0.457**	-0.410**
** p<0.01; *p<0.05				

Table 2. Summary statistics of RDA using forward selection of variables. Pearson correlations between significant environmental variables and the canonical axes are also shown.

Although a low percentage of Chironomidae variability was explained by the RDA (Table 2), canonical axes were significant in relation to the set of variables used (F=1.23, p<0.01) as MonteCarlo permutation tests (999 permutations) indicated. The first axis using the five significant variables explained 8% of the total variability in the species data. This axis was positively correlated with pH and area, and negatively correlated with altitude and percentage of siliceous rocks in catchment (Table 2) and differentiated sites located in mainly siliceous headwater streams (with lower pH and small basin area) from samples of mid-altitude streams with larger basin areas and higher pH. The second axis explained 4.3% of the variance, and was negatively related to longitude, altitude and catchment area, mainly differentiating streams located in the south-east and at lower altitudes infrom the south-west and north-west areas were the highest peaks are found (Figure 2). Despite the low values of cumulative percentage variances, species environment correlations were high for all axes.

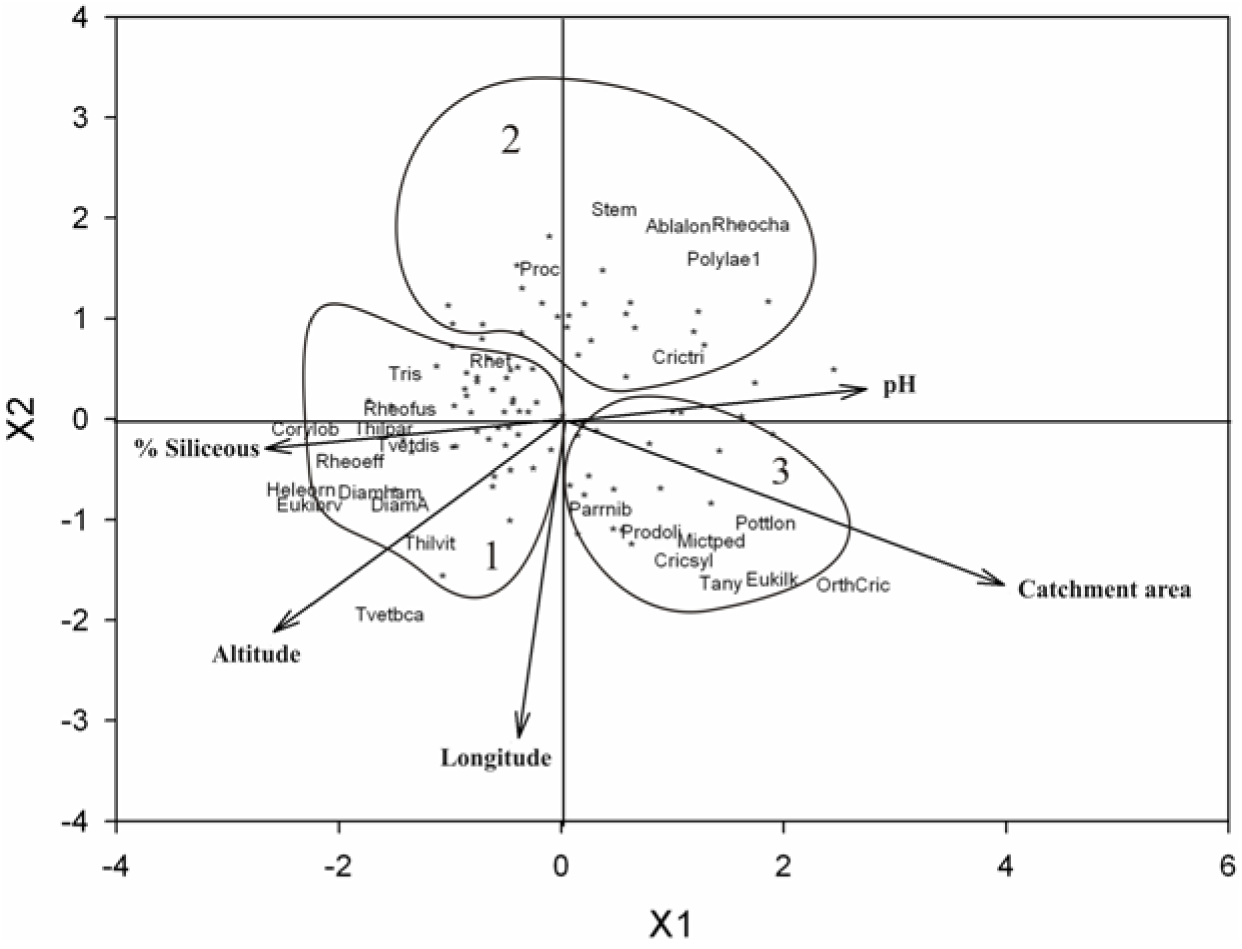


Figure 2. Constrained ordination biplot (RDA) of chironomidae assemblages, in 63 Mediterranean reference sites. Environmental variables were selected using foward selection and Monte Carlo permutation test. Only IndVal preferential Chironomidae taxa labels are shown. Ellipses show distribution among the three groups of sites ((1) Siliceous headwater streams, (2) Middle altitude streams with small catchment areas, (3) Medium sized calcareous streams).

Chironomidae assemblages

The two first canonical axes were used in the classification of sites by applying a K-means cluster to all samples, because they include the maximum variability expressed by environmental variables (Table 2). As a result, three distinct groups of sites with similar Chironomidae assemblages were identified: (1) Siliceous headwater streams; (2) Mid-altitude streams with small areas (mixed siliceous and calcareous) and (3) Medium-sized calcareous streams (Figure 2).

Group 1		**Group 2**	
Taxa	**IV**	**Taxa**	**IV**
Eukiefferiella brevicalcar	72.5	*Rheocricotopus chalybeatus* group	57.8
Tvetenia discoloripes	53.7	*Rheotanytarsus* spp.	47
Tvetenia bavarica-calvescens	52.3	*Ablabesmyia longistyla*	35.1
Trissopelopia spp.	37.9	*Polypedilum laetum* group *sp.1*	29.2
Thienemanniella partita	36.8	*Procladius* spp.	27
Rheocricotopus fuscipes	36.5	*Cricotopus (Cricotopus) trifascia*	19.3
Thienemanniella vittata	31.4	*Stempellina* spp.	16.2
Heleniella ornaticollis	31	**Group 3**	
Corynoneura lobata	26	**Taxa**	**IV**
Rheocricotopus effusus	25	*Orthocladius -Cricotopus*	76.4
Diamesa sp.A	22.7	*Microtendipes pedellus* group	37.2
Diamesa hamaticornis type	16	*Eukiefferiella ilkleyensis*	37.2
		Cricotopus (Isocladius) sylvestris group	20.2
		Potthastia longimana	20
		Paracricotopus niger	19.6
		Prodiamesa olivacea	15.9
		Tanytarsus spp.	14.7

Table 3. Indicator values for each group of k-means samples.

Group 1 comprised 25 headwater sites mainly from the basins of the north-east (Pyrenees and Montseny ranges) and of south-east (Sierra Nevada basins) (Figure 1), and was characterized by the highest percentage of siliceous rocks (61.27 ± 47.48) and altitudes (942.64 ± 506.24), and the lowest values of catchment areas (33.74 ± 48.25) and pH (7.63 ± 0.65). These sites were differentiated by the presence of 13 indicator taxa, on the basis of IndVal results (Table 3). Indicator taxa present in this group generally were associated with low-temperature habitats such as *Eukiefferiella brevicalcar, Tvetenia bavarica-calvescens, Tvetenia discoloripes, Trissopelopia* spp. and *Eukiefferiella ilkleyensis.* Group 2 included 18 sites from the south-west and central part of the study area (e.g. Guadiaro, Guadalhorce and Segura catchments); they had intermediate altitudes (484.94 ± 436.28) with intermediate catchment areas (168.61 ± 370.9) and with a low percentage of siliceous rocks (38.5 ± 38.63). This group was characterized by seven indicator taxa such as *Rheocricotopus chalybeatus* group*, Rheotanytarsus* spp. and *Ablabesmyia longystila*. Finally, group 3 included 20 sites, mainly calcareous, and belonging to basins of the north-east and of the central Mediterranean coast (Ter, Llobregat, Palancia and Segura catchments); they had higher catchment areas (812.89 ±

1270.4) and pH values (8.33 ± 0.38) and intermediate altitudes (558 ± 261.02). The *Orthocladius-Cricotopus, Microtendipes pedellus* group and the *Cricotopus sylvestris* group were some of the indicator taxa present here, generally associated with highly mineralized waters. Most of indicator taxa from each group had indicator values (IV) higher than 25 (Table 3), showing that these species were present in at least 50% of one group and that their relative abundance in that group reached at least 50% (Dufrêne & Legendre, 1997).

Optima and tolerances

We assume that taxa with a certain optimum tend to be most abundant in streams with values of the environmental variable close to this optimum. Figures 3 and 4 illustrate the optima and tolerances of altitude and surface catchment area for the 59 most frequent taxa collected in the study area and occurring in at least ten samples. We considered to figure values for altitude and catchment area because they represent the major environmental gradients relevant to this study, but optima and tolerance were also calculated for many other factors. Of the selected taxa, *Heleniella ornaticollis, Diamesa* sp. A and *Eukiefferiella brevicalcar* had the highest optimum for altitude (>1000 m), whereas *Phaenopsectra* spp., *Virgatanytarsus* spp.*, Cricotopus* group *sylvestris* and *Paramerina* spp. were restricted to lower altitudes (< 500 m). In general, taxa that had lower optima catchment area (<200 km^2), such as *Stempellinella* spp., *Corynoneura lobata* and *Paratrissocladius excerptus,* had narrow values of tolerances for this variable, indicating that these taxa were restricted to small catchments. In contrast, taxa with higher optima values for area (>800 km^2), such as *Orthocladius rivulorum*, *Microtendipes pedellus* group and *Virgatanytarsus* spp.*,* presented wider preferences and showed a preference for mid-reaches.

Optima and tolerance values allowed the study of niche specificity for some of the congeneric species found. Results of optima and tolerances from taxa belong to the same genus are shown in Table 4, and for four of the most relevant environmental variables for chironomid assemblage composition identified previously in the RDA: altitude, percentage of siliceous rocks, catchment area, pH, together with temperature and discharge.

Significant differences were found between species of the same genus. Two *Diamesa* species, *D. hamaticornis* type and *D.* spA *(sensu* Schmid 1993), showed a preference for fast flowing waters at higher altitudes in siliceous rock catchments, with low temperatures and pH, while another two species of the same genus, *D. hamaticornis* and *D.* group *zernyi,* were found mainly in headwaters of calcareous slow-flowing streams. In another genus *Corynoneura*, preferences for the *C. lobata* and *C. scutellata* group were similar: headwater (mid-high altitudes) mainly siliceous streams, with low temperatures. In contrast, *C. coronata* showed a preferential distribution in intermediate altitudes, in sites with higher temperatures, discharge and percentage of carbonates, and a wide ecological tolerance for catchment area. A total of six *Eukiefferiella* taxa were differentiated at larval stage, showing variable values for optima and tolerances of the environmental descriptors studied. *E. brevicalcar* and *E. coerulescens* were found mostly at higher altitudes mainly in siliceous catchments. However, while *E. brevicalcar* was restricted to fast-flowing streams but in sites with wide ranges of catchment's areas, *E. devonica* and *E. minor-fittkaui* larvae inhabited mid-altitudes but were not found exclusively in calcareous sites. Finally*, E. gracei* and *E. ilkleyensis* were clearly differentiated from other taxa of *Eukiefferiella* by their distribution in relatively lower altitudes and sites with a higher percentage of carbonates and higher temperature, discharge and catchment area. Another genera, *Rheocricotopus effusus* presented optima typical of headwaters of siliceous streams, with wide tolerances of catchment areas, while *R. fuscipes* was more restricted to small, mid-altitude, mineralized and slow-flowing streams. A distinct pattern was observed in the *R. chalybeatus* group, which was present in fast-flowing lower altitude streams, mainly calcareous with variable catchment areas. *Thienemanniella partita* and *T. vittata* were found in siliceous headwaters, being the first one restricted to smaller catchment areas, while the remaining species of *Thienemanniella* showed a preference for mid-altitude streams with similar optima to the other environmental variables. However, *T.* sp.1 showed carbonate preferences compared to *T. clavicornis*. The two taxa of *Microtendipes* detected had clearly distinct ecological requirements; while the *M. pedellus* group was found mostly at relatively lower altitudes, with higher carbonate concentrations and temperatures and covering a wide range of catchment areas; the *M. rydalensis* group showed a preferential distribution in headwater streams at high altitudes, with lower catchment areas. Finally, *Polypedilum* species presented similar patterns, *P. breviantenatum* differing the most different, with preference for sites located in larger streams (but with high tolerance values of area) in basins dominated by calcareous rocks. In contrast, *P. pedestre* had a wide distribution range of altitudes, temperatures and percentage of siliceous rocks, but showed a preference for fast-flowing streams and higher altitudes than the other *Polypedilum* taxa found.

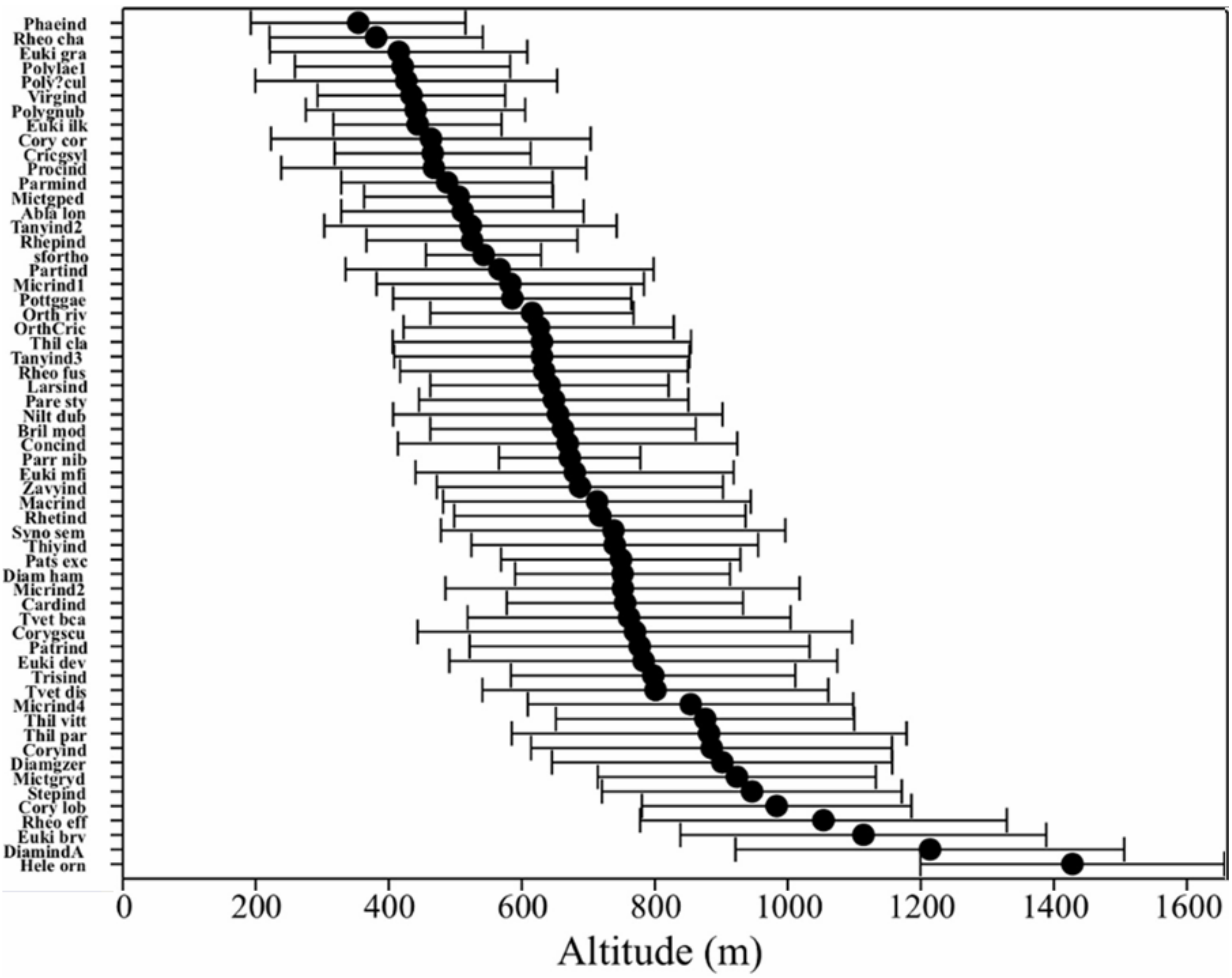


Figure 3. Scatter plots of optima with error bars indicating the tolerance of taxa present at >10% of sites. Y axes are arranged according to increasing optima in altitude. Codes of taxa are listed in appendix 3.

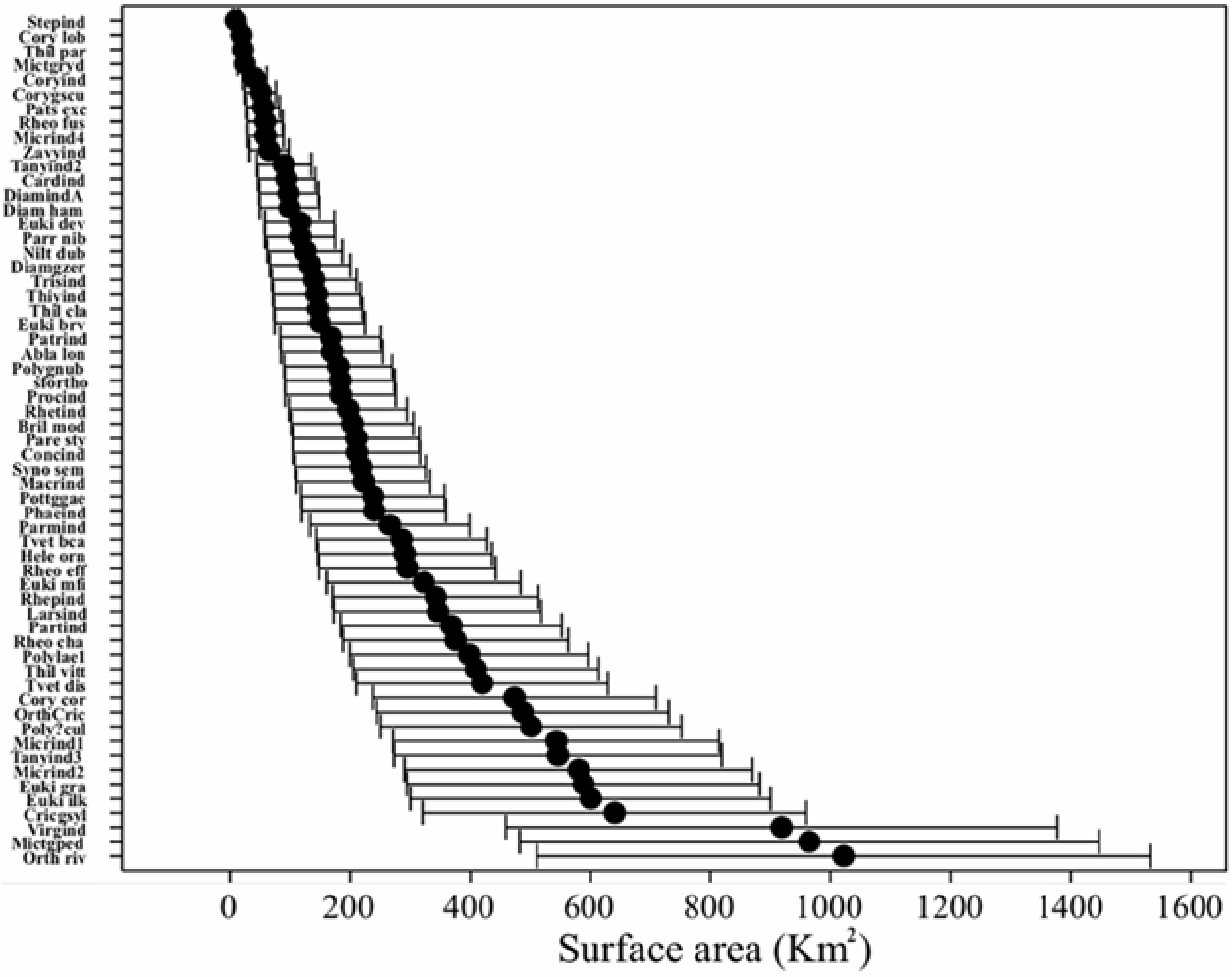


Figure 4. Scatter plots of optima with error bars indicating the tolerance of taxa present at >10% of sites. Y axes are arranged according to increasing optima in surface area. Codes of taxa are listed in appendix 3.

		Altitude (m)		Siliceous (%)		Temperature (ºC)		Area (Km^2)		Discharge (l/s)		pH	
Genus	**Taxa**	O	T	O	T	O	T	O	T	O	T	O	T
Diamesa	*Diamesa hamaticornis*	751.47	323.14	32.57	43.18	10.18	2.5	99.15	222.54	0.88	2.18	8.1	0.71
	Diamesa ?hamaticornis	1582.06	331.15	100	37.91	6.35	1.24	44.44	32.33	4.67	3.43	6.64	0.65
	Diamesa zernyi group	901.18	511.46	53.57	45.35	9.44	2.75	133.12	247.71	2.3	3.35	7.8	0.93
	Diamesa sp A sensu Schmid	1213.69	584.69	90.99	32.01	7.51	3.63	97.51	246.19	1.86	3.31	7.4	0.76
Corynoneura	*Corynoneura coronata*	463.39	480.87	35.68	35.76	14.41	4.9	473.19	553.94	4.7	4.34	7.98	0.44
	Corynoneura lobata	983.21	405.01	61.44	45.56	9.59	3.01	18.68	26.73	0.51	1.56	7.6	0.63
	Corynoneura scutellata group	770.06	653.03	61.39	46.53	10.54	4.4	51.35	76.56	0.16	0.21	7.72	0.65
Eukiefferiella	*Eukiefferiella brevivalcar*	1113.51	550.3	76.3	44.05	9.05	3.92	149.16	718.38	2.48	3.23	7.42	0.69
	Eukiefferiella coerulesencs	988.69	451.64	89.54	30.21	8.75	2.62	15.9	20.69	0.44	0.58	7.99	0.59
	Eukiefferiella devonica	782.82	583.5	39.94	48.85	12.03	4.53	115.98	272.34	1.59	2.51	7.75	0.69
	Eukiefferiella gracei	414.77	386.92	18.02	12.7	13.63	4.76	588.26	522.29	5.38	4.18	8.31	0.34
	Eukiefferiella ilkleyensis	443.22	252.89	28.42	32.91	12.6	3.38	600.28	1020.09	2.29	2.94	8.28	0.33
	Eukiefferiella minor-fittkaui	679.42	478.46	39.67	41.11	11.8	4.04	322.35	926.83	1	1.33	8.06	0.58
Rheocricotopus	*Rheocricotopus chalybeatus* group	380.95	320.8	33.94	37.88	14.98	4.26	375.25	833.44	2.46	3.45	8.19	0.47
	Rheocricotopus effusus	1053.48	551.45	83.99	34.26	8.83	3.36	294.87	1017	1.71	2.12	7.41	0.62
	Rheocricotopus fuscipes	633.39	432.67	60.9	44.79	11.53	3.21	58.78	189.07	0.23	1.25	7.82	0.47
Thienemanniella	*Thienemanniella clavicornis*	630.16	448.44	73.73	41.93	14.48	4.41	146.48	318.11	1.03	2.61	8.07	0.46
	Thienemanniella partita	881.62	593.52	57.88	46.98	10.89	3.82	21.25	20.21	0.48	0.82	7.6	0.76
	Thienemanniella vittata	875.47	448.52	64.93	46.61	10.34	2.95	409.06	1069.83	0.87	1.25	7.98	0.57
	Thienemanniella sp.1	415.73	95.31	41.67	45.87	13.71	3.36	199.72	363.1	0.77	0.95	8.54	0.31
Microtendipes	*Microtendipes pedellus* group	504.95	284.29	22.44	32.8	12.92	2.89	964.48	1481.86	2.1	2.05	8.03	0.49
	Microtendipes rydalensis group	923.2	417.89	71.6	29.08	9.82	5.47	24.21	28.42	1.01	2	7.93	0.57
Polypedilum	*Polypedilum pedestre*	974.33	683.76	75.16	59.59	10.22	6.77	208.2	419.25	5.34	4.13	7.59	1
	Polypedilum cf. cultellatum	426.27	453.8	38.22	40.79	15.26	5.3	501.15	570.08	4.41	4.33	8.42	0.23
	Polypedilum breviantenatum	362.1	325.44	17.23	30.98	15.88	3.68	1119.94	1237.32	3.97	2.79	8.27	0.32
	Polypedilum nubeculosum group	440.3	330.04	63.49	46.29	13.27	4.25	179.95	355.82	0.2	0.37	8.04	0.56
	Polypedilum laetum group_sp1	420.64	323.72	32.51	34.86	14.72	4.88	397.5	922.75	1.83	2.95	8.32	0.38

Table 4. Optima (O) and Tolerances (T) of Chironomidae taxa (genus with more than one species) for six environmental variables.

Discussion

Establishing the effects of wide-scale and local environmental factors is a prerequisite for a comprehensive understanding of the processes that determine structural and functional features of stream communities (Sandin & Johnson, 2004). Several factors determine the assemblage structure of biological communities, such as the influence of spatial patterns, dispersal capacity, historical effects, climatic constraints, or spatial variation in local environmental conditions (Minshall, 1988; Townsend, 1989; Bonada *et al*., 2005). Our study examined Chironomidae distributions across the Mediterranean region of the Iberian Peninsula, over a large area with strong environmental gradients. A spatially extensive sampling allows analysis of the contribution of environmental factors to structuring Chironomidae communities in undisturbed streams. A number of earlier studies applied the variance decomposition technique (Magalhaes *et al*., 2002; Soininen *et al.,* 2004; Johnson *et al.,* 2007) to explore the combination of spatial and environmental variables (combining regional and local factors) that play a significant role in structuring biological communities in streams. Our finding that local environmental factors explained the highest amount of variance (23.3%) in Chironomidae community structure is consistent with the results of a number of previous studies that addressed several groups of organisms, such as benthic diatoms (Soininen *et al.,* 2004), macroinvertebrates (Death & Joy, 2004; Mykra *et al*., 2007), fish (Magalhaes *et al.,* 2002) and macrophytes (Johnson *et al*., 2007). In contrast, other studies have reported that large scale factors are the best predictors of stream communities (Richards *et al.,* 1996; Urban *et al.,* 2006). These disagreements regarding the importance of local or large scale variables in stream communities might also result from differences in the study design or may be caused by artifacts of the classification of variables at distinct spatial scales (Sandin & Johnson, 2004). Our results show that the proportion of variation explained by spatial effects accounted for a considerable component of variability in Chironomidae assemblages (8.5 %), and was similar to the percentage explained by regional factors (8%). The geographical pattern of distribution may reflect historical and climatic factors that are largely independent of environmental variables (Sandin & Johnson, 2000). However, a low percentage of the explained variability (4.1%) was described by the interaction of the three explanatory variable groups. Thus, the groups of variables used in our study were poorly related in comparison with other studies (Sandin & Johnson, 2004). Furthermore, the total explained variability (48.3%) was higher than values described previously, for instance, Bonada *et al.* (2005) reported 24.83 % of variance explained in same Mediterranean streams. In contrast, the relatively high percentage of unexplained variation found (51.7%), typical of noisy data sets with large numbers of taxa and many zero values (Borcard *et al.,* 1992), could be the result of unmeasured factors such as species interactions, food resources, dispersal, sampling variability or measurement errors. Overall, our results show the contribution importance of distinct groups of variables that act at local or regional spatial scales to explaining among-site differences in community composition of the biological dataset studied. As expected, current distributions of chironomids result from a series of filters ranging from local to regional scales (Poff, 1997).

Little information is available on the environmental factors and mechanisms that regulate the composition and distribution of Chironomidae taxa in Mediterranean streams (Calle-Martínez & Casas, 2006; Puntí *et al*., 2007). Our data indicate that longitudinal zonation is the strongest environmental gradient underlying distribution patterns in Iberian Mediterranean streams, followed by geographical position, which was closely related to community patterns along the secondary axes of the RDA. pH is also an important driver of community assembly and is directly related to other regional components such as catchment geology. This pattern is consistent with the findings of other authors, who have demonstrated that Chironomidae composition changes along the river continuum, associated with altitude, stream order and channel width (Ward & Williams, 1986; Lindegaard & Brodersen, 1995; Inoue *et al.,* 2005). The importance of altitudinal gradient has been reported in the Mediterranean region as well as in other parts of the world (Coffman, 1989; Casas & Vílchez-Quero, 1993). For instance, in our study, headwater siliceous streams (Pyrenees and Sierra Nevada) show similarities, in spite of the geographical distances between them. Differences in altitude may result in considerable changes in the local climate and other physical conditions, thereby affecting assemblage structure. However, despite similar morphologies, differences at population level measured with molecular taxonomy techniques, may indicate in the future that populations of the same morphological species (like *Diamesa* or *Eukiefferiella*) located at large distances (Pyrenees and Sierra Nevada) differ, as reported for Thrichoptera in the same area (Bonada *et al*., submitted). It would be of interest to use molecular ecology in future studies to analyze the importance of mountain isolation versus the larger dispersal capacity of midges in comparison with caddisflies.

Our data show that three distinct Chironomidae assemblages provide a broad meaningful ecological interpretation for reference conditions in Mediterranean streams. Although chironomids show many adaptations for dispersal and colonization (Armitage, 1995), many species exhibit regionally restricted distributions and ecological preferences, thereby corroborating the optima and tolerances reported in the present study. Thus, several indicator taxa belonging to group 1, such as *T. bavarica-calvescens*, *H. ornaticollis* and *R. effusus* are typically associated with low temperature-torrential mountain streams. These taxa together with *Brillia bifida* and *P. excerptus* have been recorded in the Sierra Nevada range (Casas & Vílchez-Quero, 1993) and the Pyrenees (Prat *et al*., 1983; Puntí *et al*., 2007), and they are representative of headwater systems but not restricted only to upper altitudes. In contrast, *Diamesa* is regarded as a characteristic genus with narrow ecological niche, presenting mainly cold-stenothermal species (Rossaro, 1995; Maiolini & Lencioni, 2001) and found mainly in siliceous headwater streams. It is of interest to note that even in this cold-stenothermic adapted genus, several differences in optima and tolerances were observed at species level. For instance, the *D. zernyi-thienemanni* group and *D. hamaticornis* showed a preference for headwater streams at lower altitudes and were not restricted to siliceous geology. This finding contrasts with *Pseudodiamesa branickii* and *D. bertrami,* which are typical of non-glacial conditions of alpine running waters (Lods-Crozet *et al*., 2001). Diamesinae maintain relatively dense populations at mean water temperatures of around 5ºC (Maiolini & Lencioni, 2001); however, our results indicate that many species have a higher temperature optimum than the values reported for alpine streams, while pH values are very similar to those described by Rossaro *et al*. (2006).

Most of the species of *Eukiefferiella* were widely distributed along the altitudinal gradient studied, E. *brevicalcar*, *E. devonica* and *E. coerulescens* being some of the species found at higher altitudes. A similar pattern was observed by Casas & Vílchez-Quero (1993), who analyzed altitudinal distribution of Chironomidae in the Sierra Nevada Mountains and found that *Eukiefferiella* was one of the dominant genera specifically and numerically in headwater streams. Overall, we found lower altitudes for same taxa in comparison with the values reported in other studies (Casas-Vílchez-Quero, 1993; Laville & Vinçon, 1991), because many of the headwater streams studied here fell in a lower altitudinal range.

In contrast, assemblages in mid-altitude streams (group 2) are characterized by more ubiquitous species with short life cycles that may tolerate more lentic habitats and are mainly warm water-adapted, Chironominae and Tanypodinae being dominant (Garcia & Laville, 2000). Chironominae species are more abundant when water temperature increases (Castella *et al*. 2001), which would account for the observation that they were distributed mainly in mid-reaches. For instance, most of the *Polypedilum* species recorded showed preferences for mid-altitude mountain and foothill sites.

In medium-sized calcareous streams (group 3), it was difficult to select strict reference sites because of high human pressure on the lowland, and consequently these sites cannot be considered true reference conditions. These streams were characterized by the abundance of *Orthocladius* and *Cricotopus*, which are tolerant and opportunistic genera generally associated with mineralized waters (Calle-Martínez & Casas, 2006), and their presence or absence is not clearly related to a well defined range of environmental variables.

Our data confirm the importance of identification at species level in order to provide information about the ecological requirements of chironomids in reference streams. Our results are consistent with the observations than species belonging to the same genus often have a clearly distinct ecological niche. However, we must take into account that when optima and tolerances are obtained from field data, large data sets are required to determine species auto-ecology with certainty as a weak sampling effort may lead to some species not being related to a well defined range of variables. Thus in future research, given the strong seasonality of Mediterranean streams, more data sets from other seasons are required to obtain large data sets integrating space and time. Under the future scenarios of climatic change, as temporary conditions increase (Bonada *et al*., 2007a) and cold water habitats are at risk (Rossaro *et al.,* 2006), a better understanding of the ecological requirements of these species in Mediterranean regions is required to ensure the preservation of these particular and highly diverse ecosystems.

Acknowledgements

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Appendix 1. Main characteristics of catchments sampled in the Mediterranean region of Spain.

	Area (km^2)	Perimeter (km)	Discharge (m^3s^{-1})	Maximum altitude (m)	Medium altitude (m)	Siliceous (%)	Carbonate (%)	Evaporite (%)	Number of sites
BASINS OF NORTH-EAST									
Muga	795	740	4.8	1399	276	58.1	36.2	5.7	2
Fluvià	1039	745	9.1	1543	466	60.8	34.3	4.9	3
Ter	2994	2271	25.7	2825	720	73.3	21.8	4.9	7
Tordera	892	632	5.7	1633	341	76.4	17.3	6.3	3
Besòs	1038	762	4.1	1317	371	46.3	40.4	13.3	2
Llobregat	4995	2932	24.8	2435	636	19.2	57.9	22.9	11
Foix	315	281	0.8	987	381	17.3	66.6	16.1	1
Francolí	857	632	1.7	1157	457	24.8	65.6	9.5	1
BASINS OF CENTRAL MED-COAST									
Palancia	972	219	2.2	1607	662	2.4	88.2	9.4	2
Mijares	4026	1884	9.7	1998	943	4.1	89.0	6.9	2
Turia	6245	2551	11.6	1987	1016	5.8	83.4	10.8	2
Júcar	18136	7063	52.0	1826	819	9.7	77.9	12.4	2
SEGURA BASIN									
Segura	14657	4518	23.0	2031	696	14.6	75.9	9.5	5
SIERRA NEVADA BASIN									
Adra	743	148	1.8	2737	1075	60.9	37.3	1.9	2
Guadalfeo	1300	966	6.0	3435	1263	53.0	45.6	1.3	3
Genil	8198	3998	28.4	3304	708	16.6	72.8	10.6	3
Chillar	54	69	0.2	1761	748	1.9	98.1	0.0	1
BASINS OF SOUTH-WEST									
Verde	157	62	2.0	1862	665	82.4	16.8	0.8	1
Jara	58	40	0.6	772	246	0.4	73.8	25.8	1
Guadalhorce	3147	1689	13.4	1781	515	20.6	66.5	12.9	1
Guadina menor	6532	2691	14.7	3108	1089	21.3	66.4	12.3	3
Guadiaro	1416	747	20.4	1747	538	13.7	70.7	15.6	5

Appendix 2. Selected environmental variables measured at geographical, regional and local scale included in the analysis. (63 sites, spring 2003). n.s. (non significative) $p>0.05$; *$p<0.05$; **$p<0.0001$

Group of variables	**Variable**	**$\mu \pm \sigma$**	**Min - Max**	**Transformation**	**$\lambda 1$**	**p-value**
Geographical	Latitude	39.83 ± 2.3	36.10 - 42.43		0.033	*
	Longitude	-0.49 ± 2.89	- 5.63 - 23.02		0.034	*
	Latitude^2	1594.10 ± 182.22	1303.46 - 1800.52		0.033	*
	Latitude x Longitude	-12.31 ± 112.57	-203.33 - 128.31		0.034	*
	Longitude^2	2573819.82 ± 570798.59	1699001.14 - 3241885.8		0.033	*
	Latitude^3	63955.22 ± 10807.98	47059.28 - 76400.87		0.033	*
	Latitude^2 x Longitude	-240.04 ± 4384.85	-7356.92 - 5443		0.034	*
	Latitude x Longitude^2	103888696.49 ± 28308388.68	61339762.1-137561574.9		0.033	*
	Longitude^3	-20.47 ± 52.32	-178.64 - 27.68		0.041	**
Regional	Catchment area (km ^{2})	315.49 ± 801.27	2 - 4290	Log$_{10}$	0.066	**
	% Carbonate	54.25 ± 38.82	0 - 100		0.041	**
	% Evaporite	6.25 ± 10.66	0 - 36.96		0.021	n.s.
	% Siliceous	39.5 ± 42.02	0 - 100		0.045	**
	% forest & bushland	91.44 ± 10.81	50.93 - 100	Square-root arcsin	0.026	**
	% cropland	7.53 ± 10.41	0 - 48.42	Square-root arcsin	0.016	n.s.
	% pasture	0.85 ±2.18	0 - 12.51	Square-root arcsin	0.019	n.s.
	% other land uses	0.19 ±0.37	0 - 1.83	Square-root arcsin	0.046	*
Local	Alkalinity (meq L^{-1})	3.11± 1.81	0.10 - 7.08		0.039	**
	Chloride (mg L^{-1})	67.28 ± 263.03	1.23 - 1850.99	Log$_{10}$	0.021	n.s.
	Conductivity (μS cm^{-1})	674.92 ± 1359.22	15.8 - 10500	Log$_{10}$	0.040	**
	Dissolved oxygen (mg L^{-1})	10.35 ± 1.85	6.66 - 15.94	Log$_{10}$	0.032	*
	pH	8.00 ± 0.59	5.8 - 8.81		0.043	**
	Sulphates (mg L^{-1})	191.07 ± 660.62	20 - 4033.7	Log$_{10}$	0.022	n.s.
	Water temperature (ºC)	12.10 ± 4.44	4 -23	Log$_{10}$	0.041	**
	Discharge (l s^{-1})	1.50 ± 2.42	0 - 11.5	Log$_{10}$	0.043	**
	Altitude (m)	686.42 ± 461.65	12 - 1940	Log$_{10}$	0.041	**
	Stream order	1.66 ± 1.00	1 - 5	Log$_{10}$	0.052	**
	Heterogeneity elements	6.65 ± 1.99	2 - 10		0.031	*
	Embeddedness	8.41 ± 4.56	0 - 20		0.026	*
	Riffles vs .pools	8.98 ± 1.86	2 - 10		0.031	*
	Shade	7.59 ± 2.65	3 - 10		0.025	*
	Substrate habitat	14.89 ± 2.42	9 -20		0.022	n.s.

Group of variables	Variable	μ±σ	Min - Max	Transformation	λ1	p-value
	Flow and depth regimes	7.97 ± 1.59	4 - 10		0.023	*
	Temporality	0.56 ±1.48	0 - 6		0.019	n.s.
	Dry period %	27.17_29.51	0 - 97	Square-root arcsin	0.013	n.s.
	Riparian Quality	23.75 ± 2.95	10 - 25		0.011	n.s.
	Riparian Cover	21.95 ± 5.24	0 - 25		0.014	n.s.
	Riparian Structure	21.48 ± 4.24	10 - 25		0.018	n.s.
	Riparian Naturality	23.13 ± 4.24	5 -25		0.019	n.s.
	Channel width (m)	9.10 ± 7.51	1.03 - 43.33	Log_{10}	0.04	**
	Channel depth (m)	0.21± 0.15	0.02 - 0.8	Log_{10}	0.028	*

Appendix 3. Chironomid taxa collected in streams from Mediterranean region of Spain with relative abundance and number of sites where the taxa was present.

Taxa	Code	Relative abundance	Number of sites
Subfamily Podonominae			
Paraboreochlus minutissimus (Strobl, 1984)	Para min	0.019	1
Subfamily Tanypodinae			
Ablabesmyia longistyla (Fittkau, 1962)	Abla lon	0.809	13
Conchapelopia (Fittkau, 1957)	Concind	1.806	24
Krenopelopia (Fittkau, 1962)	Krenind	0.088	5
Larsia (Fittkau, 1962)	Larsind	0.487	10
Macropelopia (Thienemann, 1916)	Macrind	0.288	9
Nilotanypus dubius (Meigen, 1804)	Nilt dub	0.434	13
Paramerina (Fittkau, 1962)	Parmind	0.204	7
Procladius (Skuse, 1889)	Procind	1.245	8
Rheopelopia (Fittkau, 1962)	Rhepind	1.296	27
Thienemannimyia (Fittkau, 1957)	Thiyind	1.321	21
Trissopelopia (Kieffer, 1923)	Trisind	1.028	18
Zavrelimyia (Fittkau, 1962)	Zavyind	1.064	15
Subfamily Diamesinae			
Diamesa bertrami (Edwards, 1935)	Diam ber	0.253	1
Diamesa cf. sp. A *sensu* Schmid'93	Diam?indA	0.101	1
Diamesa hamaticornis (Kieffer, 1924)	Diam ham	0.292	7
Diamesa hamaticornis type	Diam?ham	0.145	4
Diamesa latitarsis group	Diamglati	0.012	1
Diamesa sp. A *sensu* Schmid'93	DiamindA	0.273	7
Diamesa zernyi-thienemanni group	Diamgzer	1.776	16
Potthastia gaedii group (Meigen)	Pottggae	2.883	24
Potthastia longimana (Kieffer, 1922)	Pott lon	0.076	4
Pseudodiamesa branickii (Nowicki, 1873)	Psed bra	0.019	2
Subfamily Prodiamesinae			
Prodiamesa olivacea (Meigen, 1818)	Prod oli	0.129	5
Subfamily Orthocladiinae			
? Chaetocladius	? Chae	0.05	2
? Eukiefferiella	? Euki	0.009	1
Brillia bifida (Meigen, 1830)	Bril bif	1.705	6
Brillia longifurca (Kieffer, 1921)	Bril lon	0.059	24
Cardiocladius (Kieffer, 1912)	Cardind	0.921	9
Corynoneura coronata (Edwards, 1924)	Cory cor	0.223	7

Taxa	**Code**	**Relative abundance**	**Number of sites**
Corynoneura indet.	Coryind	0.536	9
Corynoneura lacustris (Edwards, 1924)	Cory lac	0.029	1
Corynoneura lobata (Edwards, 1924)	Cory lob	1.029	15
Corynoneura scutellata group	Corygscu	0.957	11
Cricotopus (Cricotopus) indet.	Criccri	0.386	4
Cricotopus (Cricotopus) trifascia (Edwards, 1929)	Cric tri	0.213	6
Cricotopus (Isocladius) indet.	Criciso	0.021	1
Cricotopus (Isocladius) sylvestris group	Cricgsyl	0.252	8
Cricotopus (Isocladius) trifasciatus (Meigen, 1813)	Cric trd	0.023	1
Epoicocladius flavens (Malloch, 1915)	Epi fla	0.207	4
Eukiefferiella brevicalcar (Kieffer, 1915)	Euki brv	3.405	23
Eukiefferiella cf. lobifera sensu Schmid'93	Euki?lob	0.029	2
Eukiefferiella claripennis (Lundbeck, 1898)	Euki cla	0.046	3
Eukiefferiella clypeata (Kieffer, 1923)	Euki cly	0.096	3
Eukiefferiella coerulescens (Kieffer, 1926)	Euki coe	0.077	6
Eukiefferiella devonica (Edwards, 1929)	Euki dev	0.625	12
Eukiefferiella fuldensis (Lehmann, 1972)	Euki ful	0.031	3
Eukiefferiella gracei (Edwards, 1929)	Euki gra	1.164	13
Eukiefferiella ilkleyensis (Edwards, 1929)	Euki ilk	0.603	20
Eukiefferiella indet.	Eukiind	0.127	4
Eukiefferiella lobifera (Goetghebuer, 1934)	Euki?lob	0.198	1
Eukiefferiella minor-fittkaui	Euki mfi	1.193	23
Eukiefferiella similis (Goetghebuer, 1939)	Euki sim	0.04	2
Eukiefferiella tirolensis (Goetghebuer, 1938)	Euki tir	0.19	3
Heleniella ornaticollis (Edwards, 1929)	Hele orn	0.358	11
Heleniella sp.1	Heleind1	0.016	1
Heterotrissocladius marcidus (Walker, 1856)	Hete mar	0.11	3
Krenosmittia camptophleps (Edwards, 1929)	Kren cam	0.17	1
Limnophyes (Eaton, 1875)	Limnind	0.08	4
Metriocnemus fuscipes group (Meigen)	Metrgfus	0.003	1
Metriocnemus indet.	Metrind	0.019	2
Metriocnemus eurynotus group (Holmgren)	Metr obs	0.069	2
Nanocladius bicolor (Zetterstedt, 1838)	Nano bic	0.004	1
Nanocladius rectinervis (Kieffer, 1911)	Nano rec	0.035	2
Orthocladiinae indet1	sfortho1	0.01	1
Orthocladiinae indet2	sfortho2	0.037	1

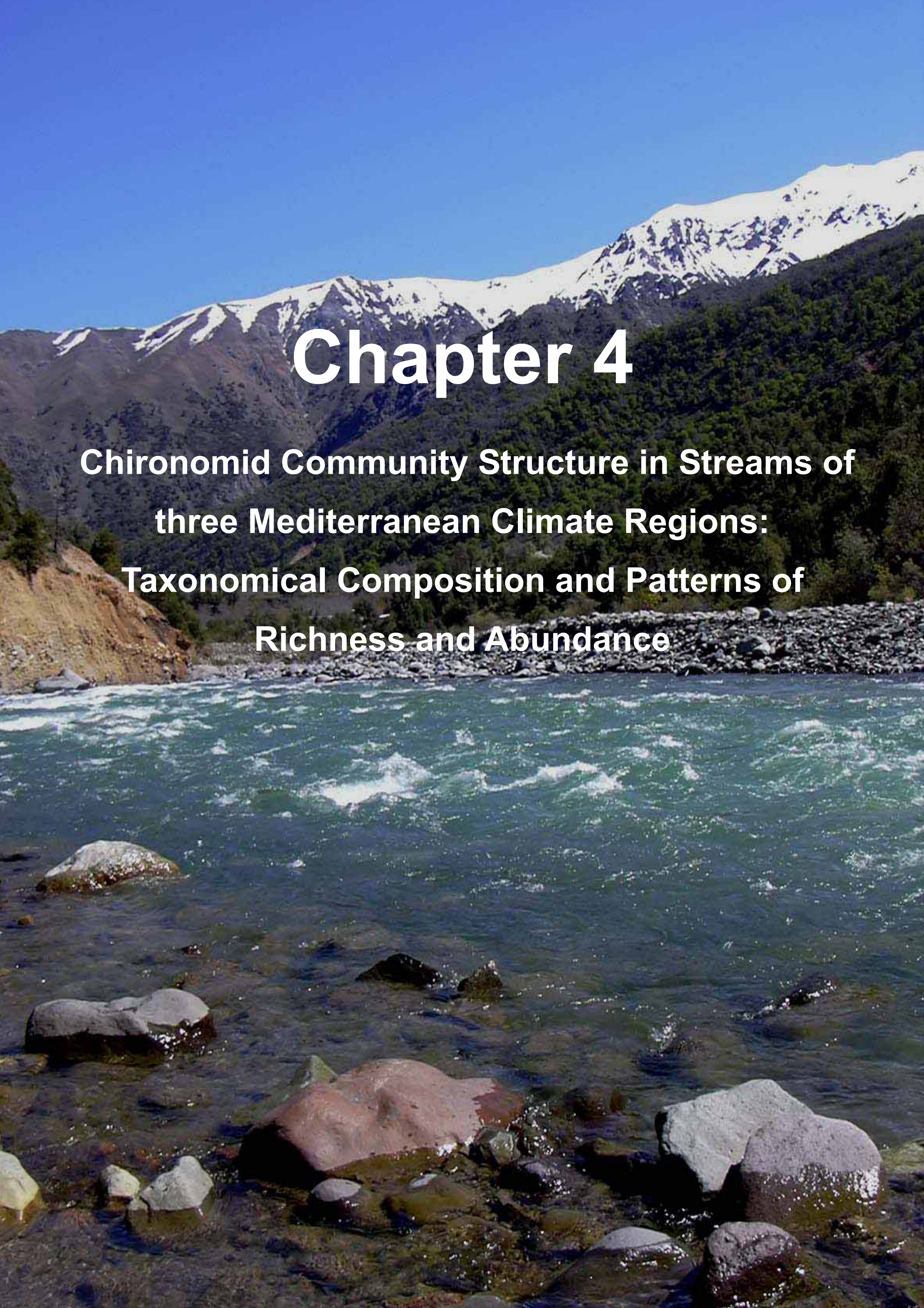
Taxa	**Code**	**Relative abundance**	**Number of sites**
Orthocladiinae indet3	sfortho3	0.01	1
Orthocladiinae unknown	sfortho	0.197	9
Orthocladius (Euorthocladius) indet.	Ortheuo	0.113	5
Orthocladius (Euorthocladius) rivulorum (Kieffer, 1909)	Orth riv	0.645	14
Orthocladius-Cricotopus	OrthCric	16.924	55
Paracladius conversus (Walker, 1856)	Parl con	0.095	4
Paracricotopus niger (Kieffer, 1913)	Parr nib	0.594	9
Parakiefferiella cf. coronata sensu Schimd'93	Park?cor	0.054	2
Parakiefferiella cf. gracillima sensu Schimd'93	Park?gra	0.075	2
Parametriocnemus stylatus (Kieffer, 1924)	Pare sty	3.672	44
Paraphaenocladius pseudirritus (Strenzke, 1950)	Parh pse	0.097	4
Paratrichocladius (Santos Abreu, 1918)	Patrind	3.449	31
Paratrissocladius excerptus (Walker, 1856)	Pats exc	0.754	15
Psectrocladius (Allopsectrocladius) obvius (Walker, 1856)	Psec obv	0.123	4
Psectrocladius (Psectrocladius) sordidellus group (Zetterstedt, 1838)	Psecgsor	1.227	2
*Pseudorthocladius (*Goetghebuer)	Pseoindet	0.124	3
Pseudosmittia holsata (Thienemann & Strenzke, 1940)	Pses hol	0.016	1
Rheocricotopus chalybeatus group	Rheo cha	2.271	26
Rheocricotopus effusus (Walker, 1856)	Rheo eff	0.552	12
Rheocricotopus fuscipes (Kieffer, 1909)	Rheo fus	2.664	20
Rheocricotopus indet.	Rheoindet	0.03	1
Smittia (Holmgren, 1869)	Smitind	0.042	2
Symposiocladius lignicola (Kieffer & Potthast, 1915)	Symp lig	0.102	2
Synorthocladius semivirens (Kieffer, 1909)	Syno sem	0.312	17
Thienemannia (Kieffer, 1909)	Thieind	0.013	2
Thienemanniella acuticornis (Kieffer, 1912)	Thil acu	0.012	1
Thienemanniella clavicornis (Kieffer, 1911)	Thil cla	0.374	8
Thienemanniella flaviforceps group	Thilgfla	0.02	1
Thienemanniella indet.	Thilindet	0.513	5
Thienemanniella majuscula (Edwards, 1924)	Thilmaj	0.018	1
Thienemanniella partita (Schlee, 1968)	Thil par	1.686	15
Thienemanniella sp.1	Thilind1	0.093	4
Thienemanniella vittata (Edwards, 1924)	Thil vitt	1.674	21
Tvetenia bavarica-calvescens	Tvet bca	5.605	44
Tvetenia discoloripes (Goetghebuer, 1940)	Tvet dis	2.967	35
Tvetenia sp.A sensu Schimd'93	TvetindA	0.136	3

Taxa	Code	Relative abundance	Number of sites
Subfamily Chironominae			
Tribe Chironomini			
Chironomus sp.2	Chirind2	0.016	1
Chironomus sp.6	Chirind6	0.509	5
Chironomus sp.7	Chirind7	0.047	1
Cryptochironomus (Kieffer, 1918)	Crypind	0.172	5
Demicryptochironomus (Lenz)	Demiind	0.114	1
Harnischia (Kieffer, 1921)	Harnind	0.099	3
Microtendipes pedellus group (Pinder, 1976)	Mictgped	0.439	13
Microtendipes rydalensis group (Pinder, 1976)	Mictgryd	0.275	7
Paracladopelma camptolabis group (Kieffer, 1913)	Pardgcam	0.138	2
Paratendipes (Kieffer, 1911)	Patdind	0.128	5
Phaenopsectra (Kieffer, 1921)	Phaeind	1.387	11
Polypedilum albicorne (Meigen, 1838)	Poly alb	0.015	1
Polypedilum pedestre group (Meigen, 1830)	Poly ped	0.171	4
Polypedilum cf. cultellatum	Poly?cul	1.029	11
Polypedilum cf. breviantenatum group *sensu* Nocentini, 1985	Poly?gbre	0.497	20
Polypedilum nubeculosum group	Polygnub	0.404	2
Polypedilum laetum group- sp.1	Polylae1	1.676	10
Polypedilum laetum group- sp.2	Polylae2	0.565	4
Saetheria (Jackson)	Saetind	0.042	2
Tribe Tanytarsini			
Cladotanytarsus (Kieffer, 1921)	Clatind	0.248	6
Micropsectra sp.1	Micrind1	1.018	10
Micropsectra sp.2	Micrind2	0.849	17
Micropsectra sp.3	Micrind3	0.003	1
Micropsectra sp.4	Micrind4	1.223	18
Micropsectra sp.5	Micrind5	0.103	4
Micropsectra sp.6	Micrind6	0.041	3
Neozavrelia (Goetghebuer, 1941)	Neozind	0.12	4
Paratanytarsus (Thienemann & Bause, 1913)	Partind	0.368	9
Rheotanytarsus (Thienemann & Bause, 1913)	Rhetind	4.644	36
Stempellina bausei group (Kieffer)	Stemgbau	0.063	1
Stempellina indet.	Stemind	0.199	3
Stempellinella (Brundin, 1947)	Stepind	1.494	11
Tanytarsus chinyensis group	Tanygchi	0.572	6

Taxa	**Code**	**Relative abundance**	**Number of sites**
Tanytarsus sp.1	Tanyind1	0.119	5
Tanytarsus sp.2	Tanyind2	0.348	7
Tanytarsus sp.3	Tanyind3	0.636	14
Tanytarsus sp.4	Tanyind4	0.087	4
Tanytarsus sp.7	Tanyind7	0.074	1
Virgatanytarsus (Pinder, 1982)	Virgind	1.412	21

Chapter 4

Chironomid Community Structure in Streams of three Mediterranean Climate Regions: Taxonomical Composition and Patterns of Richness and Abundance



Estructura de la comunitat de quironòmids en rius de tres regions mediterrànies: composició taxonòmica i patrons de riquesa i d'abundància.

Resum

Les regions mediterrànies del món són susceptibles per testar les convergències i divergències de les comunitats biològiques, ja que estan influenciades per un mateix factor a gran escala: el clima. Molts autors han hipotetitzat que els paràmetres de la comunitat hauríen de ser semblants al comparar regions climàticament similars. D'altra banda factors històrics (escala geològica) o ecològics (a escala local o regional) poden afectar de manera important la composició taxonòmica actual o bé d'altres paràmetres com la riquesa d'espècies. En el present capítol s'analitzen les similaritats intercontinentals de les comunitats de quironòmids, estudiant-ne la composició taxonòmica, els patrons de diversitat i d'abundància de les larves de quironòmids en tres regions mediterrànies del món: la conca Mediterrània, el sud-oest d'Austràlia i la regió central de Xile. A banda d'analitzar la composició global per cada punt de mostreig es compara la composició a escala de macrohàbitat. Un total de 176 taxons es van identificar al nivell taxonòmic més detallat possible (espècies, gups d'espècies o en els casos que no era possible gèneres). Com a resultat de les anàlisis de similaritat aplicades (ex. cluster i ANOSIM), s'observen diferències clares de composició taxonòmica entre les tres regions estudiades. Tot i així, les regions de l'hemisferi sud (Xile central i el sud-oest d'Austràlia) presenten una similaritat més gran entre ells que amb la conca Mediterrània, a causa principalment dels processos històrics. En general no s'observen diferències pel que fa a les comunitats de quironòmids entre les zones reòfiles respecte les lenitíques, excepte pel cas de la conca Mediterrània. Pel que fa a la riquesa taxonòmica la conca Mediterrània és la regió més rica, seguida de Xile central i el sud-oest d'Austràlia. Els factors locals que caracteritzen cada regió (per exemple l'oligotrofisme del Sud-oest d'Austràlia i la diversitat de tipologies de la conca Mediterrània) juntament amb els factors històrics (molt importants al sud-oest australià) han influenciat aquestes diferències entre regions. Després de realitzar una primera anàlisi comparativa entre les distribucions d'abundàncies d'espècies, podem dir que el sud-oest d'Austràlia s'ajusta a una distribució log-sèries mentre que les altres dues regions segueixen un patró diferent. Com a conclusió, les diferents regions mediterrànies estudiades difereixen en els paràmetres de l'estructura de la comunitat estudiats, per l'efecte important dels factors ecològics locals, combinats amb factors biogeogràfics i històrics.

Abstract

Intercontinental similarities or differences in structure of biological communities have much interest in relation to patterns and rules in community ecology. Mediterranean Climate Regions (MCRs) are ideal to test these differences because we assume similar environmental constraints due to similar climate. However, historical, regional or local environmental conditions may be more important than climate in shaping different aspects of community structure. The present study investigates taxonomical composition and patterns of abundance and species richness over three distinct MCRs of the world (South-Western Australia, Central Chile and Mediterranean Basin), of one of the most abundant and diverse family of aquatic insects: Chironomidae (Diptera). A total of 176 taxa of larvae were identified at lowest taxonomical level possible considering that Chironomidae fauna of most of these areas, especially Central Chile, is poorly known. Similarity analysis (cluster and ANOSIM) showed clear differences of taxonomical composition (subfamily and genus level) at regional scale, with higher similarities among the southern regions. On the other hand, no differences on community composition were detected at macrohabitat scale, except for Med-Basin. Rarified regional richness was higher in Med-Basin in comparison to SW-Australia (where historical events constrained the number of taxa) and Central Chile, where insular condition and limited taxonomical knowledge could affect lower value of richness. A first comparative analysis of species abundances distributions reveals that, SW-Australia fitted well to log-series distribution, where as Med-Basin and Central Chile followed a slightly different pattern. Our results contribute to improve our knowledge of Chironomidae assemblages in Mediterranean regions, obtaining different information depending on the approach selected to study community structure. Furthermore, our data provide evidence that despite convergences of Mediterranean climate, taxonomical composition and species richness are best understood considering local environmental constraints and the past historical events.

Keywords: species richness, biogeography, Mediterranean climate, species abundance distributions, rarefaction, Chironomidae

Introduction

Intercontinental comparisons using structural characteristics (e.g taxa richness, diversity and composition) have been widely used to study how environmental characteristics contribute to convergences and divergences of biological communities between climatically similar distant regions (e.g. Lamouroux *et al*., 2002; Dynesius *et al*., 2004). In such cases, it has been hypothesized that regions with similar climatic constraints should have similar community composition. However, there are deviations from this hypothetic convergence because other factors like historical events or environmental variability at local scale have greater influence over biological communities than climatic constraints at large scale (Chase, 2003). Thus, present day diversity in an area results from the balance between these current local, and regional processes and the past geological events (Ricklefs, 1987), being difficult to discern how all those factors influence current composition (Endler, 1982). Furthermore, different patterns of biotic communities may be observed depending on the studied scale (Tonn *et al*., 1990), and for this reason the assessment of intercontinental comparisons has to be done at several scales.

Several studies focused on vegetation and terrestrial arthropods among others (Di Castri, 1991; Cowling & Witkowski, 1994; Lobo & Davies, 1999), analyzed the ecological convergence in Mediterranean climate regions (MCRs), and evidenced similarities in community structure and functional characteristics. However, few of them tested convergences of aquatic stream biota (but see: Bonada *et al*. in press). Particularities of Mediterranean streams, characterized by highly variable annual and interannual discharge regimes, with predictable seasonal floods and droughts (Gasith & Resh, 1999), involve changes at multiple spatial scales that affect taxonomic composition of biological communities (Bonada *et al*. 2007b). So, MCR provide similar environmental constraints and make rivers in these geographically distant regions ideal to test intercontinental similarities between biological communities. Concerning stream macroinvertebrates, a recent work (Bonada *et al*. in press) analyzed intercontinental similarities in MCR at different scales and concluded that at regional scale differences in similarity among regions are strongly related to evolutionary history and environmental characteristics, where as at lower spatial scales (site, macrohabitat) MCRs exhibit similarities in relation to temporariness and the prevailing lotic or lentic conditions in the site. However, some macroinvertebrate families have a great diversity of genera and species, such as the Chironomidae (Diptera). This group is one of the most abundant freshwater insect family which comprises the highest number of species both in lentic and lotic habitats (up to 12000 species are estimated world wide) (Pinder, 1986) and they have been used in biogeographical studies as an example of intercontinental connections (Brundin, 1966). Given that ecological interpretations can have different meaning using higher or lower taxonomical level, thus the highest possible taxonomical resolution should be advised (Hamada *et al*., 2002).

Up to now, most studies about chironomid larval communities have been done at local or regional scale, leading to a good knowledge of stream Chironomidae fauna in several Mediterranean Regions such as Med-Basin (e.g. Casas & Vílchez-Quero, 1993; Puntí *et al*., 2007) and SW-Australia (e.g. Storey & Edward, 1989; Edward, 1989). But this is not the case of Chile where there have been few ecological studies of chironomids, and only as part of the whole macroinvertebrate community surveys (see: Arenas, 1995; Habit *et al*., 1998). Although a considerable amount of local information is sometimes available, to our knowledge no large scale studies have been conducted comparing chironomid communities' structure between different MCR using exclusively immature stages. Thus, in this paper Chironomidae taxonomic composition was compared at regional scale, considering three regions subjected to Mediterranean climate: Med-Basin, Central Chile and South-Western Australia, and using samples collected by the same methodology and operator and following the protocol used in Bonada *et al*. in press. According to several biogeographical studies of Chironomidae (Brundin, 1966; Ashe *et al.,* 1987; Saether, 2000), we hypothesized strong regional differences related to past geological events and therefore higher similarities at generic level should be expected between regions connected for a longer geological time. For instance, larger differences in composition between northern and southern hemisphere should be expected as the early breakup of Laurasia and Gondwanaland during the Cretaceous. Conversely, in the case of the southern regions studied (Chile and SW-Australia) higher similarities should be expected due to their connections during Gondwanan times. It is well known that the chironomid midges give clear evidence of transantartic relationships developed during periods when southern lands were directly connected to each other (Brundin, 1966). Moreover, taxonomical composition was compared at macrohabitat scale defined as the lotic (riffles) and lentic (pools) conditions within a reach. Several studies have reported that macroinvertebrate taxonomic composition differs between riffles and pools (e.g. Scullion *et al*., 1982; Bonada *et al*., 2006). So, we hypothesized that Chironomidae assemblages would differ also among macrohabitats and that taxonomic composition would be more similar within the same macrohabitat type for each region.

Given that similar climate constraints operate in these regions, other structural parameters of Chironomidae communities were also analyzed expecting similar responses of them. Some studies showed that similar climatic conditions should result in relatively similar regional richness (Francis & Currie, 2003). On the contrary, Bonada *et al.* (in press) analyzed convergences of macroinvertebrates communities in MCRs, and found differences in richness, being higher in northern hemisphere and South Africa in comparison to South-Western Australia and Chile. One of the explanations of these differences is the geological events that affected each MCR in the past. In the case of Chironomidae, we should expect the same pattern as for macroinvertebrates, but influenced perhaps by the higher dispersal abilities of Chironomidae (Armitage, 1995).

Finally, a first analysis of species abundance distributions (SADs) was carried out to understand mechanisms which govern the relative abundances of species in the studied regions. An SAD is a description of the abundance for each different species encountered within a community and is one of the most basic descriptors of an ecological community (Pachepsky *et al*., 2001, McGill *et al*., 2007). Species abundance patterns reflect both evolutionary and contemporary ecological processes of community formation. Variation on their patterns may be due both to influence of random fluctuations and the different processes operating at different times or in different assemblages (Tokeshi, 1999). Ecological mechanisms generating these distributions are still unclear but these models are valuable descriptors of community structures and are directly applicable in cases were sampling methods are the same.

In summary, our aim was to compare following aspects of community structure between Chironomidae assemblages of 3 Mediterranean regions over the world (Mediterranean Basin, South Western Australia and Chile): (1) taxonomical composition, (2) species richness and (3) species abundance distributions.

Methods

Study area and sampling sites

The study was carried out in streams of the Mediterranean Basin, Central Chile and South-Western Australia, sampled always in spring between 2003 and 2005. Given the seasonal variability of Mediterranean Climate Rivers, spring was the sampling period selected because is less variable in comparison to other seasons, and especially with summer when river may dry up or be reduced to pools. A total of 41 reference sites were sampled, and several characteristics of them are summarized in Appendix 1. Considering the particular conditions of Mediterranean streams, sites were selected according to 18 criteria used to establish reference conditions in Sánchez-Montoya *et al*. (2005). Natural riparian vegetation appropriate to the river type, no significant impairment by invasive species, absence of pointed and diffuse sources of pollution, no canalization or water derivations for irrigation or other purposes, no alterations of the natural discharge regimes and instream channel naturalness are several of the criteria considered.

A total of 24 reference sites in the Mediterranean Basin were sampled during spring 2003 in Catalonia region (northern-east coastal region of Spain, Figure 1). Sites belong to 7 basins: Muga, Fluvià, Ter, Tordera, Llobregat, Besòs and Francolí. Information about characteristics of this basins and typology of sites could be found in Munné & Prat (2004) and Sánchez-Montoya *et al*. (2007). Limestone and sedimentary materials are dominant in these basins, although some siliceous areas are present in the Pyrenees and Montseny ranges. Sclerophyllous and evergreen trees are the dominant vegetation *(Quercus ilex)*, although in some areas deciduous and coniferous areas are present (*Fagus sylvatica* and *Pinus uncinata)*. Different types of rivers such as siliceous headwaters, middle-reaches and also temporary streams were sampled.

In South-Western Australia, 6 sites were sampled in spring 2004 in two catchments located in south of Perth: North Dandalup and Canning River (Figure 1). Both systems arise on the Darling Range (reaching a maximum height of about 300 metres), where esclerophyllous forest formation was present dominated by jarrah (*Eucalyptus marginata*) and with shrubby undergrowth (*Dodonea* sp. and *Banksia* sp.). The streams flow over lateritic soils and granite bedrock before descending to the Swan Coastal Plain with a substratum dominated by sand (Storey & Edward, 1989). Marked seasonality is present in this area, the temperature of streams is generally warm and winter rarely fell below 10ºC. Within the study period both permanent and temporary streams were sampled. A more detailed description of this area may be found in Bunn *et al.* (1986).

In Central Chile, 11 sampling sites belonging to 4 basins were sampled in spring of 2005: 4 located in the Maule basin, 3 located in the Itata basin, 1 located in the Andaniel basin and finally 3 located in the Biobio basin (Figure 1). Most sampled sites are located in the west side of Andes ranges with a high slope with dominance of boulders and cobbles, metamorphosed sediments, igneous batholithic rocks and volcanic sand, whereas low slopes characterize few sites located in the central valley (Arenas, 1995). Mainly, sampled rivers are oligotrophic with high discharges, dominating riffles and few lentic areas (Figueroa *et al*., 2003). Riparian vegetation is constituted mainly by a native forest with high diversity of evergreen and deciduous species of trees as: *Nothofagus dombeyi, N. obliqua, Drimys winteri, Cryptocarya alba and Luma apiculata,* and several shrubs as *Chusquea quila* and *Salix chilensis* (Rodríguez *et al.,* 1983).

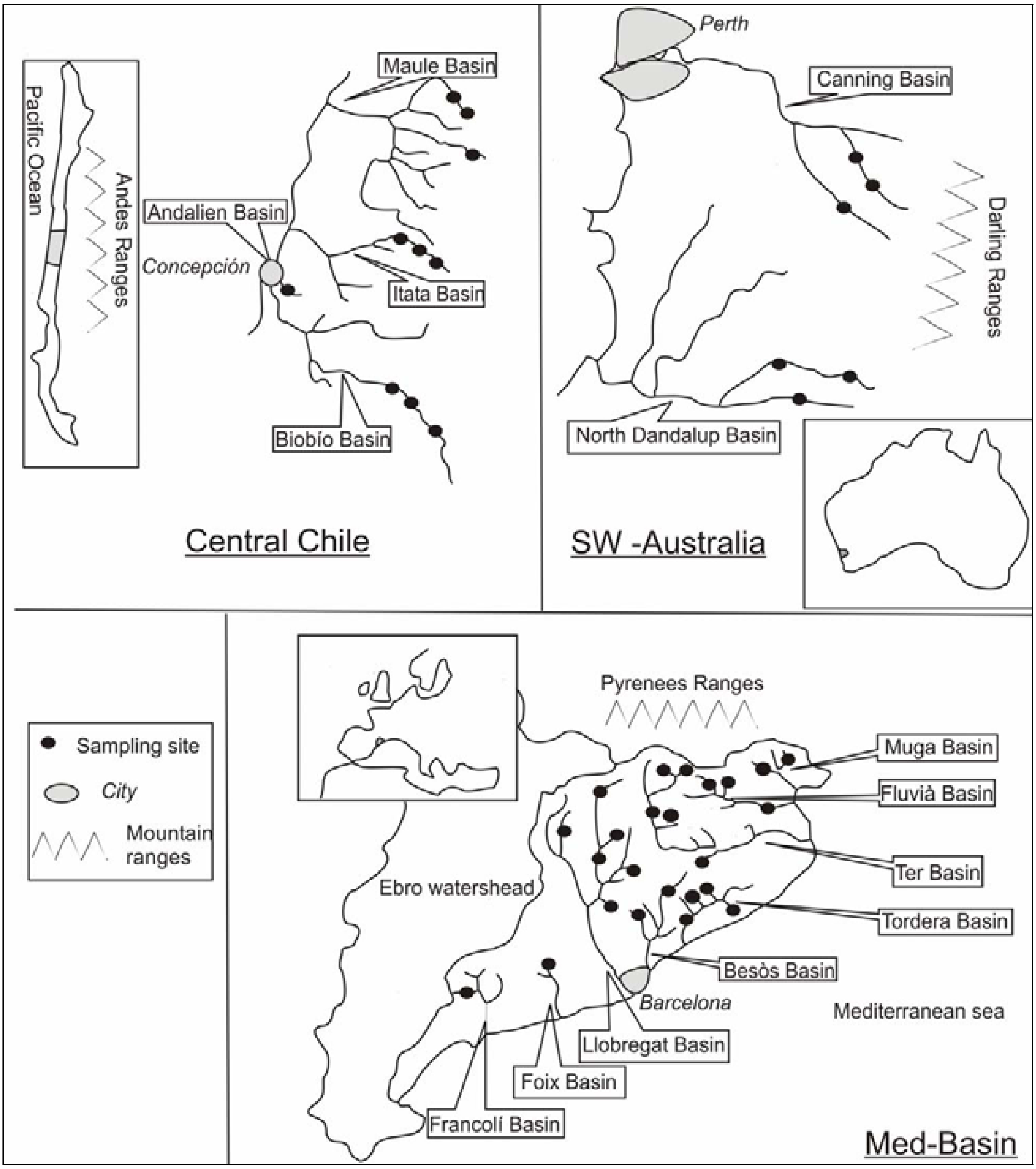


Figure 1. Location of sites for each Mediterranean Region sampled.

Sampling procedure

All reference sites were sampled using a standardized protocol developed in GUADALMED project (see: www.ecostrimed.net) (Jáimez-Cuellar *et al*., 2004; Sánchez-Montoya *et al*., 2007). Macroinvertebrate samples were collected using a circular kick-net with a mesh size of 250 µm.

Semi-quantitative samples were collected separately in two principal macrohabitats: riffles (R) and lentic (L) habitats, sampling all microhabitats present (e.g. vegetation, sand, roots). For all sampling sites we collected two samples (riffle and pool), except for two sites of Central Chile, because only riffle habitat was present. Samples were preserved in formalin 10% and sorted in the laboratory. Chironomid larvae were handsorted under a stereo-microscope, counted and determined to the lowest taxonomic level as possible (genus, species group or morphospecies whenever possible). Firstly, larvae were grouped by their similar appearance (setae present, size, instar, colour), and all (if few individuals) or a part (many individuals) were mounted on permanent slides for identification according to (Pinder, 1983). For Mediterranean basin, species description selected from the European literature was used including: Ferrarese & Rossaro (1981), Nocentini (1985), Wiederholm (1983), Schmid (1993), Rieradevall & Brooks (2001) and other miscellaneous specialised literature available for specific genus. For South-Western Australia main sources of the taxonomical identification were: Freeman (1961), Cranston (2000) and also we used previously collected voucher (V) specimens provided by D.H.D. Edward (unpublished data). For Central Chile, Brundin (1966), Trivinho-Strixino (1995), Spies & Riess (1996), Cranston & Edward (1999) and Ospina *et al.* (1999), were some of the taxonomical keys used. In few cases it was not possible to discern between some genera (e.g. Orthocladius/Cricotopus) because the great morphological similarities of 4th larval instar, made impossible to discern between several species of these genera or because larvae were in the first instars. The reasons for using larvae in this study (instead of pupal exuviae as usually is made in taxonomic works) are: 1) data obtained came from the same spatial scale and methods, 2) direct relation with habitats is possible, 3) avoid maximizing the survey of rare species or species coming from tributaries and 4) proportional abundance obtained are less dependent on specific emergence periods of the pupal exuviae.

In each site several environmental data related to water characteristics, instream habitat, riparian and basin characteristics were recorded using portable meters or applying protocols described in Pardo *et al*. (2004) (Table 1). Nutrients or other parameters related to pollution are not included because sites are in reference condition and values are always very low.

Environmental variable	Code	Description	Measurement
Conductivity	Cond	Water conductivity (µS cm $^{-1}$)	in situ; portable meter
Oxygen	Oxy	Dissolved oxygen (%)	in situ; portable meter
Temperatura	Temp	Water temperature (ºC)	in situ; portable meter
pH	pH	Water pH	in situ; portable meter
Discharge	Discharge	Water discharge (l/s)	in situ; portable meter
Embeddedness	Embed	% of embeddedness in riffles or sedimentation in pools	Pardo *et al*., 2004
Riffles vs. pools	R/L	Frequency of riffles in sampling reach: distance between riffles/stream width	Pardo *et al*., 2004
Boulders and Stones	Bou-Stones	Percentage of boulders and stones	Pardo *et al*., 2004
Pebbels and Gravels	Peb-Grav	Percentage of pebbels and gravels	Pardo *et al*., 2004
Sand	Sand	Percentage of sand	Pardo *et al*., 2004
Silt and Clay	Clay	Percentage of silt and clay	Pardo *et al*., 2004
Velocity/depth regime	Flow-depth	Number of classes present in sampling reach: slow-depth, slow-shallow, fast-depth and fast-shallow	Pardo *et al*., 2004
Shading of river bed	Shade	A score running from not shaded to completely shaded	Pardo *et al*., 2004
Heterogeneity components	Hetero	Percentage of leaf litter, presence of wood and branches, tree roots and natural dams	Pardo *et al*., 2004
Aquatic vegetation cover	Inst-veg	Types and abundance of different instream vegetation formations: % of plocon, pecton and macrophytes	Pardo *et al*., 2004
Altitude	Alti	Altitude of each site (meters a.s.l.)	Digital terrain model
Order	Order	Stream order	(Strahler method) 1:250000

Table 1. Environmental variables measured and used in the analysis.

Data analysis

To check for genus composition differences between MCRs, a clustering technique was used (flexible UPGMA) recommended by Belbin & Mcdonald (1993), using a β value of -0.6 (Van Sickle *et al*., 2006) and based on the Bray-Curtis similarity measure. For this analysis genus level of Chironomidae was used due to the large scale of this study and rare taxa were not considered because such taxa usually obscure patterns in classification analysis (Gauch, 1982). Analysis of similarities (ANOSIM) (Clarke, 1993) was used to test the differences between Med-regions. ANOSIM analysis produces an R-statistic, which contrast the similarities of sites within a MCR with the similarities of sites among MCR. The number of Monte-Carlo permutations was set at 99999. It was based on the Bray-Curtis similarities four root transformed relative abundances of genus matrix. We used the relative abundance data because present higher values of R in comparison of presence-absence matrix. Moreover, the indicator value method (IndVal) (Dufrêne & Legendre, 1997) was used to identify characteristic genus among MCRs. The indicator value (IV) of a taxon can vary from 0 to 100, the maximum value being attained when individuals of the taxon occur at all sites of one group only. The method, selects indicator species based on both high specificity for, and high fidelity to a specific group. The significance of the indicator value (IV) for each species was tested by a Monte Carlo randomisation test with 1000 permutations. Only taxa with a IV>25 (Dufrêne & Legendre, 1997) were retained.

Differences on taxonomic composition were examined using a two-way nested analysis of variance (ANOSIM) (Clarke, 1993), with MCRs and macrohabitat treated as two fixed factors. Also a single ANOSIM was performed in each region, testing differences in biological composition considering exclusively riffles versus pools communities. These analyses of similarity were performed based on Bray-Curtis similarities square root transformed relative abundances of species matrix. Data transformation was used to reduce the weight of the very abundant taxa. Finally, an IndVal analysis (now at the best available taxonomical level) was performed to determine the most representative Chironomidae taxa of macrohabitats, but only in MCR that present significative differences among R and L assemblages.

Finally, a Principal Components Analysis (PCA) was performed using environmental data recorded in each site in order to analyze environmental heterogeneity of each MCR. Cluster and IndVal analysis were performed using PC-ORD version 4.20 software (McCune & Mefford, 1999), where as ANOSIM and PCA analysis were conducted in PRIMER version 6.0. (Clarke & Warwick, 1994).

Local richness (S) is referred to the number of taxa collected per sampling site. In our case, in order to compare richness among sites, was necessary to apply the rarefaction method because different sizes of samples were found. Srar(n) is the expected species richness in a subsample of n individuals selected at random from a sample containing N individuals and S species (Hurlbert, 1971). In our case, we estimate the local richness at n=50, because is the minimum number of individuals found at one site. We calculated the rarified richness values, using the programme PRIMER version 6.0. (Clarke & Warwick, 1994). Differences between local rarefacted richness in each MCR were tested using a Kruskall-Wallis non parametric test (after finding data with non-normal distribution and non-homogeneity of variances) (Sokal & Rohlf, 1995). The Jump version 6.0. (JMP, 2005) package was used to perform this analysis.

Rarefaction technique was also needed to compare richness among different MCR, since observed species richness (S) is highly sensitive to sample-size and also to number of sites considered. Given that different number of sites were sampled among regions (see: Appendix 1) and also different size of samples (from 50 to 570 individuals) were found, richness rarefacted has estimated for each region considering both factors. In each iteration six samples were taken randomly, they were pooled and n individuals were chosen at random. Only six samples were included because is the smallest number of sites sampled in a region (SW-Australia). The number of individuals chosen, as the cut-level for inclusion in the taxon richness analysis was 790, because is the smallest number of individuals found in a region (SW-Australia). However, a small proportion of six sample combinations were smaller than n (for n>410), and these few combinations were excluded from the analysis. As a result of this calculation, an estimation of species richness was obtained for a fixed sample size and corresponding rarefaction curves for each region were obtained.

Furthermore, Species Abundances Distributions (SADs) were calculated for each MCR in order to extract information on patterns of relative species abundance without reducing this information to a single summary statistic, such as a diversity index. The graphical representation used here follows the methodology by Pueyo (2006). The expected and empirical probabilities to have a number of individuals were represented. Expected probabilities were first calculated with the assumption that they follow one of the distributions widely used in ecological studies for fitting empirical data: the log-series (Fisher, 1943) which has the form**:**

$$P(n)=\theta n^{-1} e^{\omega n} \qquad (1)$$

The parameter ω was fitted by the Maximum Likelihood Estimation (MLE)(Cachero, 1990). SADs deviating from a log-series have a slope different from 1, and in such cases the parameter β in equation 2 (Pueyo *et al.*, 2007) was also fitted by the MLE.

$$P(n)=\theta n^{-\beta} e^{\omega n} \qquad (2)$$

Equation 2 has two parts: the first is a power law and the second is an exponential function. P(n) is the probability to have a number of individuals (n), parameter β is an indicator of rarity and values close to one indicates that distribution follows a log-series, parameter ω is a measure of diversity, largely affected by sample size and parameter θ is a direct function of the other two (ω and β) and is a normalisation constant. More details on the statistics and assumptions are given in Pueyo (2006). In this work we analyze parameter β because it gives us an idea of whether empirical data is close to a log series or not, depending on whether it is close to 1. The graphical representation used here was recommended by Pueyo & Jovanni (2006), because it is more robust than the classical representation using histograms.

For all SAD calculations, the best available taxonomical resolution was used at morphospecies level. Genera whith species that could not be identified were excluded (as is the case of the complex Orthocladius/Cricotopus). In this way, we are just reducing the sample of species, while including such genera would give a biased distribution with different kinds of elements (species and genera or other taxa). For instance, if we consider genus or taxa that may include more than one species, we could make and important error underestimating the proportion of rare species.

The regional β calculated for each MCR correspond to the slope for each SAD, where as local β values were calculated from each site and the medians were obtained for each MCR. A Kruskall-Wallis non-parametric test was used to check for differences between local β values in each MCR.

Moreover, the resultant SADs were examined for goodness of fit with log series models using the chi-square and Wald-Wolfowitz non-parametric tests. The Wald-Wolfowitz or runs-test check if the relative positions of the deviations between the observed and expected values of our SADs, are distributed at random, as we would expect for the null hypothesis (logseries). This test is complementary to chi-square which considers exclusively the magnitude of the deviations between observed and expected.

The STATISTICA programme was used to perform chi-square and Wald-Wolfowitz tests (Stat Soft, 1999), where as the Jump version 6.0. (JMP, 2005) package was used to perform the Kruskall-Wallis test.

Results

Regional Composition

Chironomidae was one of the families of macroinvertebrates most important in terms of abundance for sites sampled at three MCR. They accounting for 17%, 28% and 29% at SW Australia, Med-Basin and Central Chile respectively, of the total macroinvertebrates community composition. A total of 176 taxa were identified at the highest taxonomical level as morphospecies, species groups or genus (9562 larvae examined). Frequency and occurrence of each taxon recorded for the MCR studied were given in Appendix 2. Our knowledge of Holartic genera and most species is well consolidated, also the fauna of Chironomidae larvae of Western-Australia is relatively well known thank to the papers of Edward (Edward, 1964; Edward, 1989; Storey & Edward, 1989). But is not the case for Central Chile where Chironomidae fauna is still poorly known. For instance, there were nine, four, two and one, unknown larval morphological forms of Orthocladiinae, Chironomini, Tanytarsini and Heptagyini respectively observed in Central Chile, while this number is very low for the Med-Basin and null for SW-Australia.

Subfamily	**Central Chile**	**SW-Australia**	**Mediterranean Basin**
Aphroteniinae (n=1)		0.6	
Podonominae (n=5)	10.3		0.03
Tanypodinae (n=18)	3.6	6.7	7.2
Diamesinae			
Tribe Heptagyini (n=4)	16		
Tribe Diamesini (n=5)			5.1
Prodiamesinae (n=1)			0.2
Orthocladiinae (n=89)	57	59.5	66.9
Chironominae			
Tribe Chironomini (n=27)	11	2.8	6.3
Tribe Tanytarsini (n=24)	2.2	22	14.2
Tribe Pseudochironomini (n=2)	0.1	8.1	

Table 2. Relative abundances (%) and total number of taxa (n) for Chironomidae subfamilies at each MCR

A differential composition among regions at subfamily or tribe level was found (Table 2). There was only one species of Aphroteniinae (*Aphroteniella filicornis)* from Gondwanic origin, found in SW-Australia. Moreover, *Paraboreochlus minutissimus* was the only representative of Podonominae in Med-Basin, where as four taxa of this subfamily were found in Central Chile, being one of the subfamilies most abundant in this region with *Podonomopsis* and *Podonomus* as main taxa recorded there. The contribution of Tanypodinae was low in terms of number of taxa (18) and only accounting for 3.5-7.2% of relative abundances. Four species belonged to the tribe Heptagyini, which was found exclusively in Central Chile, being *Limaya* sp. and *Paraheptagyia* sp.1 the most frequent taxa of them. On the other hand, the tribe Diamesini was found exclusively in Med-Basin, being *Diamesa zernyi-thienemanni* group and *Potthastia gaedii* the two more abundant taxa of this group. The Orthocladiinae was the most taxa rich subfamily (89 taxa) and also was the numerically dominant group in all regions accounting for 57-67% of the relative abundances for all sites. For instance, *Orthocladius-Cricotopus* is the most abundant taxa found in Med-Basin and Central Chile. Also, *Tvetenia bavarica-calvescens, Botryocladius* and *Orthocladiinae V31* are others of the most abundant and frequent Orthocladiinae taxa from Med-basin, Central Chile and SW-Australia respectively. Chironomini had 27 taxa, with higher representation in Central Chile (11%) than the other two regions, where as Tanytarsini had 24 taxa in three regions, but in this case their representation is more important in Western Australia (22%) than the other regions. Finally, two taxa of

Pseudochironomini tribe were recorded, one in Central Chile (*Pseudochironomus*) and other in Western Australia *(Riethia*).

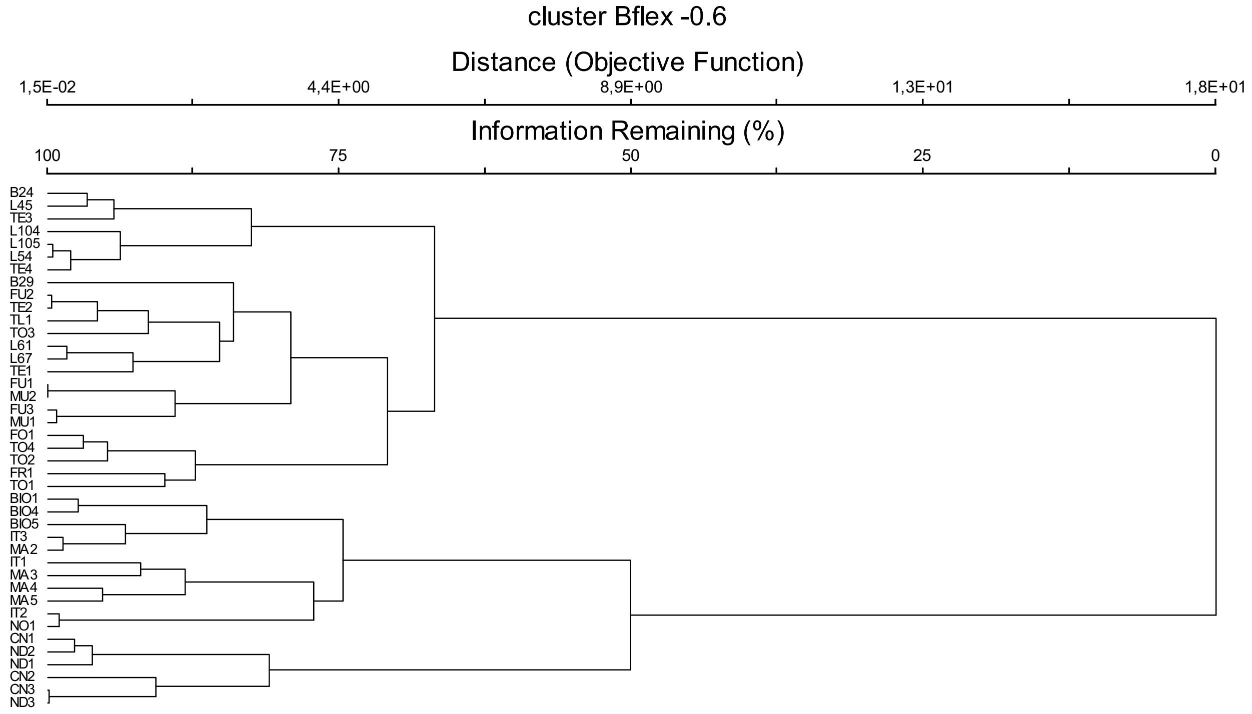


Figure 2. Cluster of 3 Med-regions sampled using relative abundance data, Bray-Curtis similarity index, B-flex=-0.6 group linkage method.

Chironomidae community composition at genus level showed higher similarity between the southern regions studied: SW-Australia and Central Chile in contrast to Mediterranean Basin (Figure 2). The SW-Australia community was closer to Central Chile with 50% Bray-Curtis similarity. Sites of SW-Australia showed a similarity of 80% between them, and sites of central Chile 75% similarity. Finally Mediterranean basin had low similarity among all sites (66%). As we expected, ANOSIM analysis evidences that 3 regions were clearly different among each-other with a global R near 1 (0.956) (P=0.01). This result indicates that similarities between sites within a MCR are higher than those between sites from different MCRs. Thus, regional composition at genus level was significantly different between MCRs.

Each MCR was clearly differentiated according to IndVal results (Table 3), showing characteristic genus for each region with IV>25. Mediterranean Basin had the highest number of indicator taxa (21), mainly Orthocladiinae, such as *Tvetenia, Eukiefferiella* or *Rheocricotopus*. On the other hand, *Cricotopus, Tanytarsus* and *Paramerina*, were some of the 16 most characteristic genus of SW-Australia. In this region Chironomini are more common taxa, due to they are more associated to temporary reaches. Moreover, *Podonomus, Heptagyini unknown sp.1* and *?Botryocladius* are some of the main taxa characteristic to streams of Chilean region.

Mediterranean Basin		**SW Australia**		**Central Chile**	
Taxa	**IV**	**Taxa**	**IV**	**Taxa**	**IV**
Tvetenia	100.0*	*Cricotopus*	100.0*	*Podonomus*	72.2*
Eukiefferiella	87.5*	*Tanytarsus*	87.6*	Heptagyini unknown sp.1	63.6*
Rheocricotopus	83.3*	*Paramerina*	60.3*	?*Botryocladius*	63.6*
Parametriocnemus	70.8*	*Harrissius*	50.0*	*Limaya*	63.6*
Brillia	58.3*	*Riethia*	50.0*	*Orthocladius-Cricotopus*	63.6*
Corynoneura	54.8*	Orthocladiinae?V59	50.0*	*Larsia*	59.3*
Micropsectra	54.2*	*Stictocladius*	50.0*	*Microtendipes*	56.6*
Rheopelopia	54.2*	*Botryocladius*	37.6*	*Phaenopsectra*	51.9*
Thienemannimyia	50.0*	?ChironominiV78	33.3*	*Paraheptagyia*	45.5*
Polypedilum	45.9	?TanytarsiniV13	33.3*	*Podonomopsis*	45.5*
Conchapelopia	45.8*	?*Paratendipes*	33.3*	*Gymnometriocnemus*	29.9*
Synorthocladius	41.7*	OrthocladiinaeV43	33.3*	Chironomini unknown sp.1	27.3*
Zavrelimyia	41.7*	Tanypodinae V20	33.3*	Orthocladiinae unknownxil1	27.3*
Euorthocladius	37.5*	*Cladotanytarsus*	29.8*	*Parochlus*	27.3
Paratrichocladius	37.5*	*Stempellina*	27.9*		
Potthastia	37.5*	*Nanocladius*	27.7*		
Trissopelopia	33.3				
Diamesa	29.2				
Stempellinella	29.2				
Virgatanytarsus	29.2				
Paratanytarsus	25.0				
Paratrissocladius	25.0				

Table 3. Results of IndVal analysis for each MCR, only genus with an indicator value higher or equal to 25(IV) are presented. * p<0.05 by MonteCarlo permutation test (9999 runs).

Local factors

Results of two-way nested ANOSIM test showed significant differences in species composition between MCR across riffles and pools groups (R=0.829; p=0.01). However, testing differences between riffles and pools across all MCR, no differences were found (R=0.094; p=0.4). Moreover, one-way ANOSIM results for each region separately showed significative differences between riffles and pools in Med-Basin (R= 0.104; p=0.04), where as Chironomidae composition was not significantly different comparing macrohabitat from Central Chile (R=0.098; p=10.3) and SW-Australia (R=-0.113; p=81.4). Even tough significative differences detected among macrohabitats in Med-Basin, the low R value obtained indicates an important overlapping among biological communities of these groups. Despite of this, IndVal analysis was performed in Mediterranean Basin (Table 4). In riffles, mainly Orthocladiinae were found as indicator taxa (e.g. *T. bavarica-calvescens* and *Brillia bifida*) and also Diamesinae (*D. zernyi-thienemanni* group). In general those taxa are typical from headwater systems with low temperatures, and associated to fast-flowing systems. On the other hand, in lentic habitats, several taxa associated to these systems were found such as some Orthocladiinae (e.g. *Corynoneura scutellata* group and *Thienemanniella vittata*) together with Tanypodinae (e.g. *Zavrelimyia* spp. or *Thiennemanimyia* spp.*).*

Mediterranean Basin			
R macrohabitats		**L macrohabitats**	
Taxa	**IV**	**Taxa**	**IV**
Tvetenia bavarica-calvescens	64*	*Thienemannimyia* spp.	40*
Tvetenia discoloripes	45.7*	*Zavrelimyia* spp.	36.6*
Orthocladius-Cricotopus	41.7	*Rheocricotopus fuscipes*	28.8
Rheotanytarsus spp.	34.5	*Thienemanniella vittata*	27.9
Eukiefferiella minor-fittkaui	32.1*	*Trissopelopia* spp.	27.7*
Diamesa zernyi-thienemanni group	27.2	*Corynoneura scutellata group*	25.9
Rheopelopia spp.	24.8		
Brillia bifida	32		
Parametriocnemus stylatus	30		

Table 4. Results of IndVal analysis for R (riffles) and L (pools) in Mediterrean Basin. Only taxa with an indicator value higher or equal to 25 (IV) are presented. * p<0.05 by MonteCarlo permutation test (9999 runs).

Furthermore, PCA analysis showed that MCRs were clearly different in environmental characteristics (Figure 3). South-Western Australia differed from the others having a particular composition of substrate (with dominance of sand, clay, pebbles and gravels) and abundance of instream vegetation and heterogeneity elements (like tree roots). On the other hand, Central Chile was being associated to boulders, riffles and higher values of discharge, altitude and variety of flow-depth regimes. In addition, Mediterranean Basin had higher environmental heterogeneity in comparison with the other two regions. Overall, this MCR was associated to higher conductivity, pH and temperature, but there were sites more similar to Central Chile, with higher altitudes and dominance of boulders, and other sites with higher conductivities and lower discharges close to the condition of temporary streams.

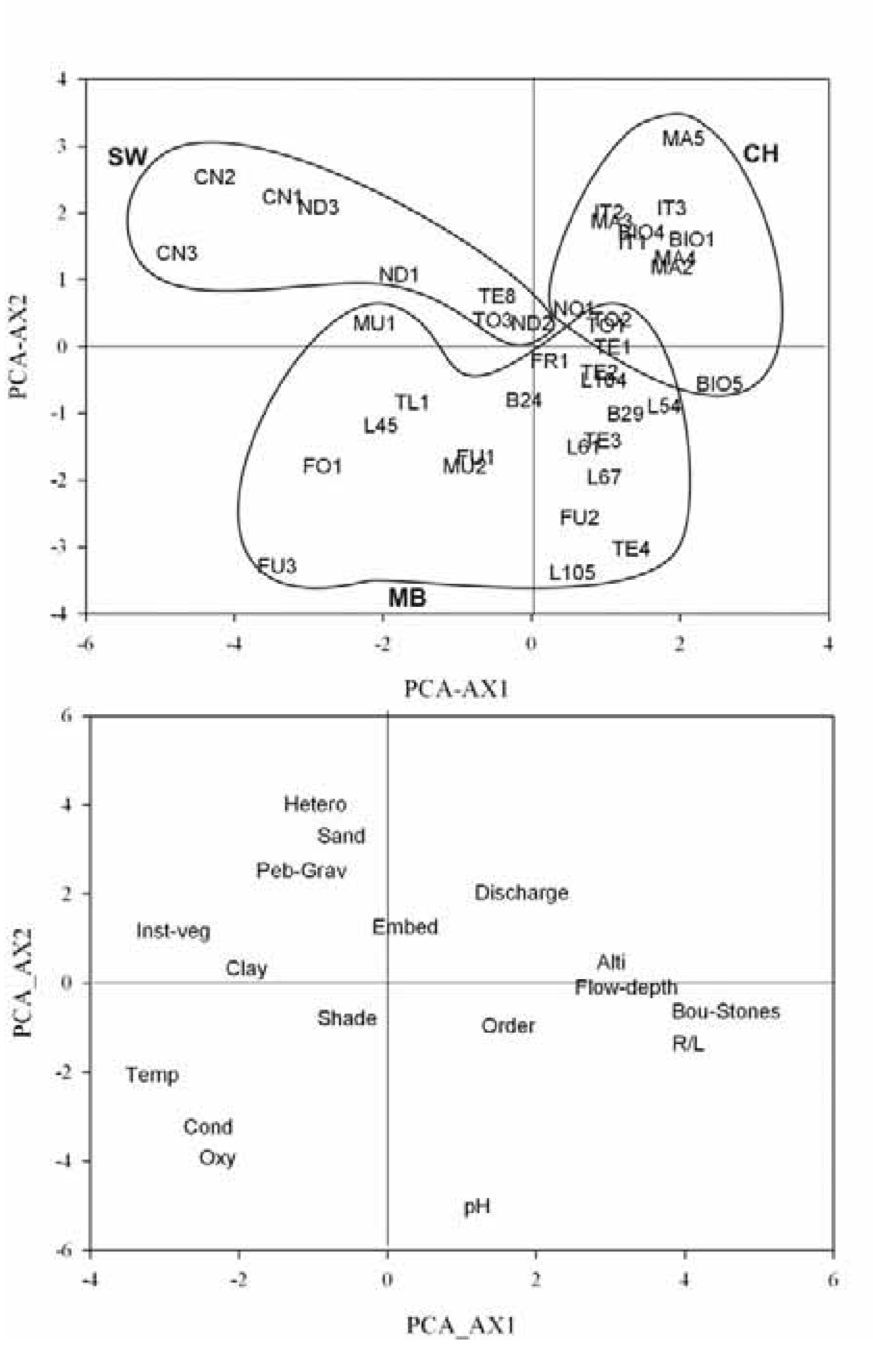


Figure 3. Results of the Principal Components Analysis (PCA) using environmental data recorded in each MCR. First and second axis explains 21.1% and 16.6% of the variance respectively. a) Sampling sites belonging to SW=South-Western Australia; MB=Mediterranean Basin and CH=Central Chile; b) position of environmental variables for first two axes, their meaning is provided in Table 1.

Species richness

Rarefacted local richness (Srar $_{n=50}$) was significantly different among regions (χ^2=13.5; p=0.001**). There were significant differences between Central Chile and Med-Basin (χ^2=10.22; p=0.001**) and also between SW-Australia and Med-Basin (χ^2=5.93; p=0.01*), but not for Central Chile and SW-Australia (χ^2=0.01; p=0.9). Overall, SW-Australia (10.14 ± 3.12) and

Central Chile (10.36 ± 2.63) had lower values of local richness in comparison to Med-Basin (13.91±2.56) (Figure 4).

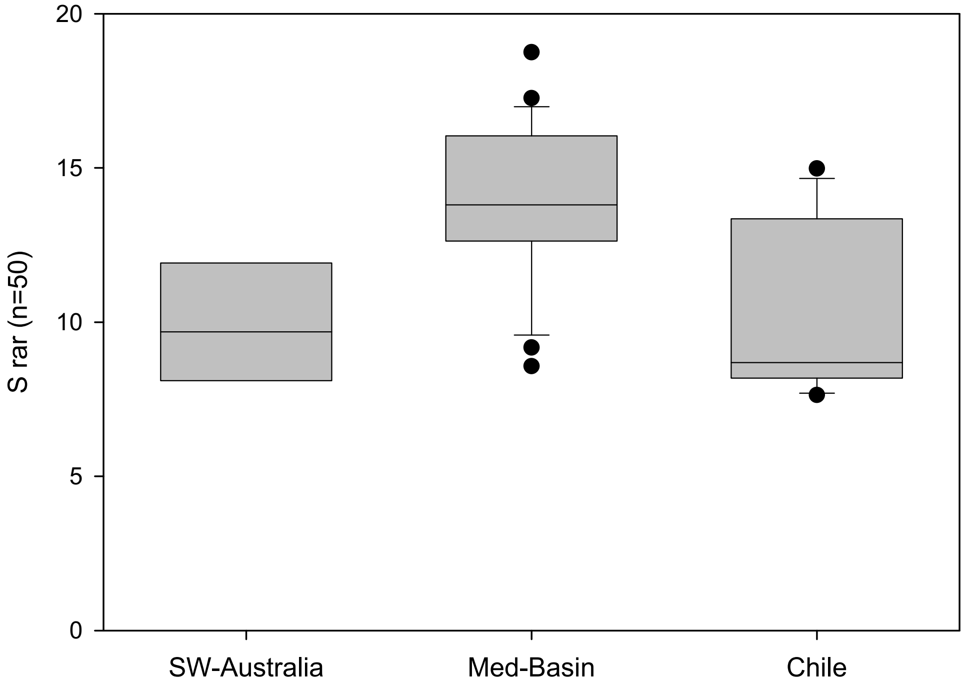


Figure 4. Box-plot of richness rarefacted between Med-regions at n=50.

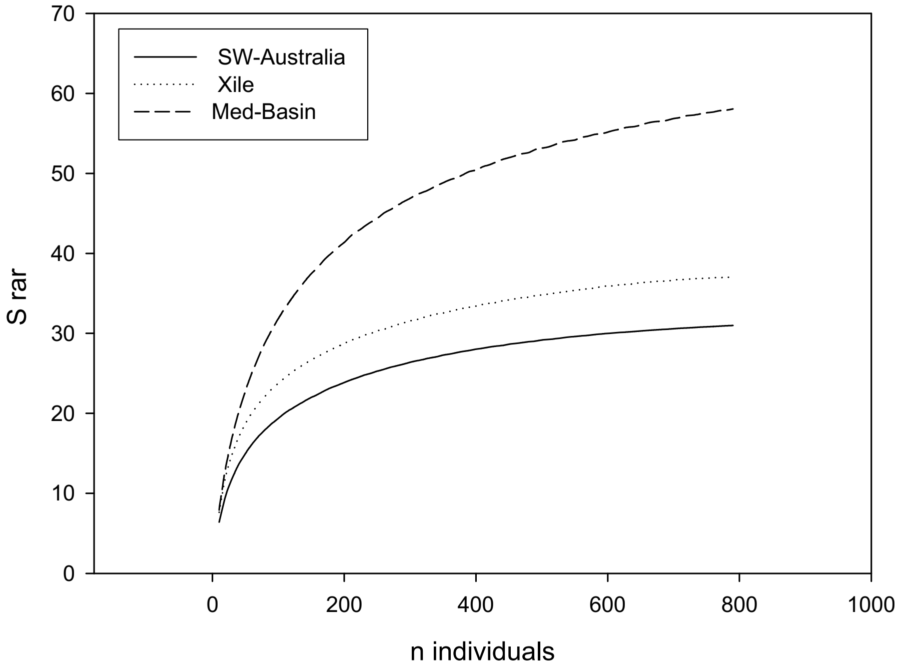


Figure 5. Rarefaction curves for Chironomidae species at three Mediterranean regions.

Species richness of Med-Basin increased faster than Central Chile and SW-Australia indicating differences between MCRs (Figure 5). For the maximum sample size calculated, Med-basin arrived at 58 taxa, where as SW-Australia and Central Chile had lower values of rarefacted richness, 31 and 37 respectively. According to those results we conclude that Med-Basin region was clearly richer than Central Chile and SW-Australia.

Species Abundance Distributions

Expected and empirical probabilities to have a number of individuals for each MCR was shown at figure 6. Differences between β at regional level were observed among regions. SW-Australia data had a slope near 1 (β=0.92), being the regional data that fit better with log-series distribution; where as Central Chile (β=0.69) and Med-basin (β=0.76) had lower values of β, suggesting a relative deviation from log-series. Distributions with β values different from 1 indicated a lower proportion of rare species, as is the case of Central Chile and Med-Basin.

	LOCAL β	
	Chi-square	**p-value**
Global (3 regions)	5.26	0.07
Med-Basin/SW-Australia	4.97	0.025*
Med-Basin/Central Chile	0.045	0.83
Central Chile/SW-Australia	3.04	0.05*

Table 5. Kruskal-Wallis test of local β among regions.

Moreover, median of local β values for each MCR, follow the same pattern as regional ones. As a result, we have obtained lower local β for Med-Basin (β=0.67) and for Central Chile (β=0.71), where as β near 1 for SW-Australia (β=1.07). Differences between β at local scale were tested (Table 5), and only we have found that SW-Australia had significant differences with the other two MCR.

Furthermore, log-series distributions were tested. First, test for goodness of fit revealed that we can't reject the hypothesis that our empirical SADs follow log-series distributions in any case: SW-Australia (χ^2=2.58; p=0.28), Central Chile (χ^2 =4.45; p=0.35) and Med-Basin (χ^2=10.34; p=0.11). According to results of Wald-Wolfowitz test**,** communities of SW-Australia (Z=1.03; p=0.3) and Central Chile (Z=1.55; p=0.12) were not significantly different from log-series model, analyzing their randomness pattern. However, for Med-Basin (Z=3.21; p=0.001) significant differences were found; this indicates a clear deviation from log-series and also that this data follows a different pattern of species abundance.

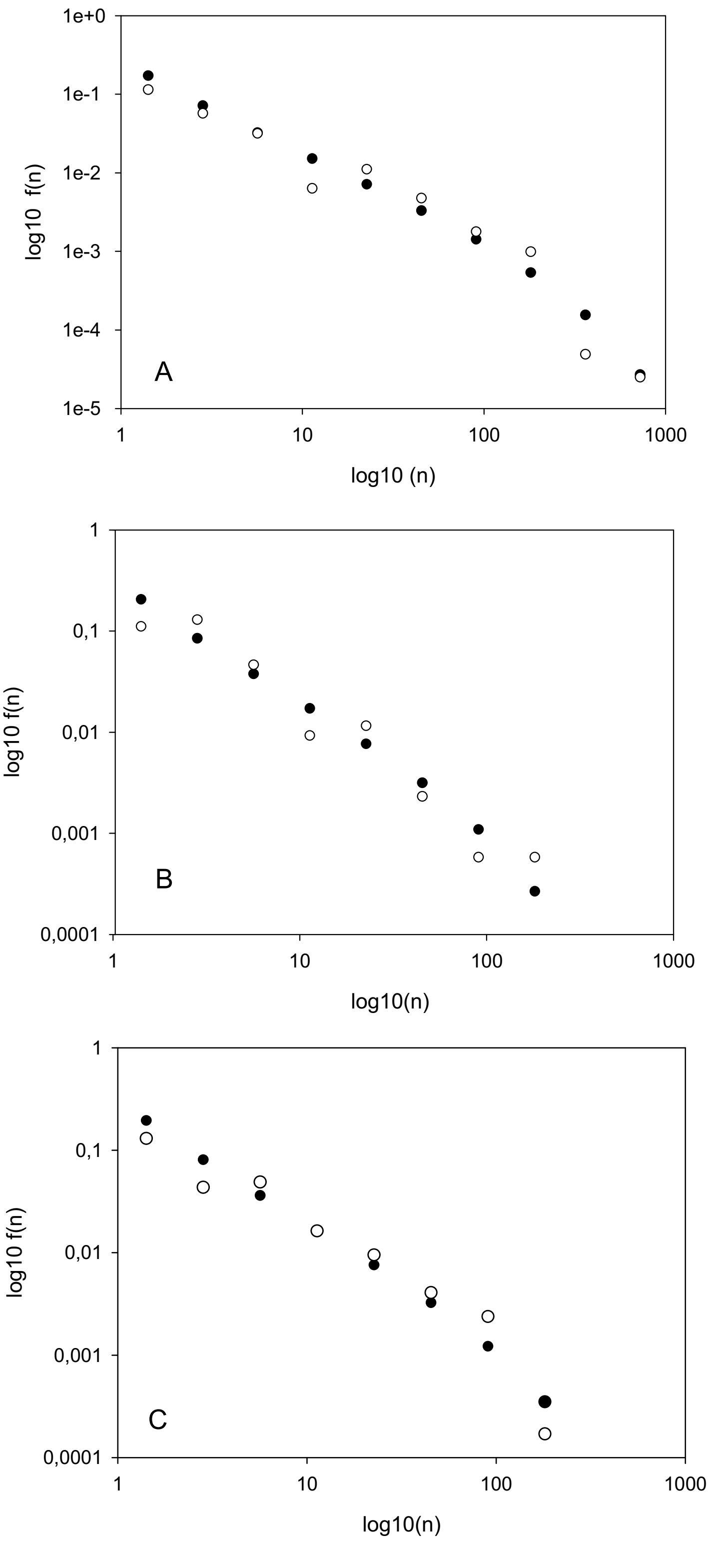


Figure 6. Species Abundances Distributions (SADs) for three Mediterranean regions studied, (A) Mediterranean Basin, B) SW-Australia and C) Central Chile). (Black circles: expected distribution log-series; empty cercles: empirical data).

Discussion

A rigorous taxonomic identification (to the lowest possible level) has been used to realize a good comparison among three MCR. Total pool of Chironomidae taxa recorded in this work (176) was remarkable, representing a gain in precision and information in responses of larvae in Mediterranean streams. In community ecology it is often difficult to discern between ecological and historical factors that explain current regional composition of any organism group (Endler, 1982), and both factors should be considered to analyze the current large-scale distribution of Chironomidae. As expected, regional taxonomic composition reflected past geological events in agreement with studies performed using macroinvertebrates at family level (Bonada *et al.*, in press). Also, our results were in concordance with Ashe *et al*. (1987), which analyzed the global distribution of Chironomidae subfamilies and genera among all zoogeographical regions of the world. Thus, the reason why Central Chile and SW-Australia showed higher similarities of composition in comparison to Med-Basin, is explained because connections between the Neotropical and Austral areas were possible until the late Cretaceous (Brundin, 1965). For instance, presence of genera *Aproteniella,* and the orthoclads *Stictocladius* and *Botryocladius,* provide a clear evidence of Gondwanan connections of south-western Australia, South-America and New-Zealand (Edward, 1989; Cranston & Edward, 1992; Cranston & Edward, 1999). We expected to find three of the subfamilies of Chironomidae: Podonominae, Aphroteniinae and Diamesinae (tribe Heptagyini), in both southern regions sampled, because they reflect a clear evidence of austral relationships (Cranston & Edward, 1992). But, Podonominae and Diamesinae were absent of SW-Australia, which could be attributed to the Mediterranean characteristics of the sampled rivers, with the frequent seasonal drought and high summer temperatures, which prevent the presence of the cold-stenothermic species of those subfamilies. In SW-Australia, relatively recent climate constraints seem to be more important than old geological events. In contrast, Podonominae play a prominent role in Central-Chile with 10% of the total abundance, although our results are not so high than Brundin (1966) states (38% for southern Andes flowing waters), which may be also an indicator of the effects of Mediterranean climate in the streams of the particular chilean region sampled. South American Podonominae fauna is concentrated to non Mediterranean climate mountain streams of South Chile and Patagonia, where this subfamily is relatively more abundant than in any other area of the world (Ashe *et al.*, 1987)**.** In the case of Aphroteniinae, they are recorded in mountain streams in the southern temperate zone (Ashe *et al*. 1987), adapted to warmer water streams, being more warm tolerant than most Podonominae species (Brundin, 1966). In our study they were recorded exclusively in SW-Australia stressing again the importance of actual climate constraints in the rivers of this region. SW-Australia is a clearly defined and distinctive region with freshwater groups of invertebrates composed predominantly of southern elements with Gondwanic distributions (Austin *et al.,* 2004), and chironomid taxa recorded here are one example of it.

Diamesinae have a widespread distribution through the Paleartic region but with a narrow ecological niche, mainly cold-stenothermal species, and is a very pertinent group for zoogeographic and ecological research (Rossaro, 1995). For instance in Med-basin region, Diamesinae of tribe Diamesinii was a group very abundant in headwater-systems with developing strategies for cold environments. In contrast, in Central Chile most austral Diamesinae belong to the tribe Heptagyini, and these large-sized midges play a prominent role in the cool mountain streams of most southern lands. This is the case of Chilean streams sampled when they represented a 16% of the total composition. In this case, we assist to a clear case of vicariance in two far distant areas. Overall, predominance of Orthocladiinae obtained in three regions sampled agrees with the general pattern reported previously in lotic ecosystems from the northern hemisphere (Coffman, 1973) and also from southern rivers of South-America (Reiss, 1977).

In summary, both large temporal scales (geological events) and regional environmental features are shaping the chironomid communities of the three areas with more ore less importance, but also current local environmental conditions may limit the presence of some taxa (Lobo & Davies, 1999). We know that in Mediterranean streams, seasonal variability affects deeply local environmental conditions (e.g. stream velocity) and this fact influence greatly the composition of macroinvertebrates communities at macrohabitat scale (Bonada *et al*., 2006; Bonada *et al*., in press). Accordingly, we hypothesized that Chironomidae composition should differ between macrohabitat, but only in Mediterranean-Basin region differences between riffles and pools were found. As we have seen in PCA performed, three regions are separated in function of different environmental characteristics, different substrate composition and different current velocity conditions, and this may be the reason of lacks of differences in taxonomic composition between macrohabitats in Central Chile and SW-Australia.

If local, regional and large temporal facts affect communities, should we expect similar richness in all three regions? Species richness represents the most basic measure of coexisting species and is widely used to estimate diversity of a system (Tokeshi, 1999). In this study we have used solely rarified local richness as an estimator of local diversity, because other indices (e.g. Shannon-Wiener or Simpson) are highly dependent of sample size (Pueyo, 2003). Accordingly, richness values obtained here are fully comparable after to rarify number of individuals. Our results are in agreement with macroinvertebrate patterns found by Bonada *et al*. (in press) being Med-Basin richer than Central Chile and SW-Australia for the same number of individuals. Probable reasons for the rarefacted richness to be lower in Central Chile are the insular condition of this region, located between the Andes, Atacama Desert and Pacific Ocean (Brundin, 1966), but also the limited knowledge of immature stages present in this region (Figueroa *et al*., 2007). Thus, our results must be regarded with caution because richness could be underestimated at certain degree since most of the taxa recorded could only be identified as morphospecies.

On the other hand, reasons of the poorer species richness observed in SW-Australia were well documented (Bunn & Davies, 1990; DeDeckker, 1986). For instance, ancestral species were widely spread across Australia during humid climates and they became isolated in Western Australia during a later arid phase. As a consequence, the fauna remained isolated and speciated in response to hydrological oscillations. Moreover, low productivity present in these systems, due to the low availability of algal resources, presence of lateritic soils and allocthonous inputs of energy of poor quality, influences the lower richness found. In an earlier study, Bunn & Davies (1990) found a reduction in number of Chironomidae species and also the absence of entire families of macroinvertebrates in comparison to SE Australia due to the reasons explained before.

Furthermore, we have evidenced the high environmental heterogeneity of the Mediterranean Basin in comparison to the other two regions. This high variability of environmental conditions (e.g. headwaters with nival influence, karsts and temporary streams); together with several historical events could influence the higher richness present in this region (Conacher & Sala, 1998). Our results agree with previous evidence of a relationship of Chironomidae richness and ecological heterogeneity (including substrate, habitat type, biotic interactions and also regional factors), as Coffman (1989) states, where “the maximally heterogeneous streams for the greatest number of variables are likely to be associated with the greatest species richness”.

Substantial differences appear in species richness, but other factors not considered (e.g. competition …) that could influence also community structure of Chironomidae has been explored using SADs. Several models have been proposed to describe SADs (McGill, 2007) and in our study we have applied a robust methodology proposed recently by Pueyo (2006). For instance, graphical representation used here has more advantages than other conventional statistical approaches (e.g. rank abundance diagram) and is statistically sound (Pueyo & Jovanni, 2006). Log-series is widely used in ecological studies to fit empirical data and corresponds to random abundances because of either demographic noise or species heterogeneity (Pueyo *et al*., 2007). Accordingly, we notice that SW-Australian chironomids had a SAD that fitted well with log-series with β close to one, as Pueyo (2006) found for Mediterranean marine diatoms. This indicates that Australian chironomids had a random assemblage with no evidence of a dominant factor. On the other hand, the observed pattern of distribution in Med-Basin did not adjust to log-series, because significant differences were found. In this case proportion of rare species was lower than was expected by chance. Our results suggest that for Central-Chile the resulting abundance distribution did not fit to a log-series because beta differs from one, but we can not guarantee it with certainty because differences found were not significant. In addition, same pattern of β at local (community) and regional (metacommunity) was observed. These results suggest that there were some mechanism that regulates communities of Med-Basin and Central Chile in a different way than SW-Australia, for both observed scales (local and regional). This is in contrast to the neutral theory, which expects systematically a logseries at large scale and a lower proportion of rare species at smaller scales (Hubbell, 2001). Although rarity is an intricate concept (Kunin & Gaston, 1993), it is possible to relate this low proportion of rare species to different rate of colonization (common versus rare species) or higher frequency of perturbations. Accordingly, we suggest that due to frequently perturbations (e.g. floods) only few species are able to recolonize streams during the short periods between disturbances events, and these systems have a dominance of r-selected species (Pianka, 1970). But, for now it may be best to assume our lack of knowledge regarding mechanisms that affects distribution patterns and corresponding values of β, and additional studies should be necessary to validate distributions obtained here.

Present work contributes to improve our understanding of chironomid diversity and assemblage structure in Mediterranean Regions. But, further studies are required to improve taxonomy of immature stages almost in Central Chile, as is recommended also by (García & Suárez, 2007; Roque *et al*., 2007). Moreover, it would be highly proposed long-term studies, before understanding the real meaning of these distributions, due to importance of seasonal changes of fauna in Mediterranean streams (Bunn *et al*., 1986; Gasith & Resh, 1999).

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Appendix 1. Location and general characteristics of sampled sites in 3 Mediterranean regions (for units see Table 1).

Mediterranean region	Sampling site	Basin	Stream	X_UTM	Y_UTM	Altitude	Conductivity
SW-Australia	ND1	North Dandalup	Wilson	410325	6399416	225	239
	ND2	North Dandalup	North Dandalup	413058	6400025	225	230
	ND3	North Dandalup	Cronin	413008	6402858	250	205
	CN1	Canning	Canning	421039	6433657	225	394
	CN2	Canning	Poison Gully	424113	6442611	225	386
	CN3	Canning	Death Adder	422948	6444142	250	484
Central Chile	BIO1	Biobio	Chaquilvin	300219	5785687	878	39
	BIO4	Biobio	Pangue	269919	5802468	897	51
	BIO5	Biobio	Queuco	264426	5809749	347	73
	MA2	Maule	Colorado	324307	6034421	749	62
	MA3	Maule	Lircay	307412	6061999	720	20
	MA4	Maule	Claro	319368	6072216	1084	28
	MA5	Maule	Claro	315616	6074105	1056	34
	NO1	Andaniel	Longuen	679106	5917663	72	65
	IT3	Itata	Ñuble	264896	5955663	370	43
	IT2	Itata	Ñuble	272152	5950809	470	18
	IT1	Itata	Ñuble	279123	5946594	534	14
Med-Basin	B24	Besòs	Riera de Caldes	924550	4628550	601	741
	B29	Besòs	Riera d'Avencó	941550	4642550	809	46
	FO1	Foix	Torrent de l'Albereda	882550	4595550	488	2120
	FR1	Francolí	Brugent	846550	4581550	531	618
	FU1	Fluvià	Riera de Beget	953550	4698550	474	378
	FU2	Fluvià	Llierca	961550	4695550	338	532
	FU3	Fluvià	Fluvià	985550	4686550	61	1034
	L104	Llobregat	Aigua d'Ora	885550	4667550	696	497
	L105	Llobregat	Riera de Postius	923550	4651550	674	929
	L45	Llobregat	Riera de Mura	915550	4627550	586	550
	L54	Llobregat	Llobregat	903550	4687550	875	373
	L61	Llobregat	Riera de Merlès	913550	4664550	540	528
	L67	Llobregat	Llobregat	905550	4650550	323	584
	MU1	Muga	Orlina	995550	4714550	204	187
	MU2	Muga	Riera d'Anyet	989550	4714550	314	131
	TE1	Ter	Ter	936550	4707550	1517	60
	TE2	Ter	Ritort	943550	4706550	1271	146
	TE3	Ter	Ges	939550	4677550	1001	409
	TE4	Ter	Ter	930550	4677550	617	258
	TE8	Ter	Riera Major	947550	4645550	840	110
	TL1	Calonge	Torrent de Calonge	998550	4652550	157	348
	TO1	Tordera	Tordera	947550	4638550	660	66
	TO2	Tordera	Riera de Gualba	953550	4639550	1264	29
	TO3	Tordera	Fuirosos	964550	4629550	237	172

Appendix 2. Frequency (%) and occurrence (number of sites) of taxa found for 3 regions sampled.

	SW-Australia		**Meditteranean basin**		**Central Chile**	
Taxa	%	Sites	%	Sites	%	Sites
Subfamily Aphroteniinae						
Aphroteniella filicornis (Brundin, 1966)	0.63	1	0.00	0	0.00	0
Subfamily Podonominae						
Paraboreochlus minutissimus (Strobl, 1984)	0.00	0	0.03	1	0.00	0
Parochlus	0.00	0	0.00	0	0.19	3
Podonomopsis	0.00	0	0.00	0	6.85	5
Podonomus	0.00	0	0.00	0	2.92	8
Podonominae unknown sp.1	0.00	0	0.00	0	0.24	2
Subfamily Tanypodinae						
Ablabesmyia longistyla (Fittkau, 1962)	0.00	0	0.30	5	0.00	0
Conchapelopia	0.00	0	1.54	11	0.00	0
Djalmabatista	0.00	0	0.00	0	0.05	1
Krenopelopia	0.00	0	0.03	1	0.00	0
Larsia	0.00	0	0.39	4	3.06	7
Macropelopia	0.00	0	0.24	4	0.00	0
Nilotanypus dubius (Meigen, 1804)	0.00	0	0.27	5	0.00	0
Paramerina levidensis	3.77	4	0.00	0	0.00	0
Paramerina sp. VBM3	0.13	1	0.00	0	0.00	0
Paramerina	0.00	0	0.36	6	0.00	0
Pentaneura sp. V10	0.38	2	0.00	0	0.00	0
Pentaneura	0.00	0	0.00	0	0.49	4
Procladius	0.00	0	0.30	3	0.00	0
Rheopelopia	0.00	0	1.09	13	0.00	0
Tanypodinae sp.V20	2.64	2	0.00	0	0.00	0
Thienemannimyia	0.00	0	1.18	12	0.00	0
Trissopelopia	0.00	0	0.79	8	0.00	0
Zavrelimyia	0.00	0	0.75	10	0.00	0
Subfamily Diamesinae						
Tribe Heptagyini						
Heptagyini unknown sp.1	0.00	0	0.00	0	5.93	7
Limaya	0.00	0	0.00	0	2.87	7
Paraheptagyia sp.1	0.00	0	0.00	0	5.00	5
Paraheptagyia sp.2	0.00	0	0.00	0	2.28	1
Tribe Diamesini						
Diamesa hamaticornis (Kieffer, 1924)	0.00	0	0.63	6	0.00	0
Diamesa zernyi-thienemanni group	0.00	0	3.01	7	0.00	0
Diamesa sp.A *sensu* Schmid, 1993	0.00	0	0.16	2	0.00	0
Potthastia longimana (Kieffer, 1922)	0.00	0	0.03	2	0.00	0

	SW-Australia		Mediterranean basin		Central Chile	
Taxa	%	Sites	%	Sites	%	Sites
Potthastia gaedii group	0.00	0	1.30	9	0.00	0
Subfamily Prodiamesinae						
Prodiamesa olivacea (Meigen, 1818)	0.00	0	0.21	2	0.00	0
Subfamily Orthocladiinae						
Botryocladius bibulmun (Cranston & Edward, 1999)	2.64	2	0.00	0	0.00	0
Botryocladius freemani (Cranston & Edward, 1999)	1.38	2	0.00	0	0.00	0
Botryocladius	0.00	0	0.00	0	5.88	5
?Botryocladius	0.00	0	0.00	0	4.52	7
Brillia longifurca (Kieffer, 1921)	0.00	0	0.04	2	0.00	0
Brillia bifida (Meigen, 1830)	0.00	0	2.44	13	0.00	0
Corynoneura coronata (Edwards, 1924)	0.00	0	0.69	4	0.00	0
Corynoneura lobata (Edwards, 1924)	0.00	0	0.89	7	0.00	0
Corynoneura scutellata group	0.00	0	1.79	8	0.00	0
Corynoneura	0.00	0	0.21	3	0.92	3
Cricotopus annuliventris (Skuse, 1889)	2.26	6	0.00	0	0.00	0
Cricotopus (Isocladius) trifasciatus (Meigen, 1813)	0.00	0	0.10	1	0.00	0
Cricotopus (Cricotopus) trifascia (Edwards, 1929)	0.00	0	0.06	1	0.00	0
Cricotopus (Isocladius) sylvestris group	0.00	0	0.51	4	0.00	0
Epoicocladius flavens (Malloch, 1915)	0.00	0	0.04	2	0.00	0
Eukiefferiella brevicalcar (Kieffer, 1915)	0.00	0	1.40	10	0.00	0
Eukiefferiella claripennis (Lundbeck, 1898)	0.00	0	0.24	3	0.00	0
Eukiefferiella coerulescens (Kieffer, 1926)	0.00	0	0.16	6	0.00	0
Eukiefferiella devonica (Edwards, 1929)	0.00	0	0.64	6	0.00	0
Eukiefferiella fuldensis (Lehmann, 1972)	0.00	0	0.10	3	0.00	0
Eukiefferiella gracei (Edwards, 1929)	0.00	0	2.16	4	0.00	0
Eukiefferiella ilkleyensis (Edwards, 1929)	0.00	0	0.91	9	0.00	0
Eukiefferiella minor-fittkaui	0.00	0	0.60	10	0.00	0
Eukiefferiella similis (Goetghebuer, 1939)	0.00	0	0.03	1	0.00	0
Eukiefferiella tirolensis (Goetghebuer, 1938)	0.00	0	0.39	3	0.00	0
Eukiefferiella cf. lobifera sensu Schmid, 1993	0.00	0	0.25	2	0.00	0
?Georthocladius	0.00	0	0.00	0	1.26	2
?Gymnometriocnemus	0.38	2	0.00	0	0.97	4
Heleniella ornaticollis (Edwards, 1929)	0.00	0	0.06	2	0.00	0
Heleniella sp.1	0.00	0	0.01	1	0.00	0
Heterotrissocladius marcidus (Walker, 1856)	0.00	0	0.01	1	0.00	0
? Limnophyes pullulus	0.13	1	0.00	0	0.00	0
Limnophyes	0.00	0	0.06	3	0.00	0

	SW-Australia		Mediterranean basin		Central Chile	
Taxa	%	Sites	%	Sites	%	Sites
Lopescladius	0.00	0	0.00	0	1.90	2
Metriocnemus eurynotus group	0.00	0	0.18	1	0.00	0
Metriocnemus fuscipes group	0.00	0	0.01	1	0.00	0
Metriocnemus	0.00	0	0.01	1	0.83	1
Nanocladius bicolor (Zetterstedt, 1838)	0.00	0	0.01	1	0.00	0
Nanocladius rectinervis (Kieffer, 1911)	0.00	0	0.07	2	0.00	0
Nanocladius sp.2 WA	0.38	2	0.00	0	0.00	0
Orthocladius (Euorthocladius) rivulorum (Kieffer, 1909)	0.00	0	0.51	8	0.00	0
Orthocladius-Cricotopus	0.00	0	14.68	20	27.75	11
Orthocladius (Euorthocladius) indet.	0.00	0	0.31	4	0.00	0
?Paralimnophyes	0.00	0	0.00	0	0.39	2
Parametriocnemus stylatus (Kieffer, 1924)	0.00	0	2.24	17	0.00	0
Paraphaenocladius pseudirritus (Strenzke, 1950)	0.00	0	0.01	1	0.00	0
Parakiefferiella sp. VSC9	9.17	3	0.00	0	0.00	0
Parakiefferiella cf. coronata sensu Schimd'93	0.00	0	0.10	1	0.00	0
Parakiefferiella cf. gracillima sensu Schimd'93	0.00	0	0.10	2	0.00	0
Parakiefferiella	0.00	0	0.00	0	5.59	5
Paracladius conversus (Walker, 1856)	0.00	0	0.07	2	0.00	0
Paracricotopus niger (Kieffer, 1913)	0.00	0	1.12	5	0.00	0
Paratrichocladius	0.00	0	3.21	14	1.36	2
Paratrissocladius excerptus (Walker, 1856)	0.00	0	0.34	6	0.00	0
Pseudosmittia holsata (Thienemann & Strenzke, 1940)	0.00	0	0.01	1	0.00	0
Rheocricotopus chalybeatus group	0.00	0	2.89	10	0.00	0
Rheocricotopus effusus (Walker, 1856)	0.00	0	0.49	5	0.00	0
Rheocricotopus fuscipes (Kieffer, 1909)	0.00	0	7.22	13	0.00	0
Rheocricotopus	0.00	0	0.15	1	0.00	0
Orthocladiinae unknown	0.00	0	0.22	7	0.44	6
Orthocladiinae unknown borer V22	0.13	1	0.00	0	0.00	0
Orthocladiinae V31	26.76	5	0.00	0	0.00	0
Orthocladiinae V43	0.25	2	0.00	0	0.00	0
Orthocladiinae ?V59	4.90	3	0.00	0	0.00	0
Orthocladiinae unknowncat1	0.00	0	0.73	3	0.00	0
Orthocladiinae unknownxil1	0.00	0	0.00	0	0.34	3
Orthocladiinae unknownxil2	0.00	0	0.00	0	0.19	2
Orthocladiinae unknownxil3	0.00	0	0.00	0	0.05	1
Orthocladiinae unknownxil4	0.00	0	0.00	0	0.24	2
Orthocladiinae unknownxil5	0.00	0	0.00	0	0.34	2

	SW-Australia		**Meditteranean basin**		**Central Chile**	
Taxa	%	Sites	%	Sites	%	Sites
Orthocladiinae unknownxil6	0.00	0	0.00	0	0.05	1
Orthocladiinae unknownxil7	0.00	0	0.00	0	0.05	1
Orthocladiinae unknownxil8	0.00	0	0.00	0	1.31	2
Orthocladiinae unknownxil9	0.00	0	0.00	0	0.44	3
Smittia	0.00	0	0.10	2	0.00	0
Stictocladius uniserialis (Freeman, 1961)	3.77	2	0.00	0	0.00	0
Stictocladius ?V35	0.88	1	0.00	0	0.00	0
Symposiocladius lignicola (Kieffer & Potthast, 1915)	0.00	0	0.01	1	0.00	0
Synorthocladius semivirens (Kieffer, 1909)	0.00	0	0.28	10	0.00	0
Thienemannia	0.00	0	0.03	2	0.00	0
Thienemanniella acuticornis (Kieffer, 1912)	0.00	0	0.01	1	0.00	0
Thienemanniella clavicornis (Kieffer, 1911)	0.00	0	0.89	6	0.00	0
Thienemanniella partita (Schlee, 1968)	0.00	0	2.21	7	0.00	0
Thienemaniella sp.V19	6.03	5	0.00	0	0.00	0
Thienemanniella vittata (Edwards, 1924)	0.00	0	2.30	11	0.00	0
Thienemanniella sp.1	0.00	0	0.04	1	0.00	0
Thienemanniella	0.00	0	0.55	1	1.94	7
Thienemanniella majuscula (Edwards, 1924)	0.00	0	0.04	1	0.00	0
Tvetenia bavarica-calvescens	0.00	0	9.30	24	0.00	0
Tvetenia discoloripes (Goetghebuer, 1940)	0.00	0	2.46	11	0.00	0
Subfamily Chironominae						
Tribe Chironomini						
Chironomini ? genus? sp.V78	0.38	2	0.00	0	0.00	0
Chironomini unknown sp.1	0.00	0	0.00	0	0.24	3
Chironomini unknown sp.2	0.00	0	0.00	0	0.44	2
Chironomini unknown sp.3	0.00	0	0.00	0	0.10	1
Chironomini unknown sp.4	0.00	0	0.00	0	0.05	1
Chironomus aff.alternans Walker	0.25	2	0.00	0	0.00	0
Chironomus	0.00	0	0.00	0	0.10	1
Chironomus sp.2	0.00	0	0.07	1	0.00	0
Dicrotendipes ? conjunctus	0.25	1	0.00	0	0.00	0
Dicrotendipes	0.00	0	0.00	0	0.29	2
Harnischia	0.00	0	0.03	1	0.00	0
Harrissius	1.01	3	0.00	0	0.00	0
Microtendipes pedellus group	0.00	0	0.25	5	0.00	0
Microtendipes rydalensis group	0.00	0	0.04	2	0.00	0
Microtendipes	0.00	0	0.00	0	3.79	7
? Paratendipes sp. V12	0.50	2	0.00	0	0.00	0

	SW-Australia		Meditteranean basin		Central Chile	
Taxa	%	Sites	%	Sites	%	Sites
Phaenopsectra	0.00	0	0.18	5	4.28	6
Polypedilum	0.00	0	0.00	0	0.58	1
Polypedilum watsoni (Freeman, 1961)	1.13	5	0.00	0	0.00	0
Polypedilum cf. cultellatum (Goetghebuer, 1931)	0.00	0	1.13	4	0.00	0
Polypedilum nubeculosum group	0.00	0	1.25	6	0.00	0
Polypedilum pedestre group	0.00	0	0.04	1	0.00	0
Polypedilum laetum group- sp.1	0.00	0	2.18	8	0.00	0
Polypedilum laetum group- sp.2	0.00	0	1.13	1	0.00	0
Saetheria	0.00	0	0.03	1	0.00	0
Chironomini unknown	0.00	0	0.00	0	1.17	4
Tribelos	0.00	0	0.00	0	0.05	1
Tribe Pseudochironomini						
Pseudochironomus	0.00	0	0.00	0	0.10	1
Riethia sp.	8.04	3	0.00	0	0.00	0
Tribe Tanytarsini						
Tanytarsini ?genus V13	0.63	2	0.00	0	0.00	0
Tanytarsini unknown sp.1	0.00	0	0.00	0	0.19	1
Tanytarsini unknown sp.2	0.00	0	0.00	0	0.15	1
Cladotanytarsus	1.01	2	0.13	2	0.00	0
Micropsectra sp.1	0.00	0	2.25	4	0.00	0
Micropsectra sp.2	0.00	0	1.06	8	0.00	0
Micropsectra sp.3	0.00	0	0.01	1	0.00	0
Micropsectra sp.4	0.00	0	0.49	5	0.00	0
Micropsectra sp.5	0.00	0	0.40	3	0.00	0
Micropsectra sp.6	0.00	0	0.03	1	0.00	0
Neozavrelia	0.00	0	0.25	3	0.00	0
Paratanytarsus	0.00	0	0.34	6	0.00	0
Rheotanytarsus	2.76	4	5.02	14	1.36	4
*Stempellina australiensis (*Freeman, 1961)	0.75	2	0.00	0	0.00	0
Stempellina bausei group	0.00	0	0.24	1	0.00	0
Stempellinella	0.00	0	1.10	7	0.00	0
Tanytarsus sp. V6	16.71	6	0.00	0	0.00	0
Tanytarsus chinyensis group	0.00	0	0.07	1	0.00	0
Tanytarsus	0.00	0	0.00	0	0.49	4
Tanytarsus sp.1	0.00	0	0.36	3	0.00	0
Tanytarsus sp.2	0.00	0	0.58	6	0.00	0
Tanytarsus sp.3	0.00	0	1.01	8	0.00	0
Tanytarsus sp.4	0.00	0	0.34	3	0.00	0
Virgatanytarsus	0.00	0	0.57	7	0.00	0

Discussió General i Conclusions

DISCUSSIÓ GENERAL

En aquesta discussió general es recullen les principals conclusions derivades d'aquesta tesi, i que ja s'han comentat en cada capítol de manera independent. La discussió s'ha estructurat en tres apartats generals que aborden les principals qüestions d'aquesta tesi presentades de forma transversal. El primer apartat tracta la importància dels factors ambientals i les comunitats de quironòmids a diferents escales espacials. Malgrat la variabilitat temporal no s'ha estudiat molt extensament en aquesta tesi, també hem cregut necessari discutir-ho en un apartat independent. I finalment es presenten les conclusions relacionades amb els aspectes més aplicats, per respondre si hi ha una concordança entre les comunitats biològiques i els ecotipus definits en la regió mediterrània de la Península Ibèrica.

Per tal d'obtenir tots aquests resultats, s'ha hagut de fer un gran esforç en la identificació dels estadis larvaris de quironòmids, per tal d'arribar a la resolució taxonòmica més detallada possible: espècies, grups d'espècies o bé gèneres. Això ha suposat la recol·lecció i comptatge manual de 20266 larves, i la preparació i muntatge per a la seva observació al microscopi òptic de 8495 espècimens. En total s'han identificat 227 taxons de quironòmids que representen la riquesa d'aquest grup per les tres zones mediterrànies estudiades. Així no obstant, un estudi a partir d'altra material que permetés una resolució taxonòmica més detallada i que inclogués més mostrejos al llarg de l'any, segurament mostraria valors de riquesa superiors.

Els factors ambientals i els quironòmids, i la seva relació amb les escales espacials

L'estructura local d'una comunitat biològica és resultat de la influència de processos locals (a petita escala) i d'altres biogeogràfics i històrics (a gran escala) (Ricklefs, 1987), per tant les comunitats estan estructurades per processos que actuen a múltiples escales espacials (Minshall, 1988; Li *et al.*, 2001). Per tal d'analitzar la importància dels factors ambientals actuals, ho hem fet estudiant escales espacials petites (Capítols II i III). En canvi, per tal d'estudiar la influència dels factors històrics sobre les comunitats de quironòmids, s'ha utilitzat una escala espacial més gran (Capítol IV). Realitzant una comparació intercontinental entre les tres regions mediterrànies mostrejades: Xile central, sud-oest d'Austràlia i la conca Mediterrània, s'han trobat diferències de riquesa i de composició taxonòmica entre les tres regions. Les diferències pel que fa a la composició taxonòmica eren d'esperar, a causa dels processos a gran escala que històricament han determinat el *pool* d'espècies mediterrànies presents a les diferents regions mediterrànies. Però les diferències de riquesa les expliquem a causa d'una combinació de factors històrics i també a causa de la heterogeneïtat dels factors locals. També es troben diferents patrons de les distribucions d'espècies d'abundàncies en les tres regions, tot i que en aquest cas no s'han pogut identificar quins són els mecanismes que les regulen.

Per estudiar quines són les variables ambientals que afecten els patrons de distribució de les comunitats de quironòmids en rius mediterranis en condicions de referència ens hem centrat amb el *pool* d'espècies mediterrànies de la conca Mediterrània de la Península Ibèrica (Capítols II i III). Una de les conclusions del treballs és que les variacions en l'estructura de les comunitats de quironòmids en rius de referència està principalment explicada per la zonació longitudinal (per exemple: altitud, àrea de conca, temperatura…). Els factors claus obtinguts concorden en gran manera amb d'altres treballs realitzats en rius mediterranis (Prat *et al.*, 1983; Casas & Vílchez-Quero, 1993) o d'altres regions climàtiques del món (Coffman, 1989; Lindegaard & Brodersen, 1995).

A més a més, s'ha quantificat la importància de diferents grups de variables ambientals: locals, regionals i geogràfiques (capítol III). Com en d'altres estudis (Death & Joy, 2004; Mykra *et al.,* 2007) obtenim que els factors locals són els que expliquen una proporció més gran de la variança, en comparació amb d'altres escales espacials. Però tot i això, tal i com esperàvem per tal d'entendre la composició d'una comunitat biològica, és necessària una combinació tant de factors locals com regionals, i també és important el component geogràfic (espacial) que representa un descriptor sintètic d'alguns factors ambientals no mesurats explícitament.

L'importància de l'escala temporal

Els canvis estacionals en la composició de les comunitats de quironòmids en rius mediterranis han estat poc estudiats (Prat *et al.,* 1983; Langton & Casas, 1999). En el nostre treball s'han diferenciat quatre grups de quironòmids en relació amb la influència de les variables ambientals (capítol II). En alguns grups (per exemple: capçaleres silíciques) no s'aprecien canvis de les comunitats biològiques entre primavera i estiu. Mentre que en d'altres grups (per exemple: rius petits calcaris de mitjana altitud), si que s'han detectat canvis estacionals molt importants, tenint per la mateixa localitat una composició biològica totalment diferent a la primavera i a l'estiu. Amb aquest treball hem comprovat que els quironòmids és un grup sensible que ens pot servir per detectar canvis estacionals en la composició biològica de les comunitats. Per tant, aquest és un aspecte a considerar per la conservació i la gestió dels rius mediterranis, per exemple alhora de plantejar-nos quina és la millor època per mostrejar.

D'altra banda, aquest és un resultat que haurem de tenir present davant dels futurs escenaris del canvi climàtic global (Arnell, 1999), ja que l'augment de temperatures i el clima més sec que es preveu, pot fer que les comunitats descrites a la primavera (tant en el capítol II com en el III) siguin totalment diferents en un futur proper, i per tant més semblants a les comunitats presents a l'estiu. A més a més conèixer els requeriments específics de les espècies (òptims i toleràncies) tal i com hem fet al capítol III, pot ser una eina molt útil alhora de predir la resposta de les diferents espècies cap a variables significatives que poden canviar en el futur, com la temperatura o variables hidrològiques com el cabal. En aquesta línia s'han desenvolupat diferents estudis en sistemes alpins per detectar els canvis de les comunitats biològiques, per exemple analitzant si hi ha una disminució de les espècies més adaptades al fred com a resposta a l'adaptació de l'augment de temperatura global (Lodz-Crozet *et al.*, 2001; Rossaro *et al.,* 2006).

Els ecotipus dels rius mediterranis i la validació amb les comunitats de macroinvertebrats i de quironòmids

Aquesta tesi s'emmarca en un projecte de recerca (GUADALMED) de caire aplicat i que té com a rerafons la DMA, a on la tipificació fluvial és un dels principals objectius a tenir en compte. D'aquí l'interès de conèixer les comunitats fluvials presents en els rius i si és possible diferenciar-les per ecotipus fluvials. Usant les directrius de la DMA, s'han definit cinc ecotipus fluvials utilitzant una combinació de variables hidrològiques, geològiques, morfològiques i climàtiques: (1) rius temporals, (2) trams mitjos de rius de geologia preferentment evaporítica-calcària, (3) capçaleres silíciques d'elevada altitud, (4) capçaleres calcàries de mitjana a elevada altitud i (5) trams mitjos-baixos (capítol I). El número d'ecotipus obtinguts és petit si el comparem amb d'altres treballs realitzats a la conca Mediterrània. Per exemple, Munné & Prat (2004) fan una anàlisi semblant a la regió de Catalunya i obtenen 10 tipus de gestió fluvial. En aquest cas que esmentem, la tipificació obtinguda potser és excessivament variada, i per tant l'assignació dels estats de referència i dels corresponents objectius de qualitat seria més difícil, que amb el número d'ecotipus que obtenim nosaltres. Sigui quina sigui la regionalització que s'utilitzi, alhora de fer gestió s'hauran de tenir en compte quins resultats s'ajusten més a l'heterogeneïtat de la zona d'estudi. És per aquest motiu que la classificació de rius basada amb les variables ambientals ha de ser rigorosament validada per les comunitats biològiques, per tal que la puguem utilitzar posteriorment com una eina eficient per a la gestió a llarg termini (Soininen *et al.*, 2004; Verdonschot, 2006). Recentment, alguns estudis han testat la concordança entre classificacions ambientals i comunitats biològiques (Moog, 2004; Snelder *et al.*, 2004). Per exemple a Europa, s'han realitzat estudis on s'ha testat la validesa de les tipologies de rius obtingudes amb els macroinvertebrats (Verdonschot & Nijboer, 2004). Normalment alhora d'estudiar les diferències entre ecotipus i les comunitats biològiques, s'ha fet utilitzant tota la comunitat de macroinvertebrats (Sandin & Johnson, 2000). En aquesta tesi a més de fer-ho amb els macroinvertebrats a nivell de família (capítol I), s'han testat les diferències amb els quironòmids a nivell específic (capítol II). Hem partit de la hipòtesis que les localitats que pertanyen a un mateix ecotipus haurien de tenir unes comunitats relativament homogènies, en comparació amb les comunitats de localitats presents en d'altres ecotipus (McCreadie & Adler, 2006). Dels quatre ecotipus que validem per les comunitats de macroinvertebrats n'hem obtingut els corresponents taxons indicadors, i això és un resultat rellevant que reforça la idea que aquests ecotipus en part tenen significat ecològic. A grans trets hem obtingut uns resultats molt semblants, tant amb les comunitats de macroinvertebrats com amb les de quironòmids, malgrat que amb aquests últims no s'ha pogut realitzar una validació definitiva i contrastada dels rius temporals (ecotipus 1) per manca de punts de mostreig. Així doncs, les comunitats biològiques corresponents als diferents ecotipus es diferencien clarament entre elles, llevat de les comunitats dels ecotipus 2 i 4, les quals són molt semblants. A més a més, els rius temporals presenten una variabilitat biològica de les comunitats de macroinvertebrats molt més gran que en d'altres ecotipus i s'hauria d'analitzar molt més detalladament què passa amb els quironòmids, ja que per exemple es coneix que en tipologies de rius com les ramblas (Moreno *et al.,* 1997) i els karsts (Álvarez & Pardo, 2007), trobem unes comunitats biològiques particulars. Aquests resultats ens seran útils quan s'hagin d'establir els rangs de qualitat de l'estat ecològic per cadascun dels ecotipus tal i com indica la DMA. En el nostre cas i de cara a la gestió, tant pel que fa als macroinvertebrats com als quironòmids, les comunitats dels ecotipus 2 i 4 no seria necessari de mantenir-les separadament en el moment de definir les condicions de referència.

Hi ha hagut una certa controvèrsia respecte a quina és la millor resolució taxonòmica alhora de realitzar les classificacions ambientals (Hawkins *et al*., 2000). En el nostre cas, malgrat concloure que els quironòmids són un grup efectiu de cara a la classificació dels rius, els patrons generals obtinguts són molt semblants als dels macroinvertebrats a nivell de família. Per això, si l'objectiu del nostre treball és el de fer diagnosi de l'estat ecològic global dels rius, no caldria fer l'esforç taxonòmic de determinació a nivell específic pel cas dels quironòmids, tot i que això no significa que hagi de ser igual amb d'altres grups de macroinvertebrats. Diferents autors han suggerit també que s'hauria de comparar l'eficiència de diferents classificacions de tipus fluvials a diferents escales espacials, per tal de veure com es caracteritza la variació de les comunitats de macroinvertebrats (Li *et al*., 2001; Snelder *et al.,* 2004). De totes maneres tot i les diferències de comunitats entre alguns ecotipus, els resultats mostren que seria més apropiada una classificació ambiental basada amb les comunitats biològiques (*bottom-up*) que no pas una classificació basada amb les variables ambientals (*top-down*).

Propostes per una recerca futura

La recerca duta a terme en aquesta tesi ha originat algunes qüestions que caldrà plantejar de cara al futur. Algunes d'aquestes preguntes es deriven directament dels capítols d'aquesta tesi, mentre que d'altres corresponen a mancances de coneixement amb les quals ens hem trobat i que creiem que s'haurien de treballar en un futur.

Quina és la variabilitat intra i interanual de les comunitats de quironòmids?

En aquesta tesi hem estudiat la variabilitat temporal de les comunitats de quironòmids comparant la composició biològica entre dues estacions de l'any, però en un futur s'haurien de mesurar les variacions intraanuals de manera més intensiva i també les interanuals. Analitzar la variabilitat interanual pot ser de gran interès de cara als efectes del canvi climàtic. Aquests estudis seran necessaris de cara a una futura conservació d'aquests ecosistemes tal i com recomana Álvarez-Cobelas *et al.* (2005).

Com són les comunitats de quironòmids dels rius temporals?

Després de concloure que els rius temporals presenten una elevada heterogeneïtat pel que fa a les comunitats biològiques de macroinvertebrats, seria interessant ampliar el número de punts de mostreig de rius temporals i analitzar-ne també la variabilitat dins de les comunitats de quironòmids. Per exemple, es coneix que les comunitats biològiques de les rambles són molt diverses i riques en espècies endèmiques (Gómez *et al.,* 2005). Però hi ha una manca de coneixement de la composició de les comunitats de quironòmids en aquests sistemes, en els quals s'hi podria aprofundir.

Com varien les poblacions de quironòmids estudiant caràcters de taxonomia molecular?

Aquesta seria una nova línia d'investigació interessant, ja que els estudis de fil·logènia ens permetrien de verificar les identificacions morfològiques fetes amb les larves. Amb aquesta nova informació podríem veure si les espècies identificades en base als caràcters morfològics, serien les mateixes que les espècies identificades a través dels caràcters genètics.

Quins són els requeriments ecològics de les espècies de quironòmids?

Es coneix poc sobre l'autoecologia dels quironòmids, i malgrat en aquesta tesi s'han estudiat els òptims i toleràncies per diferents espècies de la regió mediterrània, seria ideal que s'estimessin nous òptims i toleràncies tenint en compte diferents estacions de l'any, per tal de conèixer amb detall els requeriments ecològics i les distribucions dels diferents taxes. A més a més idealment s'hauria de considerar la influència de l'ambient en tots els estadis del cicle de vida combinant investigacions de camp i de laboratori.

Quins són els mecanismes que regulen les distribucions d'abundàncies d'espècies de les comunitats de quironòmids?

Per tal de contrastar els resultats obtinguts en aquest estudi, s'hauran de validar les distribucions d'espècies d'abundàncies obtingudes, amb la obtenció de noves dades. Per això serà necessari millorar el coneixement de la taxonomia dels quironòmids dels estadis immadurs, sobretot en les regions de l'hemisferi sud. A través d'aquestes noves dades empíriques, es podrà aprofundir en els mecanismes que regulen les diferents distribucions d'abundàncies i especialment amb el paràmetre β.

CONCLUSIONS

- En el present treball s'han caracteritzat les comunitats biològiques de macroinvertebrats i de quironòmids en rius mediterranis de referència a diferents escales espacials, i s'han obtingut les espècies característiques per cadascuna de les associacions biològiques definides.

- Les variacions en l'estructura de les comunitats de quironòmids en rius de referència està principalment explicada per la zonació longitudinal (altitud, àrea de conca, temperatura…) juntament amb la heterogeneïtat temporal i espacial.

- S'han calculat els òptims i toleràncies per als paràmetres ambientals significatius de les espècies de quironòmids en condicions de referència. Els nostres resultats evidencien que hi han gèneres característics d'alguns tipus de rius, com per exemple *Diamesa* el qual trobem restringit en àrees de capçalera. Mentre que d'altres gèneres estan molt més diversificats, com és el cas del gènere *Eukiefferiella*, que presenta espècies típiques de capçalera i d'altres que prefereixen trams mitjos dels rius més mineralitzats.

- Per algunes associacions de quironòmids, com per exemple els que trobem a les capçaleres silíciques, no s'observen canvis estacionals importants entre comunitats de primavera i estiu. En canvi les comunitats de quironòmids de rius petits calcaris presenten variacions temporals importants.

- S'han estudiat els filtres que modelen l'estructura de les comunitats de quironòmids de diferents regions mediterrànies del món. Per la regió mediterrània de la Península Ibèrica, s'observa que l'estructura de les comunitats de quironòmids és el resultat d'una combinació tant de factors locals, regionals i geogràfics (espacials), dels quals són les variables locals les que contribueixen de manera més important a la proporció de variança explicada.

- La comparació entre tres regions mediterrànies del món (Xile central, sud-oest d'Austràlia i la conca Mediterrània), dóna com a resultat que hi ha diferències pel que fa a l'estructura de les comunitats de quironòmids. La composició taxonòmica és el reflex predominantment dels procesos històrics, així les regions de l'hemisferi sud presenten unes similaritats més elevades entre elles. Pel que fa a riquesa taxonòmica la conca mediterrània és la regió més rica, seguida de Xile i el sud-oest d'Austràlia, resultat d'una combinació de factors històrics biogeogràfics (escala gran) i de factors locals (escala petita). També es detecten diferències pel que fa a les distribucions d'abundàncies d'espècies, per exemple les comunitats del sud-oest australià presenta una distribució log-sèries a diferència de les altres regions que segueixen un patró diferent.

- Després de realitzar una classificació *top-down* dels rius en condicions de referència seguint les directrius de la DMA s'han obtingut cinc ecotipus de rius a les conques mediterrànies de la Península Ibèrica: (1) rius temporals, (2) trams mitjos de rius de geologia preferentment evaporítica-calcària, (3) capçaleres silícIques d'elevada altitud, (4) capçaleres calcàries de mitjana a elevada altitud i (5) trams mitjos-baixos dels rius.

- La DMA suposa que cada ecotipus hauria de tenir una comunitat biològica característica. Els nostres resultats mostren que una tipologia de rius basada en les comunitats de macroinvertebrats i/o de quironòmids identifica diferents tipus de rius que en alguns casos coincideixen amb la tipologia ambiental (ecotipus 1 i 3), però en d'altres no, com és el cas dels ecotipus 2 i 4 on s'obtenen unes comunitats biològiques molt similars entre ells. Per tant per establir les comunitats de referència en els rius mediterranis la metodologia més correcte seria la utilització directe de les comunitats biològiques i no la de les característiques ambientals.

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