



# Les Crustacés souterrains, indicateurs naturels de l'aquifère alluvial de l'Ariège (France, Pyrénées)

Pascal Dumas

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Thèse présentée devant  
L'Université Paul Sabatier – Toulouse III  
En vue de l'obtention du titre de  
Docteur de l'Université

Spécialité : Hydrobiologie

**Les Crustacés souterrains,  
indicateurs naturels de l'aquifère alluvial  
de l'Ariège (France, Pyrénées)**

Pascal DUMAS

Soutenue publiquement le 25 Novembre 2000 devant la commission d'examen composée de :

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J. Durand	Directeur de Recherches CNRS – Laboratoire Souterrain de Moulis	Examinateur
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C. Bou	Professeur du Secondaire - Albi	Invité



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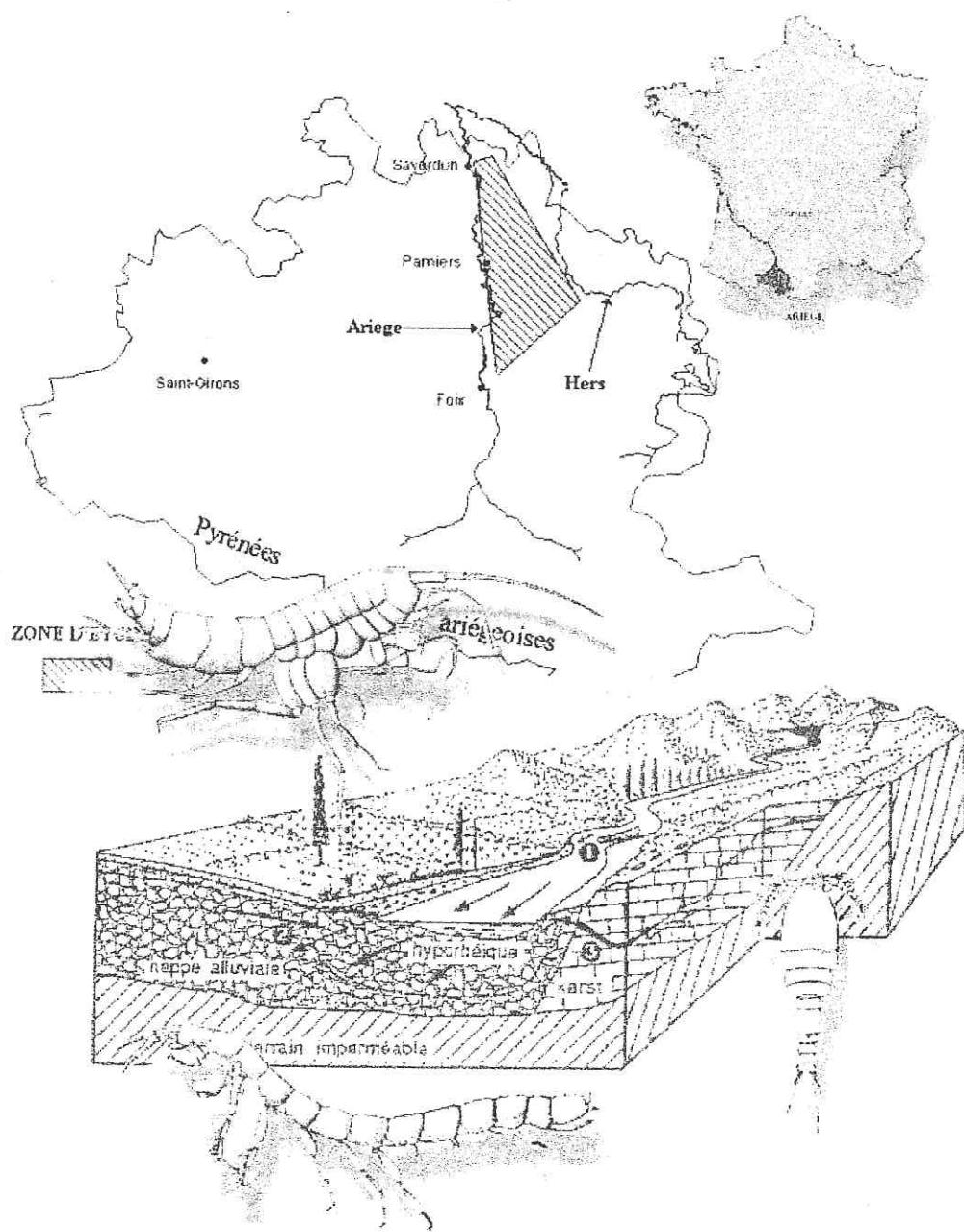
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# - INTRODUCTION -





### **Le milieu souterrain, milieu vivant.**

L'idée de la continuité fondamentale du domaine souterrain, dont le biologiste P.A. CHAPPUIS avait eu l'intuition au milieu du XX<sup>ème</sup> siècle, s'est progressivement imposée et apparaît aujourd'hui comme une évidence. D'une importance cruciale, elle a profondément modifié la conception du milieu souterrain ; elle marque le début de l'articulation d'éléments jusqu'alors juxtaposés (grottes, rivières souterraines, nappes, fissures, gours etc. considérés isolément) en systèmes hiérarchisés et cohérents, organisés selon une approche fonctionnelle. Sur le plan écologique, cette vision intégrée implique notamment la reconnaissance de la variété des écosystèmes souterrains et des processus d'interaction les unissant à diverses échelles d'observation ; les définitions récentes (JUBERTHIE & DECOU 1994) mettent ainsi l'accent sur le domaine souterrain en tant que *milieu de vie* pour les divers types d'organismes rencontrés.

Les eaux souterraines représentent un maillon essentiel du cycle de l'eau (CASTANY 1966, GIBERT et al. 1996) et leur importance en tant que ressource n'est plus à démontrer, tant sur le plan biologique ou écologique qu'économique (CASTANY 1982, U.N.E.S.C.O. 1986, BARBAULT 1990, STANFORD & SIMMONS 1992, GIBERT et al. 1994). Le réservoir souterrain renferme ainsi la quasi-totalité des eaux douces continentales directement mobilisables (CASTANY 1982). Il constitue également un ensemble d'habitats d'une grande complexité renfermant une faune abondante et variée, souvent sans équivalent par rapport aux écosystèmes de surface (cf. GINET & DECOU 1977, VANDEL 1964, CULVER 1982, synthèse dans JUBERTHIE & DECOU 1994). A ce titre, les eaux souterraines constituent un réservoir de biodiversité à l'échelle de la planète (JUBERTHIE & DECOU 1994, MALARD et al. 1997, PALMER 1997).

### **1. Historique des eaux souterraines : du karst à la plaine alluviale.**

Allant de pair avec la découverte des premiers animaux souterrains dans les grottes de Slovénie et de France, la prospection biospéologique débuta souvent dans les vastes systèmes aquatiques des zones karstiques (historique dans VANDEL 1964, GINET & DECOU 1977). Du fait de leurs caractéristiques (grande extension géographique, variété des histoires géologiques et climatiques, propriétés structurales), les aquifères karstiques livrèrent aux chercheurs des biocénoses diversifiées comprenant des espèces étroitement spécialisées (BOTOSANEANU 1986, GINET & JUBERTHIE 1987). La majorité des taxons était représentée, depuis les bactéries et protozoaires jusqu'aux invertébrés les plus évolués ; on y trouvait même des vertébrés qui, à l'instar du Protée, constituaient des exemples frappants de l'étrangeté des animaux "cavarnicoles" (synthèse dans JUBERTHIE & DECOU 1994) (fig. 1).

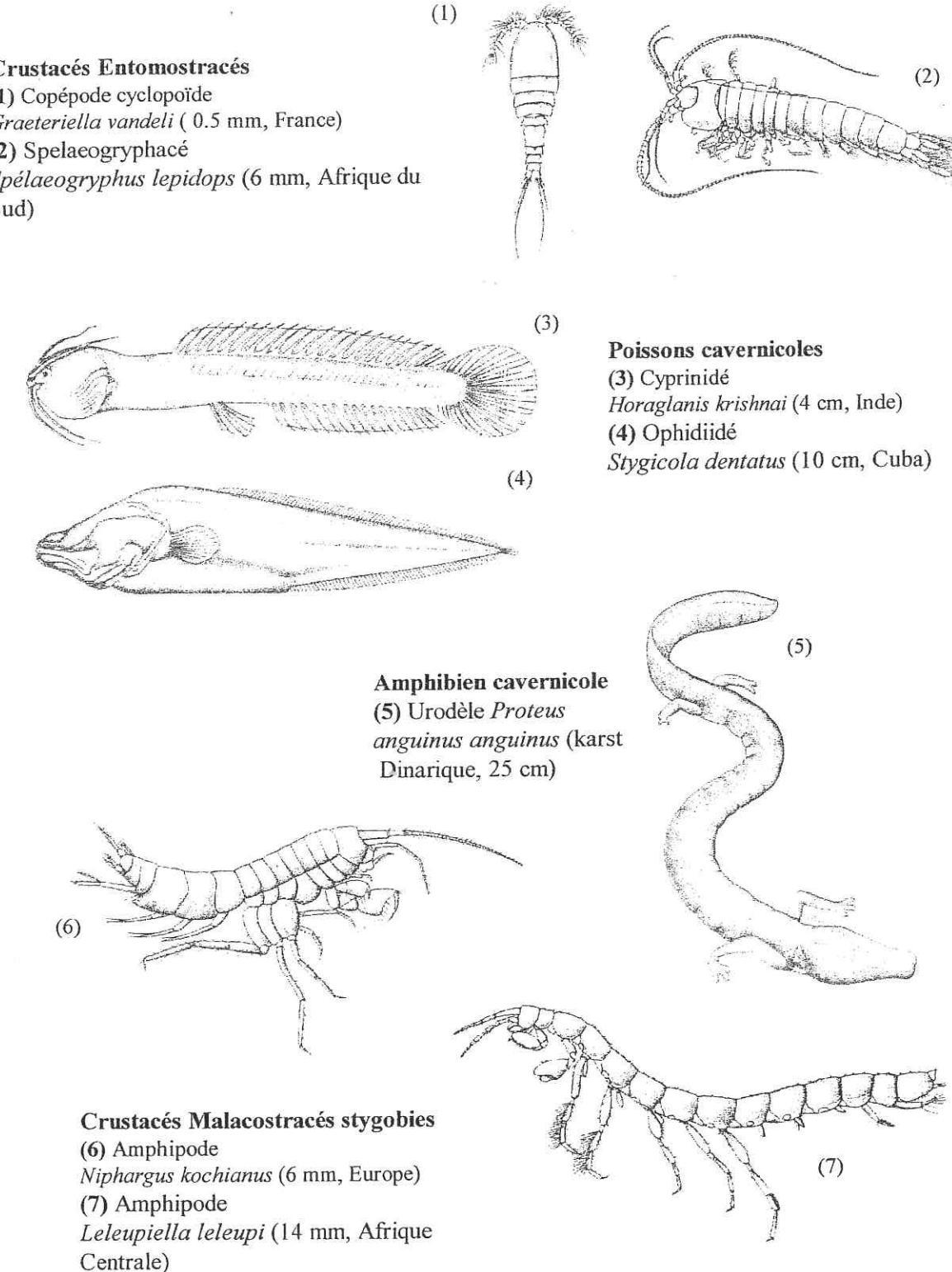


Figure 1. Quelques exemples d'organismes stygobies (échelles non respectées, d'après GINET & DECOU 1977).

A l'opposé, les milieux interstitiels et leur biocénoses ne furent prospectés que plus tardivement, les biologistes marins et limnologues quittant progressivement le domaine benthique pour scruter de plus en plus profondément l'épaisseur des sédiments des cours d'eau (historique dans DELAMARE-DEBOUTTEVILLE 1960). Initialement concentrées sur la zone sous-fluviale (biotope hyporhéique, ORGHIDAN 1959), les recherches s'élargirent par la suite à d'autres compartiments fluviaux, progressivement replacés dans un contexte fonctionnel global rivière-aquifère. Récemment, depuis l'amélioration des techniques d'échantillonnage, les recherches ne se limitent plus aux zones superficielles des aquifères mais autorisent un accès en profondeur (MATHIEU et al. 1991, MALARD et al. 1997, MAUCLAIRE et al. 1998).

## **2. Les aquifères alluviaux.**

La principale caractéristique de ces aquifères est leur liaison, plus ou moins étroite, avec un cours d'eau (CASTANY 1982). D'épaisseur et de structure variable, ils constituent des réservoirs dont les liaisons hydrauliques avec les rivières sont imposées principalement par leurs surfaces piézométriques relatives et le degré de colmatage du lit des cours d'eau. L'entité (rivière + aquifère) doit alors être considérée comme un système hydrodynamique, hydrochimique et hydrobiologique unique : il s'agit du système global rivière-aquifère (CASTANY 1985) (fig. 2).

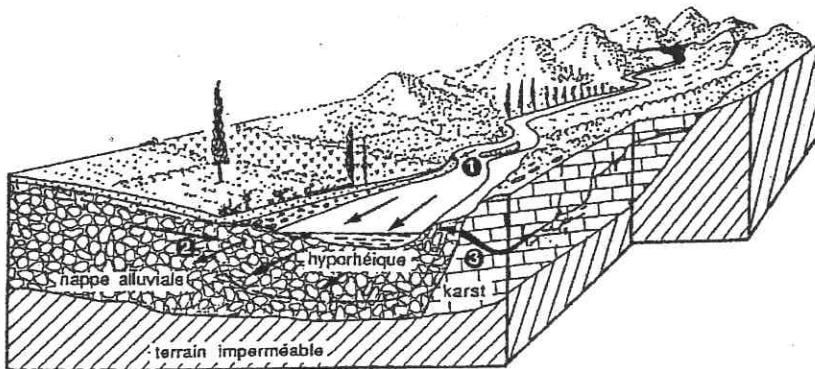
De par leur nature de réservoirs, les aquifères alluviaux remplissent une triple fonction vis-à-vis des eaux souterraines : 1) fonction capacitive (fonction d'emmagasinement), 2) fonction conductrice (fonction de transmission) et 3) fonction d'échange (hydrochimique, hydrobiologique). Leur productivité est en général d'autant plus élevée que les liaisons avec les cours d'eau sont fortes ; à ce titre, ils constituent souvent des sources privilégiées d'approvisionnement en eau douce (BODELLE & MARGAT 1980, CASTANY 1985, MARGAT 1994). Or à l'heure actuelle, la qualité des eaux dans les pays industrialisés est un facteur critique (ACADEMIE DES SCIENCES 1991, MAYER et al. 1993, GIBERT et al. 1994, MALARD 1995), les effets de contaminations venant souvent à dépasser les capacités naturelles d'autoépuration des eaux souterraines (GIBERT et al. 1994, MAUCLAIRE 1999).

Face à la vulnérabilité des aquifères, de considérables efforts de recherche ont donc été entrepris à l'échelle internationale (revue dans MAYER et al. 1993). Fondamentalement pluridisciplinaires, ces études concernent l'origine, la nature, les transferts, les impacts, le devenir des polluants en milieu souterrain et font appel à des disciplines environnementales telles que la géologie, l'hydrogéologie, l'hydrodynamique, l'hydrochimie ; plus récemment, depuis les années 80, les composantes biologiques des aquifères (macro- / microinvertébrés, biofilm bactérien) ont été intégrées dans une approche globale

## *Introduction*

de surveillance des eaux souterraines (“biomonitoring”, synthèses dans NOTENBOOM et al. 1994, DE ZWART 1995, MÖSSLACHER & GRIEBLER 1999, MÖSSLACHER & NOTENBOOM 1999a).

Une telle approche complémentaire (MÖSSLACHER & NOTENBOOM 1999b) est en effet basée sur la reconnaissance récente du rôle fondamental des organismes dans le fonctionnement des aquifères, “systèmes biologiques” (*sensu* CREUSÉ DES CHATELLIERS et al. 1991). Bien que limitée par le manque de connaissances approfondies sur les traits biologiques des espèces potentiellement indicatrices, par la complexité des systèmes mis en jeu et par les difficultés d'accès au milieu, il s'agit néanmoins d'une approche prometteuse (MÖSSLACHER & NOTENBOOM 1999b).



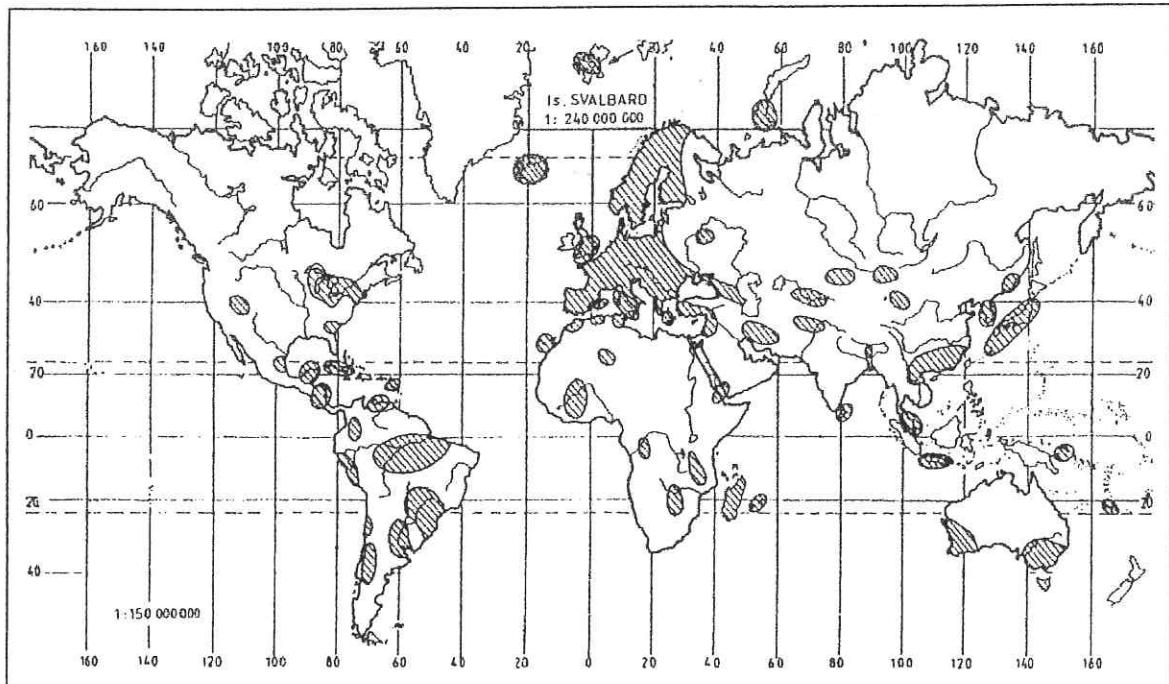
**Figure 2.** Système aquifère – rivière à l'échelle d'une plaine alluviale. Relations entre cours d'eau (1), nappe alluviale (2) et autres systèmes (système karstique, 3) (d'après JUBERTHIE & DECOU 1994)

## **2. Origine, diversité et répartition de la faune des aquifères.**

Les études biogéographiques témoignent de la présence sur l'ensemble de la planète d'organismes adaptés à la vie en milieu souterrain (BOTOSANEANU 1986, JUBERTHIE & DECOU 1994) : espèces troglophiles et troglobies se rencontrent sous toutes les latitudes entre l'équateur et la limite des glaces polaires (Groenland, Spitzberg, Islande...), à toute les longitudes sur l'ensemble des continents et certaines îles océaniques. Leur distribution verticale s'étend des grottes et fonds sous-marins jusqu'à près de 5000 m d'altitude dans les chaînes montagneuses tropicales (Cordillère des Andes, Tibet) (JUBERTHIE & DECOU 1994) (fig. 3).

A l'échelle d'un aquifère, malgré une décroissance générale de la biodiversité avec la profondeur, la présence de faune est couramment rapportée jusqu'à une centaine de mètres de profondeur (GIBERT et al. 1996). Plus exceptionnellement, quelques études attestent même l'existence d'organismes dans des nappes profondes : invertébrés dans des puits artésiens de 110 à 1000 mètres au Maroc, dans les plaines

de Fez et de Beni-Sadden (ESSAFI et al. 1998), invertébrés et vertébrés dans les forages artésiens de l'Edwards aquifer au Texas (LONGLEY 1986, KROSCHEWSKY 1990).



**Figure 3.** Un exemple de la répartition mondiale des organismes souterrains : distribution des Crustacés Copépodes hypogés (d'après JUBERTHIE & DECOU 1994).

Conséquence de ces observations, la question de l'origine de la faune souterraine constitue une interrogation récurrente qui a donné lieu à de nombreux débats depuis l'origine de la biospéologie (voir VANDEL 1964, BARR 1968, THINES 1969, ROUCH & DANIELOPOL 1987, BOTOSANEANU & HOLSINGER 1991, COINEAU & BOUTIN 1992). Si les milieux de surface constituent l'origine unanimement reconnue des espèces souterraines, les causes et les mécanismes de ce passage apparaissent d'autant plus complexes qu'ils se réfèrent implicitement à des concepts de base du monde vivant (évolution et adaptation, relations organismes / environnement, interactions biotiques) (ROUCH 1986).

Historiquement, parmi les nombreux modèles de colonisation du milieu souterrain proposés, deux grandes familles de processus ont été évoqués :

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EMBRANCHEMENT	SOUS-EMBRANCHEMENT	CLASSE	ORDRE	SOUS-ORDRE
PROTOZOAires		Flagellés Rhizopodes Ciliés		
SPONGIAires				
CNIDAires				
PLATHELMINTHES		Turbellariés	Rhabdocoeles Triclades	
NEMERTIENS				
NEMATHELMINTHES		Nématodes		
ROTIFERES				
ANNELIDES		Polychètes Oligochètes Achètes		
MOLLUSQUES		Gastéropodes Lamellibranches	Prosobranches Pulmonés	
ARTHROPODES	Chélicérates Trachéates	Arachnides Insectes	Acariens Collemboles Coléoptères Diptères	
	Crustacés (Entomostracés)	Branchiopodes Copépodes Ostracodes	Cladocères Cyclopoides Harpacticoides	
	(Malacostracés)	Syncarides Péracarides	Bathynellacés Thermosbaenacés Spélaeogriphacés Mysidacés Isopodes	Cymothoidea Anthuridea Phreaticoidea Sphaeromidea Asellotes Amphipodes Ingolfiellidae Gammaridea
		Eucarides	Décapodes	Natantia Reptantia
VERTEBRES		Actinoptérygiens Amphibiens	Urodèles Anoures	Pérennibranches Caducibranches

**Figure 4.** Principaux groupes d'organismes rencontrés dans les eaux souterraines (d'après GINET & DECOU 1977).

**1) les modèles de colonisation “ passive ”**, où la pénétration des organismes en profondeur se fait en réponse à des changements abiotiques (climat, géologie) ou biotiques (interactions spécifiques). Il s'agit alors de colonisation “ sous contrainte ” (théorie du “ milieu refuge ” retenue notamment par CHAPPUIS (1927), JEANNEL (1943), VANDEL (1964), GINET & DECOU (1977), voir synthèse critique dans ROUCH & DANIELOPOL (1987)) ;

**2) les modèles de colonisation “ active ”**, où la colonisation des biotopes hypogés s'effectue selon les mêmes principes et schémas directeurs que pour les écosystèmes de surface (disponibilité des ressources, vacance de niches écologiques, pression biotique) (initialement, « modèle de colonisation active » de ROUCH & DANIELOPOL 1987). Ces modèles se sont progressivement imposés, réfutant l'ancien paradigme du “ milieu refuge ” cher aux premiers biospéologues. Récemment, STOCH (1995) a proposé un modèle synthétique (“ Adaptive Zone Model ”) organisé en trois étapes indépendantes (colonisation / spéciation / radiation) intégrant les principaux facteurs écologiques et environnementaux expliquant l'origine et le développement de la biodiversité des eaux souterraines.

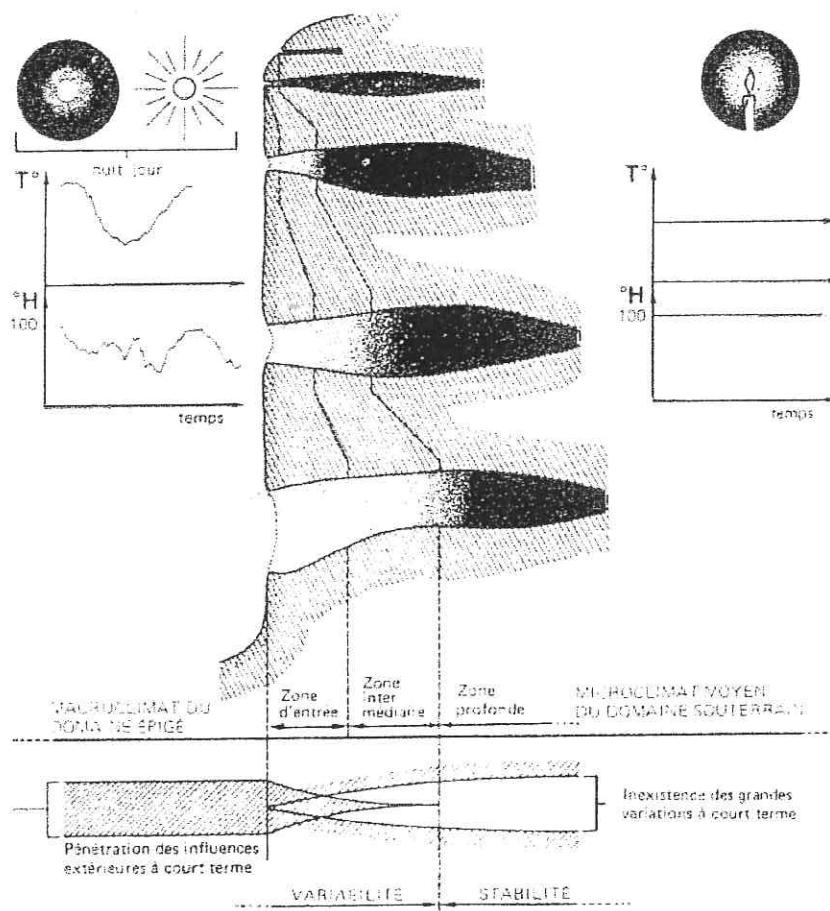
Globalement plus faible que pour les écosystèmes de surface, la biodiversité souterraine est cependant loin d'être négligeable, notamment en raison du fort taux d'endémisme rencontré (JUBERTHIE & DECOU 1994, STOCH 1995).

A l'heure actuelle, cette diversité est estimée à près de 80 espèces de vertébrés (poissons et amphibiens) et plus de 7000 espèces d'invertébrés strictement inféodés au milieu souterrain (troglobies et stygobies) ; les espèces souterraines "facultatives" (troglophiles et stygophiles) en représentent quant à elles plus du double (JUBERTHIE & DECOU 1994). Si ces chiffres apparaissent modestes en regard des espèces épigées (diversité estimée pour les sédiments des cours d'eau, taxons épigés + hypogés : 175 000 espèces, PALMER 1997), il faut cependant tenir compte des difficultés d'accès ainsi qu'à l'effort de recherche consenti sur ces milieux : STOCH (1995) démontre clairement que la richesse spécifique régionale des eaux souterraines est directement liée à l'effort d'échantillonnage et au nombre de taxonomistes en activité. Pour de nombreux taxons, le rythme de description de nouvelles espèces est élevé (REID 1992, COBOLLI SBORDONI 1990) et les espèces déjà connues ne représentent qu'un faible pourcentage du nombre total estimé (PALMER 1997).

Les données récentes tendant ainsi à prouver que la biodiversité des habitats aquatiques hypogés est grandement sous-estimée (STOCH 1995, PALMER 1997).

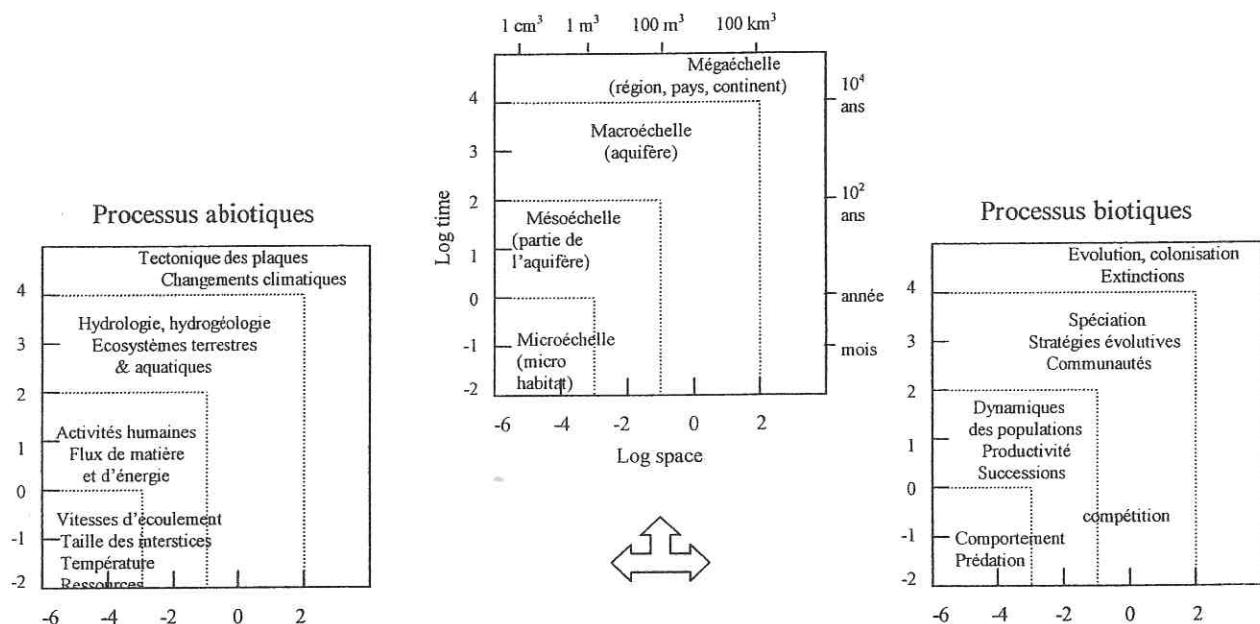
### 3. Facteurs de répartition de la faune

A l'origine, une certaine vision simplificatrice des écosystèmes souterrains se retrouvait couramment dans la littérature scientifique (voir discussion dans DANIELOPOL & ROUCH 1991) : des milieux ramenés à quelques paramètres caractéristiques - obscurité, constance, faibles ressources – peuplés par des associations simples d'espèces à métabolisme réduit, dépigmentées et aveugles. Si le milieu souterrain présente effectivement un ensemble de particularités indiscutables, cette vision réductrice a progressivement évolué à mesure que les études soulignaient l'hétérogénéité à diverses échelles des biotopes souterrains (ex. GINET & DECOU 1977, ROUCH 1992, JUBERTHIE & DECOU 1994, GIBERT et al. 1994... parmi d'autres) ainsi que la grande diversité des réponses des organismes " qui n'incite à conclure, ni à l'uniformité, ni à la simplicité. " (ROUCH 1986) (fig. 5).



**Figure 5.** Comparaison entre l'environnements souterrain et l'environnement superficiel – gradients de stabilité (facteurs climatiques et photiques) (d'après GINET & DECOU 1977).

Dans le domaine aquatique interstitiel, les recherches effectuées depuis lors donnent l'image de systèmes complexes et dynamiques, comprenant des biocénoses diversifiées structurées par un ensemble de facteurs hiérarchisés (synthèse dans GIBERT et al. 1994, WARD et al. 1998). L'explication de la dynamique spatiale et temporelle des peuplements repose sur la prise en compte de multiples échelles d'observation, organisées de façon hiérarchique et fonctionnelle (fig. 6). Cette notion d'échelle apparaît aujourd'hui fondamentale, puisqu'" il y a autant d'écologies que d'échelles d'observation " (FRONTIER & PICHOT-VIALE 1992) ou bien, ce qui revient au même, qu'" il n'y a qu'une seule écologie, laquelle comprend des processus interactifs à toutes les échelles d'espace-temps " (FRONTIER & PICHOT-VIALE, op. cit.).



**Figure 6.** Principales échelles et processus biotiques / abiotiques associés intervenant en écologie souterraine (d'après GIBERT 1994).

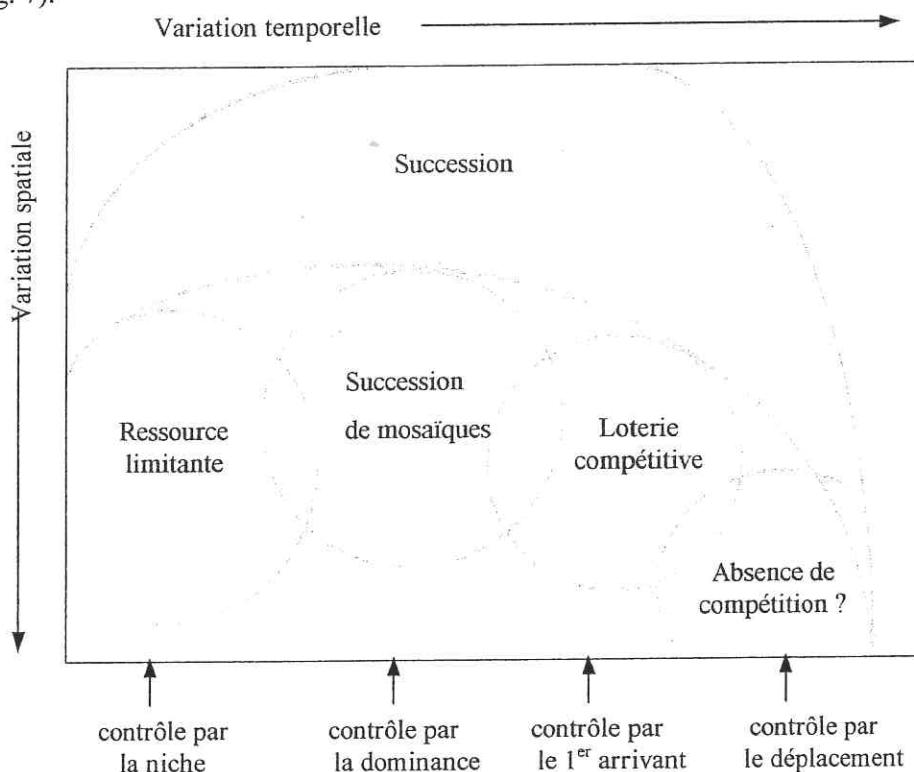
Pour les eaux souterraines, les principaux facteurs d'organisation des écosystèmes peuvent donc être classés selon l'échelle à laquelle ils interviennent (GIBERT et al. 1994, GIBERT 1999) ; on distingue :

- Les facteurs géologiques et climatiques intervenant à très large échelle spatio-temporelle (continentale ou régionale, i.e. "méga-échelle"), contribuant à la formation et aux processus de colonisation des aquifères à l'échelle des temps géologiques ;
- Les facteurs hydrologiques, hydrogéologiques et géomorphologiques à large échelle (échelle de l'aquifère, "macro-échelle"), intervenant dans la distribution, les propriétés des aquifères, les circulations générales d'eau et associés aux changements de structure des communautés biologiques selon divers processus évolutifs (spéciation) ;

- Les facteurs hydrodynamiques, les flux de matière et d'énergie intervenant à une échelle intermédiaire (échelle de l'habitat, "méso-échelle") et responsables de changements environnementaux à moyen terme (perturbations naturelles : inondations, sécheresses ... ou anthropiques : exploitation, contaminations...) suivis de modifications des peuplements (successions).
- Les facteurs locaux, de nature variée, intervenant à petite échelle ("micro-échelle") et responsables de la distribution hétérogène "en mosaïque" constatée à l'échelle métrique, décimétrique... des invertébrés (DANIELOPOL 1989).

#### 4. Faune et perturbations.

Le caractère dynamique des peuplements interstitiels apparaît aujourd'hui comme une caractéristique fondamentale des écosystèmes aquatiques (WARD et al. 1998) et de nombreux modèles conceptuels ont été développés pour rendre compte des rapports entre structure des peuplements et variabilité de l'environnement (ex. "Habitat Templet", SOUTHWOOD 1977, "Patch Dynamic Concept", TOWNSEND 1989, fig. 7).



**Figure 7.** Dynamique des écosystèmes en fonction des niveaux d'hétérogénéité du milieu : modèle du « patch dynamic concept » (d'après TOWNSEND 1989).

Ainsi, les systèmes aquatiques doivent être considérés comme des systèmes "en mosaïque" dans lesquels les communautés s'organisent en fonction des traits biologiques des espèces et du cadre de variabilité spatio-temporelle imposé par le milieu (WARD et al. 1998). A ce titre, la notion de perturbation (« disturbance ») constitue une notion clé pour rendre compte de cette dynamique et s'applique tout autant aux écosystèmes souterrains qu'à ceux de surface (GIBERT et al. 1994, WARD et al. 1998).

Historiquement, la définition du concept de perturbation et son importance dans l'organisation des biocénoses et de la biodiversité ont donné lieu à de nombreuses controverses (revues dans RESH et al. 1988, REICE et al. 1990) et ont scindé l'écologie en deux camps dans les années 50.

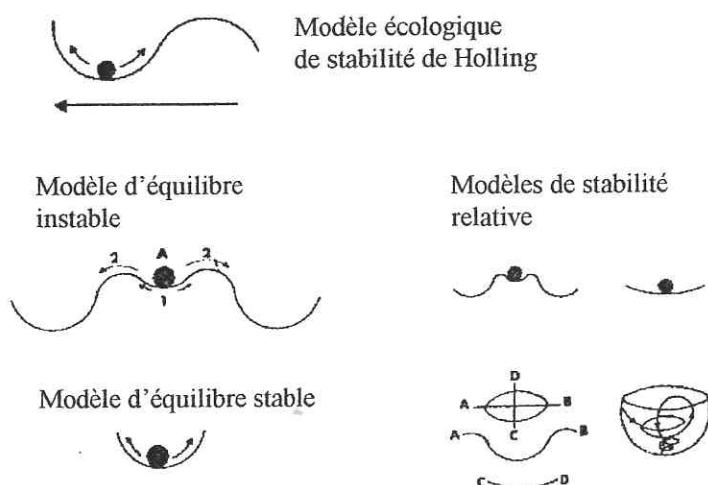


Figure 8. Modèles de stabilité écologique (modifié d'après GRAY 1977)

Ce conflit manifestait l'opposition fondamentale de deux visions des systèmes naturels, dominés pour l'une par les interactions biotiques (phénomènes de densité-dépendance) et pour l'autre par les fluctuations de l'environnement (phénomènes de densité-indépendance). L'émergence de théories reconnaissant le rôle des perturbations comme facteur structurant des écosystèmes (« Intermediate Disturbance Hypothesis », GRIME 1973, CONNELL 1978, « Dynamic equilibrium model », HUSTON 1979) annonça le déclin de la vision dominante de systèmes à l'équilibre dans un environnement quasi-constant (notamment théorie sur la diversité végétale insulaire de MACARTHUR & WILSON 1963, 1967), paradigme trouvant ses racines dans les modèles de compétition et d'interaction prédateur-proie de Lotka-Voltéra (fig. 8).

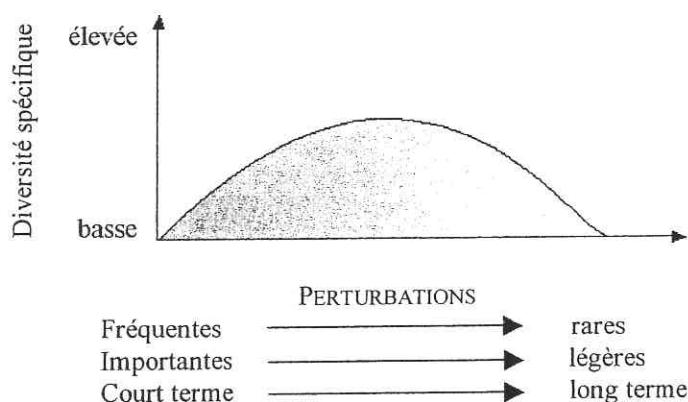
Ainsi, le rôle des perturbations dans l'évolution et le maintien des écosystèmes aquatiques de surface apparaît aujourd'hui majeur (REICE et al. 1990, SEDELL et al. 1990, TOWNSEND et al. 1997, DEATH &

## Introduction

WINTERBOURN 1994, LAMOTTE 1994) et implique la prise en compte de processus agissant à diverses échelles spatio-temporelles. Cette préoccupation se retrouve d'ailleurs dans les définitions du terme « perturbation », depuis BLANDIN & LAMOTTE en 1984 : « Il y a perturbation lorsque telle ou telle variable d'état se trouve à une certaine époque hors de la gamme de valeurs correspondantes sous l'effet de causes accidentnelles, c'est-à-dire non répétitives ou du moins irrégulières à l'échelle de temps considérée », jusqu'aux reformulations plus récentes (RESH et al. 1988) : « perturbation : any discrete event characterized by a frequency, intensity and severity outside a predictable range, that changes resources or physical environment ».

Pour les écosystèmes aquatiques souterrains, malgré une stabilité environnementale comparativement plus importante qu'en surface, les perturbations jouent un rôle majeur sur la structuration du milieu et donc des peuplements (GIBERT et al. 1994, MARMONIER & CREUSÉ DES CHATELLIERS 1991, MARMONIER et al. 1992, WARD et al. 1998, MAUCLAIRE 1999). Le remaniement des alluvions, les variations des niveaux piézométriques ou les modifications physico-chimiques des eaux peuvent ainsi se traduire par une altération des structures de peuplements : crues, inondations, sécheresses, pompages, aménagements artificiels de cours d'eau, contaminations agricoles ou industrielles ... constituent autant de perturbations naturelles ou anthropiques susceptibles d'influencer l'abondance, la diversité et l'organisation des communautés interstitielles.

La théorie des « perturbations intermédiaires » a par exemple été appliquée avec succès à certains peuplements interstitiels du Rhône (DOLE 1983, GIBERT et al. 1994, WARD et al. 1998) : elle postule une diversité maximale des biocénoses pour une stabilité intermédiaire de l'environnement, entre l'exclusion compétitive des espèces les moins performantes dans les milieux très stables et le remplacement de la faune en place par des espèces à haut potentiel de colonisation dans les milieux fortement instables (fig. 9).



**Figure 9.** Variation de la richesse spécifique en fonction des perturbations : hypothèse des perturbations intermédiaires de CONNELL (modifié d'après BARBAULT 1990).

Cette théorie, initialement développée par GRIME (1973) puis popularisée par CONNELL (1978), presuppose une hiérarchie compétitive des espèces ; elle nécessite donc une connaissance approfondie des traits biologiques des espèces qui malheureusement fait encore souvent défaut pour les espèces souterraines (DANIELOPOL 1989, GIBERT et al. 1994, DANIELOPOL et al. 1999).

L'impact des perturbations sur les biocénoses interstitielles se révèle donc complexe (PALMER 1992, BOULTON & STANLEY 1995) et apparaît modulé par de nombreux facteurs hydrogéologiques, hydrodynamiques et écologiques tels que la dynamique des échanges surface/milieu souterrain (ex. zones d'upwelling/downwelling, MARMONIER & CREUSÉ DES CHATELIERS 1991), la présence ou l'absence de refuges (discutée dans WARD et al. 1998), les traits biologiques des espèces (*idem*) ; de fait, la notion d'échelle est une fois de plus fondamentale dans l'étude et la compréhension des mécanismes de réponse des communautés interstitielles aux perturbations (GIBERT et al. 1994, WARD et al. 1998).

## **5. Structuration des peuplements dans l'aquifère.**

Comparativement à la zone hyporhéique, les biocénoses des aquifères alluviaux ont été relativement peu étudiées, en particulier à l'échelle de l'ensemble de l'aquifère (MARMONIER et al. 1992, DANIELOPOL 1991, MAUCLAIRE 1999) ; de même, les contraintes d'échantillonnage limitent généralement l'accès aux zones plus profondes de l'aquifère pour lesquelles les méthodes classiques se révèlent peu adaptées (pompages Bou-Rouch, carottages, substrats artificiels). Néanmoins, même si l'accès au milieu constitue une des principales difficultés en écologie souterraine, les recherches actuelles bénéficient du développement récent de méthodes d'échantillonnage prometteuses (ex. pompes immergées, cf. MALARD et al. 1997, pompes couplées avec des systèmes d'obturateurs pneumatiques (« packer », MAUCLAIRE 1999).

Les principaux facteurs structurant la distribution des invertébrés sont à l'heure actuelle bien connus : il s'agit essentiellement de la disponibilité des ressources énergétiques (teneurs en nutriments) et de la quantité d'oxygène, ce dernier jouant un rôle prépondérant dans le développement et l'organisation des biocénoses interstitielles (synthèse dans MALARD & HERVANT 1998).

La distribution de ces facteurs s'effectue selon l'intensité des relations milieu de surface / aquifère ; de fait, la répartition des organismes dans les nappes s'effectue selon des facteurs géologiques, hydrogéologiques, géochimiques, hydrologiques... variés, aux différentes échelles spatio-temporelles précédemment citées (DANIELOPOL 1991, DOLE-OLIVIER & MARMONIER 1992, GIBERT et al. 1994, MALARD et al. 1994, STRAYER et al. 1997, MÖSSLACHER 1998). Taille et nature des sédiments,

quantité et qualité des nutriments, mode d'alimentation de l'aquifère et transferts d'eau structurent ainsi l'abondance et la diversité des invertébrés dans les aquifères alluviaux.

## **CONTEXTE ET PRINCIPAUX OBJECTIFS DE L'ETUDE**

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Il ressort de l'ensemble de ces considérations que, si les facteurs expliquant l'origine, la répartition et l'évolution des communautés aquatiques interstitielles sont bien connus dans leur ensemble, la complexité intrinsèque des écosystèmes rend leur explication difficile dans bien des cas et nécessite un effort de recherche soutenu.

Pour les raisons historiques brièvement évoquées, principalement les difficultés d'accès au milieu, la connaissance de la faune des aquifères « profonds » (terme peu précis, mais choisi par opposition avec les zones en relation directe avec la surface, zones de transition ou écotones) a progressé moins vite que celle des aquifères superficiels.

L'objectif de ce travail est donc d'apporter des données complémentaires sur les structures de peuplement de ces milieux, et en particulier :

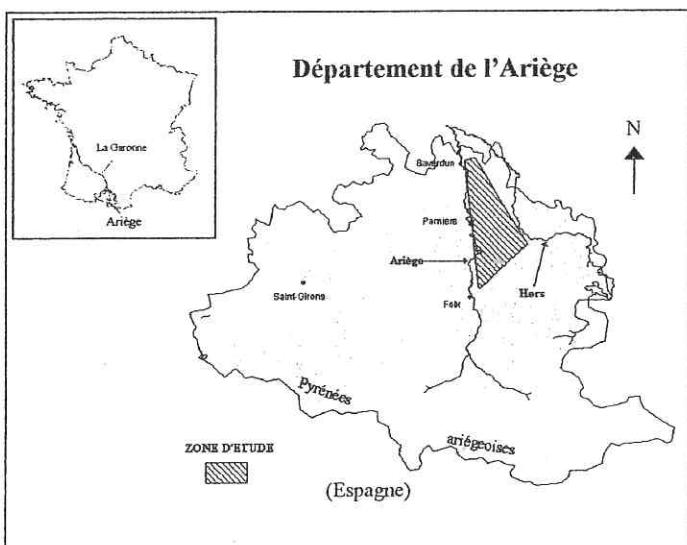
- De caractériser les assemblages et de rechercher l'existence de relations entre les structures de peuplements et les facteurs de l'environnement, dans un aquifère soumis à une pression anthropique marquée (pratiques agricoles intensives) ;
- D'étudier la variabilité spatio-temporelle de l'environnement à l'échelle de l'ensemble de l'aquifère, dans une optique d'évaluation de la stabilité globale du milieu ;
- D'évaluer la persistance temporelle des assemblages observés et la constance globale des peuplements à large échelle (celle de l'aquifère), en relation avec la variabilité environnementale ;
- D'évaluer la pertinence de l'outil potentiel que représentent les biocénoses interstitielles en tant qu'indicateurs de processus naturels ou anthropiques intervenant au sein de l'aquifère : transferts d'eau liés à la perméabilité des sédiments, qualité des eaux influencée par les pratiques agricoles, pompages à partir d'ouvrages de captage.

## ZONE D'ETUDE

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### 1. Localisation

La plaine alluviale de l'Ariège est située dans les Pyrénées ariégeoises, au sud-ouest du bassin Aquitain. Il s'agit d'une plaine au relief à peu près uniforme, s'étirant selon un axe nord-sud sur une distance d'environ 50 km. La vallée de l'Ariège possède la particularité d'être fortement encaissée dans les massifs du Plantaurel constituant le versant nord des Pyrénées ariégeoises, avant de s'élargir pour atteindre sa largeur maximale (à peu près 10 km) au niveau de la butte de Montaut. Elle est bordée par deux rivières principales, l'Ariège à l'ouest et l'Hers (ainsi que l'un de ses affluents, l'Estaut) à l'est. Elle est limitée au sud par les massifs calcaires du Plantaurel et au nord par la confluence Hers-Garonne (fig. 10).



**Figure 10.** Localisation générale de la zone d'étude. Département de l'Ariège, principaux cours d'eau de surface dans le secteur étudié (plaine alluviale de l'Ariège).

La zone étudiée couvre une superficie d'environ 250 km<sup>2</sup> et correspond à la majorité de la plaine alluviale, à l'exception de la bande nord. Elle s'étend de la cluse de Varilhes au sud jusqu'à la région de Saverdun au nord, un peu avant la confluence de l'Ariège et de l'Hers. Il s'agit d'une zone ayant fait l'objet de plusieurs études depuis les années 80 et pour laquelle un ensemble de données géologiques, pédologiques et hydrogéologiques est désormais acquis (CUGNY 1980, SEGUIN 1980) (fig. 11).

### 2. Géomorphologie

Bordée par une suite de coteaux molassiques oligo-miocènes, la plaine de l'Ariège est constituée d'un ensemble de terrasses alluviales emboîtées et séparées par des talus de hauteur variable. La morphologie de ces terrasses date du Quaternaire et fait suite aux différents épisodes climatiques ayant entraîné plusieurs phases successives de dépôt et d'érosion des alluvions.

Facilement différenciables à l'amont et dans l'ensemble de la zone d'étude, ces niveaux de terrasse tendent à se confondre vers le nord au-delà de Saverdun. On distingue, d'ouest en est (fig. 12) :

- **Le lit majeur de l'Ariège et les éléments associés.**

Il s'agit des alluvions les plus récentes, encaissées, formant d'étroites bandes de terrain continues à l'amont de la plaine avec par endroits une succession de gradins étagés qui tendent à s'estomper vers l'aval. Le lit de l'Ariège, comme celui des cours d'eau principaux de la zone, est enfoncé dans l'épaisseur des alluvions ; de fait, la rivière s'écoule par endroits directement sur le substratum molassique, à des niveaux inférieurs aux niveaux piézométriques de la nappe.

- **La basse plaine**

Jouxtant l'Ariège à l'est, elle s'étend sur une largeur moyenne de 3 km entre Pamiers et Saverdun pour atteindre ensuite sa largeur maximale (6 km). D'une superficie moyenne de 100 km<sup>2</sup>, elle domine le lit majeur de l'Ariège d'une hauteur de 10 à 20 m dans la partie amont de la nappe (entre Varilhes et Pamiers), puis cette dénivellation décroît graduellement (8 m à Saverdun) jusqu'à disparaître pratiquement au niveau de la confluence de l'Hers et de l'Ariège.

- **La basse terrasse**

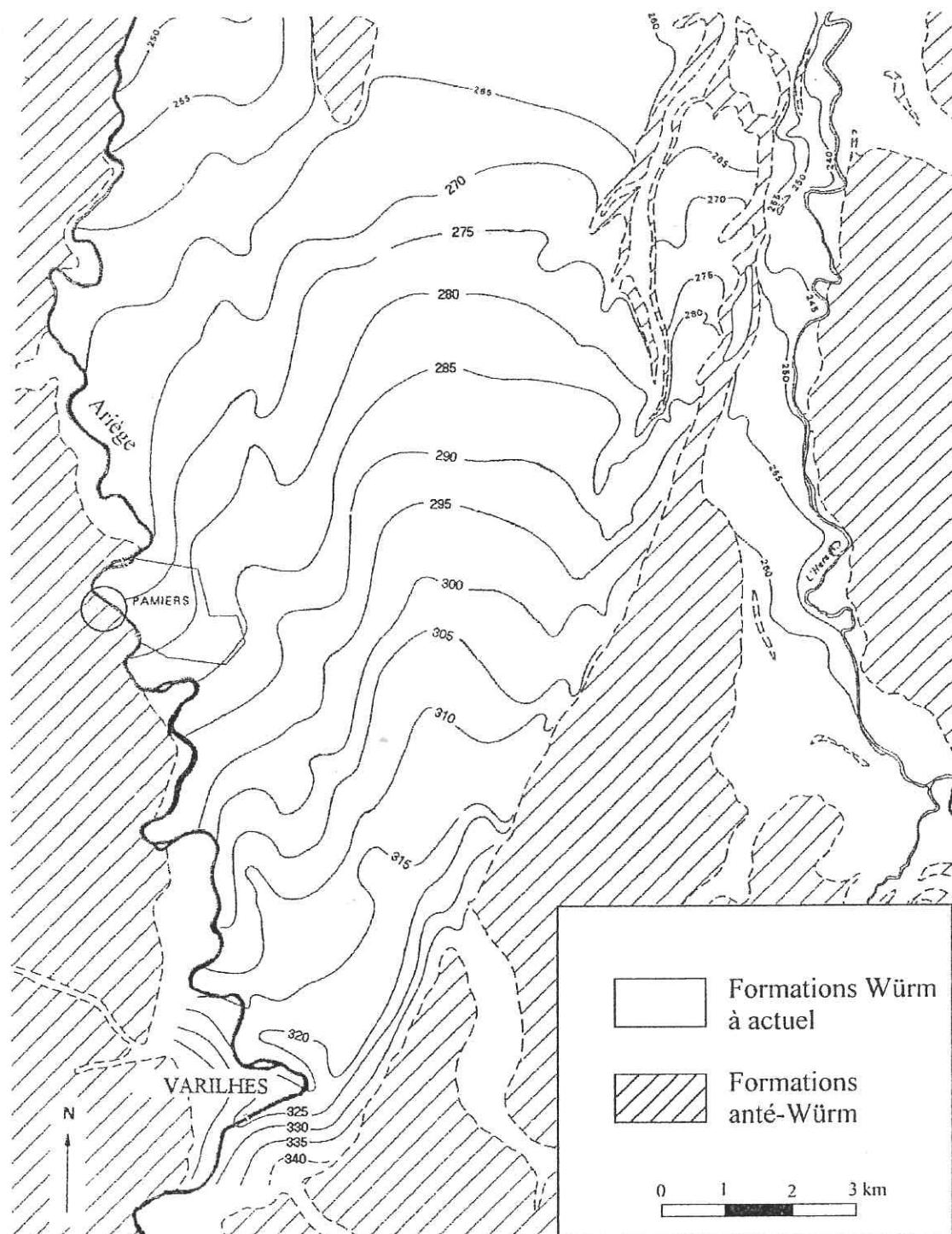
La basse terrasse (ou « basse Boulbonne ») s'étend sur 21 km du nord au sud avec une largeur maximale de 9 km. Avec une pente régulière d'environ 5% du nord vers le sud, elle domine la basse plaine de 4 m au maximum et les étages de l'Ariège de 18 à 30 m.

- **Les niveaux supérieurs**

Il subsiste dans la zone d'étude des éléments appartenant aux niveaux supérieurs plus anciens (hautes et moyennes terrasse), caractérisés par une altération poussée des alluvions contrastant fortement avec les niveaux inférieurs. Fortement étagés, ces niveaux contiennent des nappes fragmentées de faible capacité, fortement colmatées. Il n'en sera pas question dans cette étude.

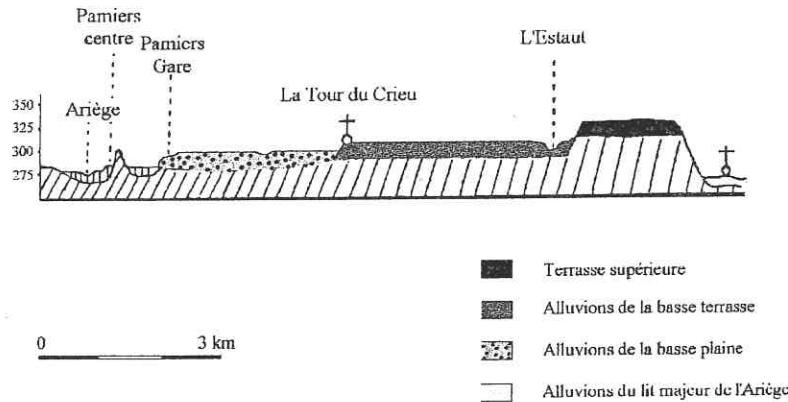
### **3. Structure de l'aquifère.**

Le substratum de l'aquifère est constitué par des dépôts argilo-calcaires oligo-miocènes disposés en couches pratiquement horizontales, avec par endroits des lentilles sableuses. Les alluvions le recouvrant sont d'âge quaternaire, leur ancienneté croissant avec l'altitude : Würm ou post-Würm selon les estimations pour la basse plaine, Riss ou Würm pour la basse terrasse. D'une manière générale, il s'agit de formations gravelo-sableuse associées ou non à une couverture limoneuse :



**Figure 11.** Surface piézométrique de la nappe alluviale de l'Ariège, période de basse eaux — Année 1998.

- lit majeur de l'Ariège : alluvions graveleuse et sableuses ;
- basse plaine : graviers et limon ;
- basse terrasse : graviers surmontés de dépôts argilo-sableux et limons.



**Figure 12.** Coupe géologique de la plaine alluviale de l'Ariège – positions respectives des principales formations (lit majeur, basse plaine, basse terrasse et niveaux supérieurs).

#### 4. Hydrodynamisme.

D'un point de vue hydrodynamique, les deux niveaux inférieurs (basse plaine et basse terrasse) constituent un aquifère unique et continu sur l'ensemble de la zone, ayant pour principales limites :

- L'Ariège et l'Hers, dont les lits encaissés sont situés très généralement au-dessous du niveau de base de la nappe et qui constituent généralement des limites de drainage, voire d'émergence de l'aquifère. Dans l'ensemble de la zone, les cours d'eau de surface peuvent la plupart du temps être considérés comme des cours d'eau drainants vis-à-vis de la nappe, bien que localement le phénomène inverse puisse se produire (zone amont, alluvions du Crieu).
- La zone de contact basse terrasse / haute terrasse à l'est (des sources de l'Estaut à la hauteur de Varilhes) et la zone de fermeture au sud des monts du Plantaurel, apparaissant en partie comme des zones d'alimentation de la nappe (cf. figure 11).

Pour l'essentiel, l'aquifère de l'Ariège peut être considéré comme un aquifère à surface libre, dont l'écoulement bidimensionnel s'effectue selon un axe préférentiel sud-nord s'infléchissant progressivement vers les deux cours d'eau principaux, l'Ariège et l'Hers. A l'échelle de la plaine, l'alimentation du réservoir se fait essentiellement à partir d'infiltations d'eau d'origine météorique, bien que localement les cours d'eau superficiels puissent jouer un rôle non négligeable (zone du Crieu par exemple).

L'étude des paramètres structuraux du réservoir fait apparaître des propriétés conductrices du milieu contrastées entre les différentes zones de l'aquifère :

- Permeabilités de l'ordre de  $10^{-3}$  m.s<sup>-1</sup> pour les alluvions du Crieu ;
- Permeabilités de l'ordre de  $10^{-4}$  m.s<sup>-1</sup> pour les alluvions de la basse plaine ;
- Permeabilités de l'ordre de  $10^{-5}$  à  $10^{-4}$  m.s<sup>-1</sup> pour les alluvions de la basse terrasse (Table 1).

Ces différences sont d'ailleurs observées sur le terrain en fonction de la nature et de l'utilisation des ouvrages de captage d'eau souterraine implantés dans la plaine : la zone du Crieu et celles de la basse plaine possédant les meilleures propriétés conductrices correspondent aux ouvrages d'irrigation à grande échelle, ne tarissant pas en été même lors de pompages soutenus. La zone de la basse terrasse est nettement moins favorable et les puits implantés servent essentiellement à couvrir les besoins domestiques des fermes situées à proximité (eau de consommation, irrigation des jardin, bétail).

**Table 1.** Valeurs de perméabilité (conductivité hydraulique) obtenues sur la zone.

Lieu-dit	Crieu	Basse Plaine	Basse Terrasse	
La Plume	$1.3 \cdot 10^{-3}$ m.s <sup>-1</sup>	-	-	
Roques	-	$5.4 \cdot 10^{-3}$ m.s <sup>-1</sup>	-	
Les Vernèzes	-	$6.6 \cdot 10^{-4}$ m.s <sup>-1</sup>	-	
Péric	-	-	$6.1 \cdot 10^{-4}$ m.s <sup>-1</sup>	
La Baudouine	-	-	$6 \cdot 10^{-5}$ m.s <sup>-1</sup>	
P4	-	-	$10^{-4}$ m.s <sup>-1</sup>	Données
P6	-	-	$10^{-4}$ m.s <sup>-1</sup>	BONHOURE (1999)

## 5. Contexte économique

Selon le découpage INSEE, la zone étudiée appartient à la région agricole de la basse vallée de l'Ariège ; elle est caractérisée par une très faible implantation industrielle et par l'absence de grosses agglomérations (moins de 25 000 habitants au total pour les communes de Foix et Pamiers au recensement de 1998).

La plaine de l'Ariège est un secteur à vocation essentiellement agricole, avec un système de culture majoritaire céréales-prairies à forte prédominance pour les céréales (maïs grain, blé tendre). Entre 1965 et 1990, de vastes efforts de modernisation ont été entrepris suivant la politique agricole communautaire d'autosuffisance alimentaire (remembrements, assainissement des terres, développement de l'irrigation).

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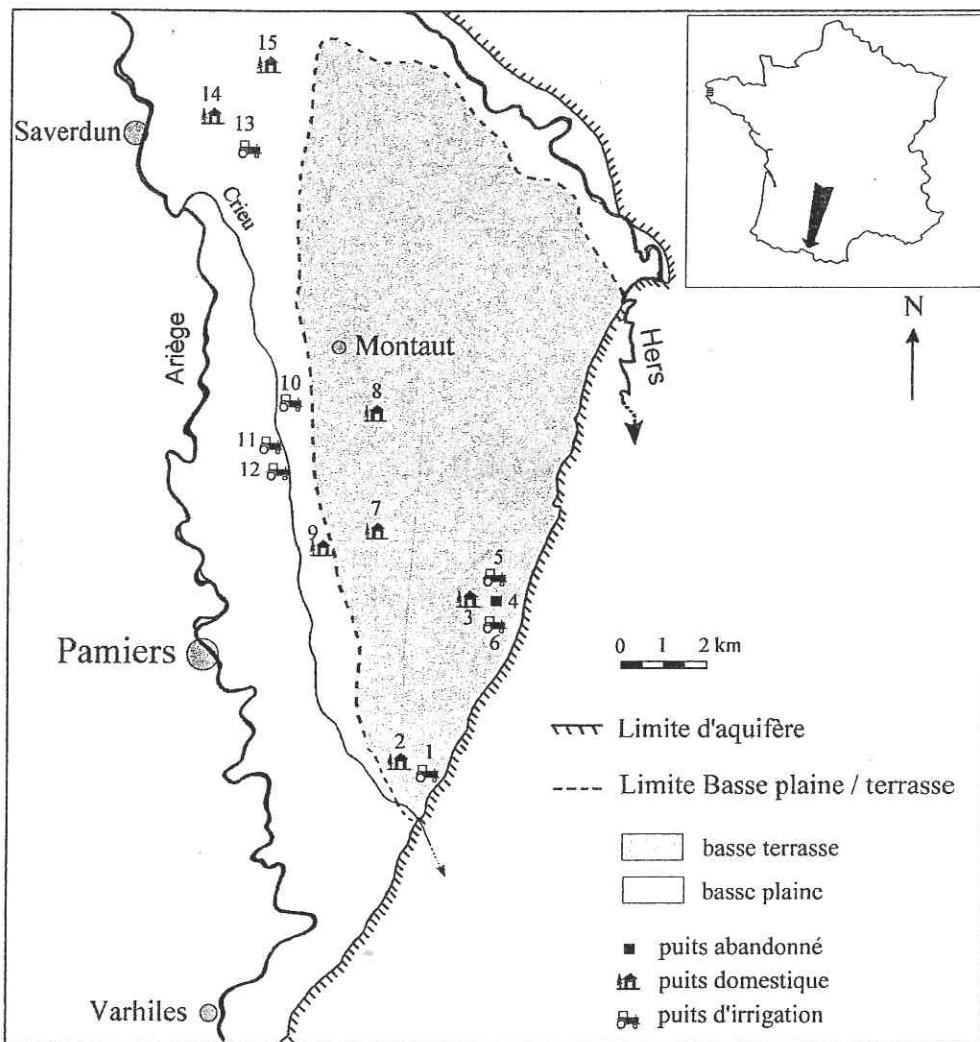
A l'origine d'une forte augmentation de la productivité, ces nouvelles pratiques ont entraîné une dégradation progressive de la qualité de l'eau dans la plaine de l'Ariège. Une augmentation sensible et progressive des taux de nitrates a ainsi été observée depuis les années 80, condamnant de nombreux captages souterrains et aboutissant à une prise de conscience brutale de l'état alarmant du réservoir souterrain. Cette contamination est notamment mise en relation avec les pratiques agricoles intensives adoptées à l'échelle de la plaine, en particulier :

- le niveau élevé de fertilisation azotée minérale employé pour la culture des céréales, souvent surestimé par rapport aux besoins réels ;
- la pratique majoritaire de cultures d'été à forte productivité (maïs) nécessitant une irrigation importante, favorisant le lessivage des éléments solubles en profondeur ;
- l'absence fréquente de couverture des sols en hiver en raison des rotations culturales pratiquées, réduisant fortement la fixation des nitrates par les végétaux.

Parmi les mesures adoptées pour tenter d'enrayer ce processus, de vastes opérations de conseil et d'optimisation des pratiques agricoles ont été mises en place (opération Ferti-Neuf dans la Basse Vallée de l'Ariège), dont les bilans sont encore difficiles à évaluer ; à l'heure actuelle, les taux de nitrates rencontrés dans la nappe souterraine de l'Ariège sont situés aux alentours de 90 – 100 mg/l, soit près de deux fois la norme de potabilité (50 mg/l). Ces teneurs élevées constituent ainsi un témoin direct de la contamination à l'échelle de l'aquifère ; néanmoins, la complexité des phénomènes mis en jeu (origines multiples, historique, transfert, devenir des polluants) constitue une gêne considérable à l'interprétation précise de ce processus.

## **6. Stations échantillonnées**

Les expérimentations ont été effectuées sur un ensemble de 15 stations réparties sur la plaine alluviale de l'Ariège, retenues sur la base de leurs caractéristiques, de leur localisation et de leur utilisation variable à l'échelle de l'ensemble de l'aquifère. Il s'agit de puits de captage des eaux souterraines de 1m à 1,50 m de diamètre et de 4,20 m à 11 m de profondeur, utilisés selon les cas pour l'irrigation des cultures (céréales, vergers), pour les besoins domestiques (consommation courante, arrosage des jardins potagers, bétail) ou abandonnés (fig. 13).



**Figure 13.** Nappe alluviale de l'Ariège : carte des stations échantillonnées, utilisation des puits et contexte hydrogéologique.

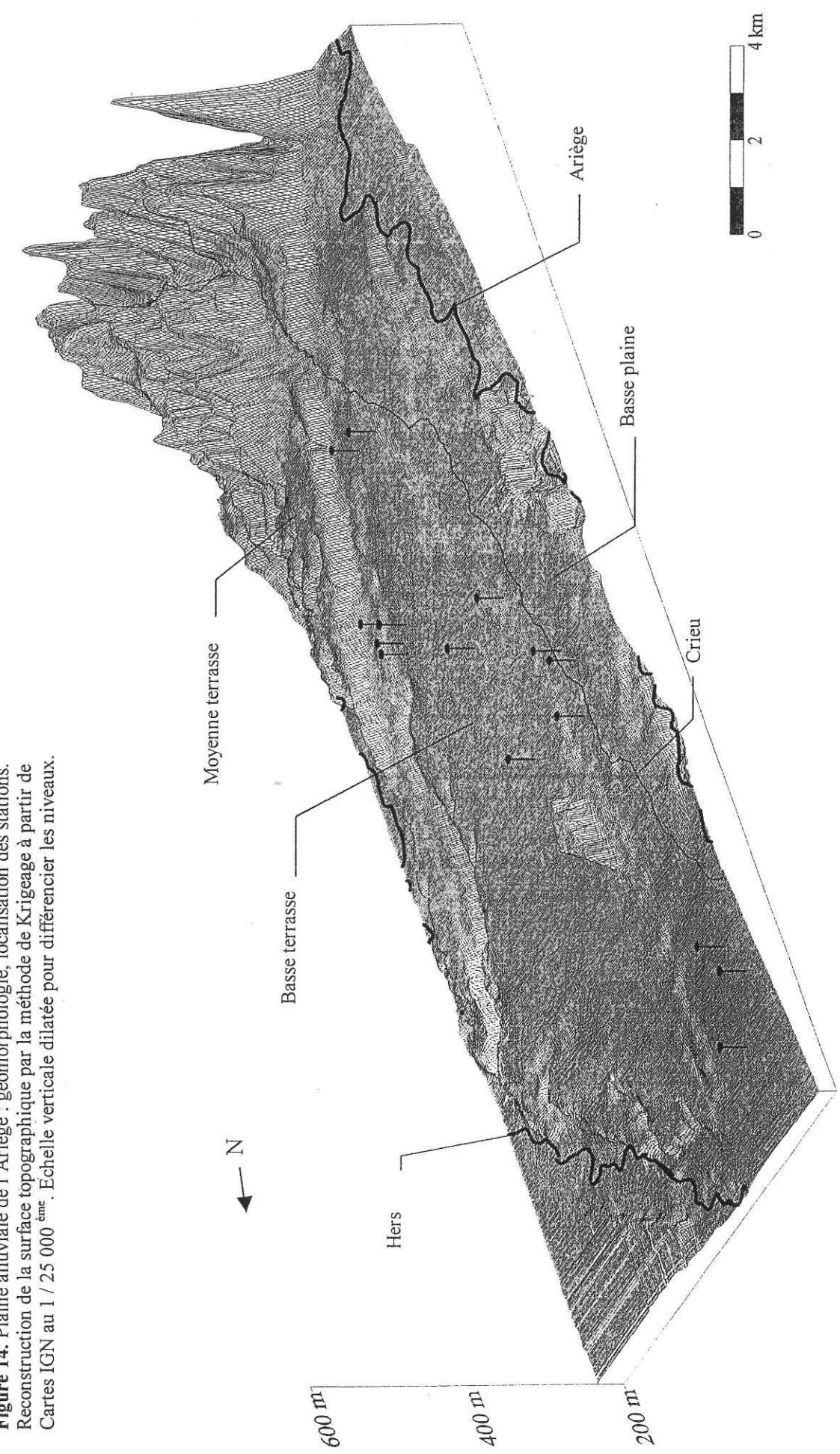
## 7. Faune hypogée

Au total, 24 taxa appartenant aux trois groupes considérés (crustacés cyclopoides, amphipodes et isopodes) ont été récoltés sur l'ensemble de la zone d'étude. La liste des espèces et les stations dans lesquelles elles ont été collectées est donnée dans le tableau 2.

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**Tableau 2.** Liste des espèces récoltées (cyclopides, amphipodes, isopodes) et stations correspondantes.  
(x) espèces récoltées en exemplaire unique. \* espèce stygobie.

CYCLOPOIDA		STATIONS
<i>Macrocylops albidus</i>	JURINE 1820	3, 4, 5, 6, 7, 9
<i>Eucyclops serrulatus</i>	FISCHER 1851	3, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15
<i>Tropocyclops prasinus</i>	FISCHER 1860	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14
<i>Paracyclops fimbriatus</i>	FISCHER 1853	1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15
<i>Acanthocyclops viridis</i>	JURINE 1820	(x)
<i>Diacyclops languidus</i>	SARS 1863	
<i>Diacyclops languidoides*</i>	LILLJEBORG 1901	
<i>Diacyclops clandestinus*</i>	KIEFER 1926	
<i>Diacyclops bicuspispidatus</i>	CLAUS 1857	12, 13, 14
<i>Diacyclops bisetosus</i>	REHBERG 1880	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15
<i>Graeteriella unisetigera*</i>	GRAETER 1910	2, 4, 7
<i>Graeteriella sp.*</i>		1, 3, 14
<i>Speocyclops sp.*</i>		2, 11
AMPHIPODA		
<i>Salentinella ginetti*</i>	BALAZUC 1957	1, 8, 10, 11, 12, 13, 14, 15
<i>Salentinella petiti*</i>	COINEAU 1963	1, 3, 5, 6, 9, 10, 12, 13, 14, 15
<i>Niphargus gineti*</i>	BOU 1965	6, 9, 10, 11, 13
<i>Niphargus pachypus*</i>	SCHELLENBERG 1933	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
<i>Niphargus robustus*</i>	CHEVREUX 1901	5, 6
<i>Niphargus gallicus*</i>	SCHELLENBERG 1935	7, 8, 13, 15
<i>Niphargus n. sp.*</i>		5
<i>Ingolfiella thibaudi*</i>	COINEAU 1968	(x)
ISOPODA		
<i>Stenasellus virei*</i>	DOLFFUSS 1897	1, 2, 3, 4, 6, 7, 8, 13, 14
<i>Microcharon rouchi*</i>	COINEAU 1968	3, 6, 14
<i>Proasellus meridianus</i>	RACOVITZA 1919	5, 6



**Figure 14.** Plaine alluviale de l'Ariège : géomorphologie, localisation des stations.  
Reconstruction de la surface topographique par la méthode de Krigage à partir de  
Cartes IGN au 1 / 25 000<sup>ème</sup>. Echelle verticale dilatée pour différencier les niveaux.



## ORGANISATION DU DOCUMENT.

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Ce mémoire est organisé en quatre chapitres, correspondant aux grands thèmes de cette étude. Ces chapitres reprennent, en les complétant, les données déjà publiées ou en cours de publication et se présentent donc sous forme d'articles.

### **CHAPITRE 1. Sampling the fauna in aquifers: a comparison of net-sampling and pumping.**

Cette première partie présente les résultats d'une étude comparative de deux méthodes utilisées pour l'échantillonnage de la faune dans les aquifères : le pompage, à l'aide d'une pompe mobile immergée, et l'utilisation d'un filet phréatobiologique.

Testées sur leur capacité à décrire les structure de diversité et de dominance des assemblages phréatiques, ces méthodes montrent une efficacité tout à fait similaire en dépit de la variabilité des situations habituellement rencontrées sur le terrain. Dans certaines limites, et en raison de nombreux avantages pratiques (simplicité, rapidité, efficacité), le filet phréatobiologique peut donc constituer une alternative performante aux dispositifs de pompage classiquement utilisés.

Une partie des résultats de ce chapitre a fait l'objet d'une publication :

DUMAS, P. & FONTANINI, G. (2000). Sampling the fauna in aquifers, a comparison of net-sampling and pumping. *Archiv für Hydrobiologie* (sous presse).

### **CHAPITRE 2. Faunal distribution in an agriculturally-impacted alluvial aquifer.**

Cette deuxième partie est consacrée à l'étude de l'influence d'une contamination d'origine agricole sur les assemblages de Crustacés interstitiels d'un aquifère.

L'analyses des paramètres écologiques des communautés (structures de diversité, d'abondance, de dominance) fait apparaître une structuration spatiale marquée des assemblages à l'échelle de l'aquifère. Cette hétérogénéité n'est pas expliquée par les distributions spatiales ou temporelles des paramètres physico-chimique des eaux souterraines. Dans l'ensemble, les variables environnementales et en particulier les nitrates ne constituent pas un facteur de structuration majeur des communautés interstitielles dans la zone étudiée.

Une partie des résultats de ce chapitre a fait l'objet d'une publication :

DUMAS, P. & LESCHER-MOUTOUÉ, F. (2000). Cyclopoid distribution in an agriculturally-impacted alluvial aquifer. *Archiv für Hydrobiologie* (sous presse).

## *Introduction*

### **CHAPITRE 3. Groundwater crustaceans as indicators of aquifer hydrodynamics and utilization.**

Ce chapitre est consacré à l'étude de l'impact des facteurs du milieu, d'origine naturelle (structure de l'aquifère) ou anthropique (exploitation du réservoir), sur les structures de peuplement observées.

L'analyse de la structure des assemblages témoigne de variations significatives de composition en fonction du contexte hydrogéologique des stations et, dans une moindre mesure, du régime d'exploitation des captages. Malgré l'existence d'une variabilité locale liée à l'hétérogénéité intrinsèque du milieu alluvial, les crustacés souterrains constituent ainsi des indicateurs naturels à l'échelle de l'aquifère.

Une partie de ces résultats fait actuellement l'objet d'une publication :

DUMAS, P., BOU, C., & GIBERT, J. Groundwater macrocrustaceans, natural indicators of aquifers.

Soumis à *International Review of Hydrobiology*.

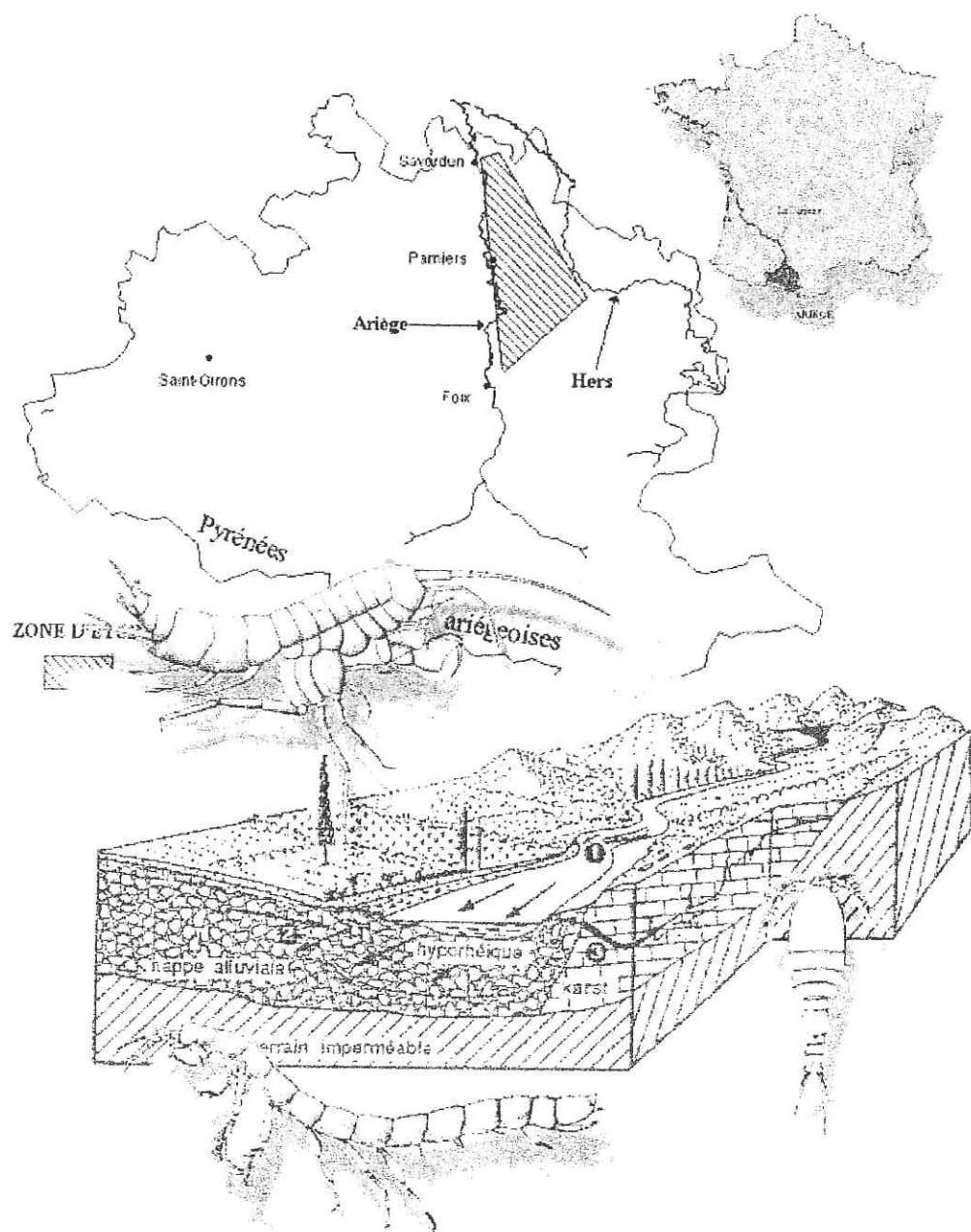
### **CHAPITRE 4. Stability of interstitial crustacean communities and environmental variability in an alluvial aquifer.**

La dernière partie de ce mémoire concerne l'étude de la stabilité temporelle des assemblages en relation avec la variabilité du milieu.

Les résultats témoignent d'une persistance très marquée des structures de diversité et de dominance des crustacés souterrains, associée à une constance des paramètres environnementaux de l'aquifère. Dans le contexte de cette étude, ces observations préliminaires suggèrent l'existence d'une relation directe entre le degré de confinement des aquifères et la variabilité temporelle des communautés interstitielles.

Un article est actuellement en préparation à partir de ces résultats.

# - CHAPITRE 1 -





## **Sampling the fauna in aquifers: a comparison of net-sampling and pumping.**

### **ABSTRACT**

Two devices frequently used for sampling the groundwater fauna in aquifers were compared: the phreatobiological net-sampler and the centrifugal pump. These two methods were tested in a French Pyrenean alluvial aquifer at two contrasting seasons. Macrocrustacean (amphipods, isopods) and microcrustacean taxa (cyclopoids) were collected from 15 wells with different characteristics (piezometric level, depth, water extraction, hydrogeological settings) in order to compare the relative abundance patterns obtained with the two methods. Very similar patterns were observed using either the phreatobiological net or the pump, whatever taxa were considered. Species characteristics (size, ecological preferences, locomotory behavior) did not influence the results. The global efficiency of the methods appeared to be dependent on the sample size, i.e. on the abundance of species collected. The advantages and disadvantages of the two methods were discussed relative to the various sampling criteria (framework, damage to fauna, ease of use, convenience). Under some conditions, the phreatobiological net-sampler could be considered a valuable alternative to pumping in aquifers.

## **INTRODUCTION**

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Despite the development of various sampling methods, collecting the fauna from deep subterranean habitats is still a difficult task. Since the beginnings of groundwater ecology, practitioners have had to overcome methodological difficulties to obtain qualitative, and then quantitative data on the groundwater invertebrate communities (GIBERT et al. 1994, MALARD et al. 1997).

While sampling techniques have been developed for a long time on shallow interstitial habitats, some authors emphasized that by comparison, little work has been done to collect the fauna from deep water-table aquifers (MATHIEU et al. 1991, MALARD et al. 1994, MAUCLAIRE et al. 1998). At present, groundwater invertebrates are usually removed from aquifers using either pumping devices (see MALARD et al. 1997), net samplers (CVETKOV 1968, BOU 1974), colonization samplers (GINET & DECU 1977, TABACCHI 1990), coring techniques (COLEMAN & HYNES 1970, STOCKER & WILLIAMS 1972, BRETSCHKO 1985) or a combination of these methods (BOUTIN & BOULANOUAR 1983). Yet, comparative studies on the efficiency of these sampling techniques still remain rare (MAUCLAIRE et al. 1998).

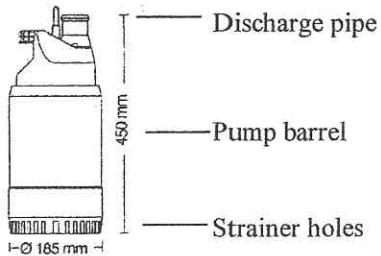
The objective of this work was to compare two methods for sampling the fauna in aquifers: net sampling (a "quantitative" technique) and pumping (a "qualitative" one). The comparative criterion was their respective suitability and efficiency in describing the structure of groundwater invertebrates communities.

We used a phreatobiological net and an immersed centrifugal pump to collect macro- and microcrustacean from fifteen low water-table wells at two contrasting seasons of the year 1999. The efficiency in describing the community structure of the phreatic assemblages was evaluated for the two devices using the relative abundances for the common taxa (amphipods, isopods, cyclopoids).

## MATERIAL AND METHODS

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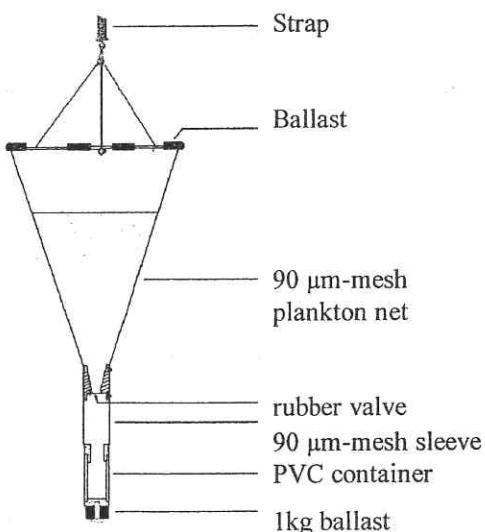
### 1. Pumping device



We used an immersed centrifugal FLYGT pump (READY 8 model) equipped with a 0.75 kW motor, originally designed for operating in domestic water supply systems. The barrel of the pump was 0.450 m long and 0.185 m in diameter. It had several 0.8 x 1.5 cm wide slots in its lower part. The pump was connected to a 4.5 cm diameter flexible PVC discharge pipe and was supplied from a 2.2 kVA portable generator. According to manufacturer's specifications, the theoretical maximum discharge rate was about  $20 \text{ m}^3 \cdot \text{h}^{-1}$ . In the field, the pump was immersed down to the bottom of the wells.

After preliminary tests, the extracted volume of water was fixed at 2  $\text{m}^3$  per well, to evaluate statistically significant crustaceans populations. The samples were strained through a 90  $\mu\text{m}$ -mesh plankton net and were immediately preserved in 70% ethanol.

### 2. Net-sampling.



A net sampler derived from the phreatobiological net originally described by CVETKOV (1968) was used in field trials (cf. BOU 1974). It was made up of :

- A 0.45 m-long, 90  $\mu\text{m}$ -mesh cone-shaped plankton net, 0.30 m in diameter;
- A small PVC container, 0.10 m long, 0.05 m in diameter, bound at the extremity of the net and ballasted with a 1 kg weight.

In the field, sampling was achieved by drawing the net sampler 10 times through the entire water column. A small rubber valve placed inside the container avoided invertebrates escape during the up-and-down movements of the device. The samples were immediately preserved in 70% Ethanol following collection.

### 3. Counting and identification of the fauna.

As other taxa exhibited very low abundances and/or occurrence, only crustaceans were considered in this study, i.e. macrocrustaceans (amphipods, isopods) and microcrustaceans (cyclopoids) that were strongly dominant in the samples. In the laboratory, all the macrocrustaceans were sorted and counted using Dolfuss chambers. Nine species were identified:

<i>Salentinella gineti</i>	BALAZUC 1957	<i>Niphargus gallicus</i>	SCHELLENBERG 1935
<i>Salentinella petiti</i>	COINEAU 1963	<i>Niphargus</i> n. sp.	
<i>Niphargus gineti</i>	BOU 1965	<i>Proasellus meridianus</i>	RACOVITZA 1919
<i>Niphargus pachypus</i>	SCHELLENBERG 1933	<i>Stenasellus virei</i>	DOLFUSS 1897
<i>Niphargus robustus</i>	CHEVREUX 1901		

For the microcrustaceans, Bengal rose was added to stain the animals and make them more distinguishable. They were sorted and counted using Dolfuss chambers under a stereomicroscope. For samples with very high animal densities, sub-sampling was used. Afterwards, the specimen were mounted in glycerine, identified and counted using a Nchet microscope.

Eight species were identified at species levels. Some other species were not easily recognizable without dissection, so identification was performed at genus level for *Speocyclops* sp. and *Graeteriella* sp. In the same way, *Diacyclops languidus*, *Diacyclops languidoides* and the very rare *Diacyclops clandestinus* were assigned together to *D.* spp.:

<i>Macrocylops albidus</i>	JURINE 1820	<i>Diacyclops bicuspidatus</i>	CLAUS 1857
<i>Eucyclops serrulatus</i>	FISCHER 1851	<i>Diacyclops bisetosus</i>	REHBERG 1880
<i>Tropocyclops prasinus</i>	FISCHER 1860	<i>Graeteriella unisetigera</i>	GRAETER 1910
<i>Paracyclops fimbriatus</i>	FISCHER 1853	<i>Graeteriella</i> sp.	
<i>Acanthocyclops viridis</i>	JURINE 1820	<i>Speocyclops</i> sp.	
<i>Diacyclops languidus</i>	SARS 1863		
<i>Diacyclops languidoides</i>	LILLJEBORG 1901		
<i>Diacyclops clandestinus</i>	KIEFER 1926		
			Assigned to <i>Diacyclops</i> spp.

#### **4. Sampling sites.**

Sampling took place in April and October 1999 (high water period / low water period). The sampling stations were scattered over an alluvial floodplain located close to the northern border of the French Pyrénées, the Ariège river floodplain.

All the stations were agricultural or domestic wells, 1-1.50 m in diameter, with depth ranging from 8 to 15 m. Piezometric levels in the area were between -0.50 to about -8.50 m below the soil for the periods considered. Overall, two groups of stations could be distinguished by their hydrogeological characteristics: wells located on the most transmissive part of the aquifer (the low plain, with average transmissivity about  $10^{-3} \text{ m}^2 \cdot \text{s}^{-1}$ ), and wells located on the lower transmissive area (the low terrace, with transmissivity about  $10^{-4} \text{ m}^2 \cdot \text{s}^{-1}$ ).

Water extraction differed from site to site in relation to the general use of ground water: some wells were exclusively for cereal irrigation, with strong, seasonal water extraction from July to September. Some other wells were for everyday home and cattle consumption, with permanent, moderate water extraction. Some wells were abandoned, groundwater extraction having been stopped for 2-3 years. At each station, the invertebrates were collected using the two devices tested.

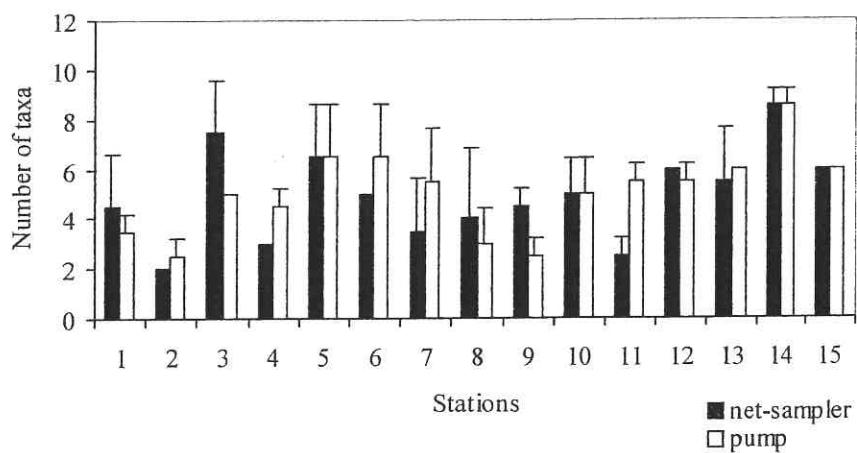
## RESULTS

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### 1. Collected taxa.

In the area, the phreatic communities were dominated by microcrustacean taxa. Harpacticoids exhibited very low abundances (1.4 % of copepod abundance), thus they were not considered in this study. Cyclopoids dominated the fauna with epigean taxa (*P. fimbriatus*, *T. prasinus*, *E. serrulatus*) associated with hypogean genera or species (*Graeteriella*, *Speocyclops* or *Diacyclops* spp.) (DUMAS & LESCHER-MOUTOUÉ 2000). They were found at all the study sites and represented 98.3 % of the total abundance. Macrocrustaceans were represented by isopod and amphipod taxa, these latter being found in higher abundances and occurrence (amphipods: 97.3 % of the total macrocrustacean abundance, found at all the sites / isopods: 2.7 % of macrocrustacean abundance, found at 7 of the 15 sites). Eight of the nine collected species were exclusively groundwater-dwelling animals (stygobites). The only stygoxen species was the isopod *Proasellus meridianus*, collected at 2 shallow water-table stations.

**Figure 1.** Taxonomic richness in the 15 stations of the Ariège alluvial aquifer (number of cyclopoid, amphipod and isopod taxa per sample collected with the phreatobiological net-sampler and with the centrifugal pump.) Means and standard deviations (vertical  $\pm$  symbol).



In the wells, the taxonomic richness was higher for the microcrustacean species than for macrocrustaceans (Table 1). The total number of taxa collected using the two different methods was plotted on Fig. 1. The mean number of collected taxa per sample was very similar using either the pump or the net-sampler (Student' T test,  $n = 15$ ), whatever faunal groups were considered (isopods, amphipods or cyclopoids).

**Table 1.** Taxonomic richness per sample in the 15 wells for (mean number of taxa per sample collected using the phreatobiological net and the centrifugal pump, variance, Student's T test coefficient and probability.)

Collected taxa	Microcrustaceans		Macrocrustaceans	
	Net-sampler	Pump	Net-sampler	Pump
Mean	3.07	3.2	1.87	1.87
Variance	1.26	0.69	0.98	0.78
T		0.36		$3 \cdot 10^{-7}$
$\alpha$ probability		0.72		1
Test		non significant		non significant

## 2. Abundance patterns for microcrustaceans

### 2.1 Relative abundance patterns

In order to evaluate their efficiency in describing the community structure of the phreatical assemblages, the two sampling devices were compared using the relative abundances of the cyclopoid species in the 15 wells (Fig. 2a for the net-sampler and fig. 2b for the pump). The very rare species in term of abundance and occurrence were not considered (abundance  $\leq 0.1\%$ , species found in a sole station: *Acanthocyclops viridis*, *Diacyclops bicuspis*, *Speocyclops* sp., *Graeteriella unisetigera*, *Graeteriella* sp.).

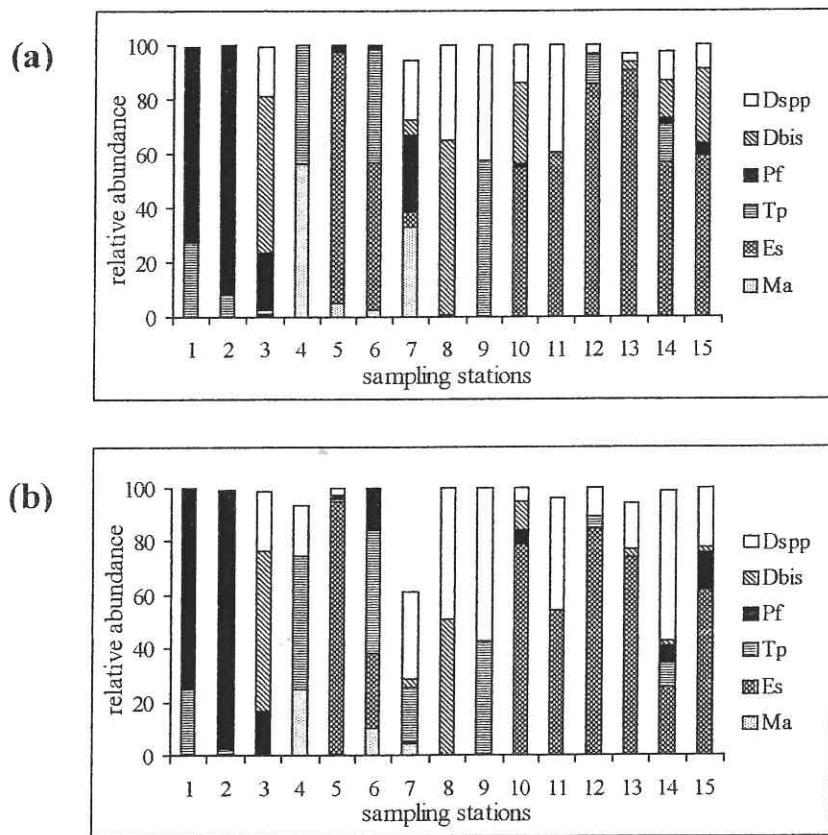
For most sites, the abundance patterns obtained exhibited only small differences whatever the sampling method. In particular, samples collected using either the net-sampler or the pump in wells 1, 2, 3, 5, 11, 12 could on the whole be considered similar (maximum differences in relative abundances  $< 10\%$ ). Samples from wells 8, 9, 13 also exhibited strong resemblance (maximum differences  $\sim 15\%$ ) while the samples from wells 4, 6, 10, 15 showed similar trends with higher differences (25-30%). Samples 7 and 14 were the exceptions, with cyclopoid abundance patterns that were significantly different for the phreatobiological net and the pump. These samples were also characterized by the lowest abundances of cyclopoids (mean individual number per sample for all the wells  $n = 1538$ ; well 7:  $n = 64$ ; well 14:  $n = 127.5$ ).

### 2.2 Multivariate analysis

In order to investigate more precisely the effects of each sampling method on the resulting patterns, the collected samples were analysed using factorial analyses (factorial correspondence analysis, FCA

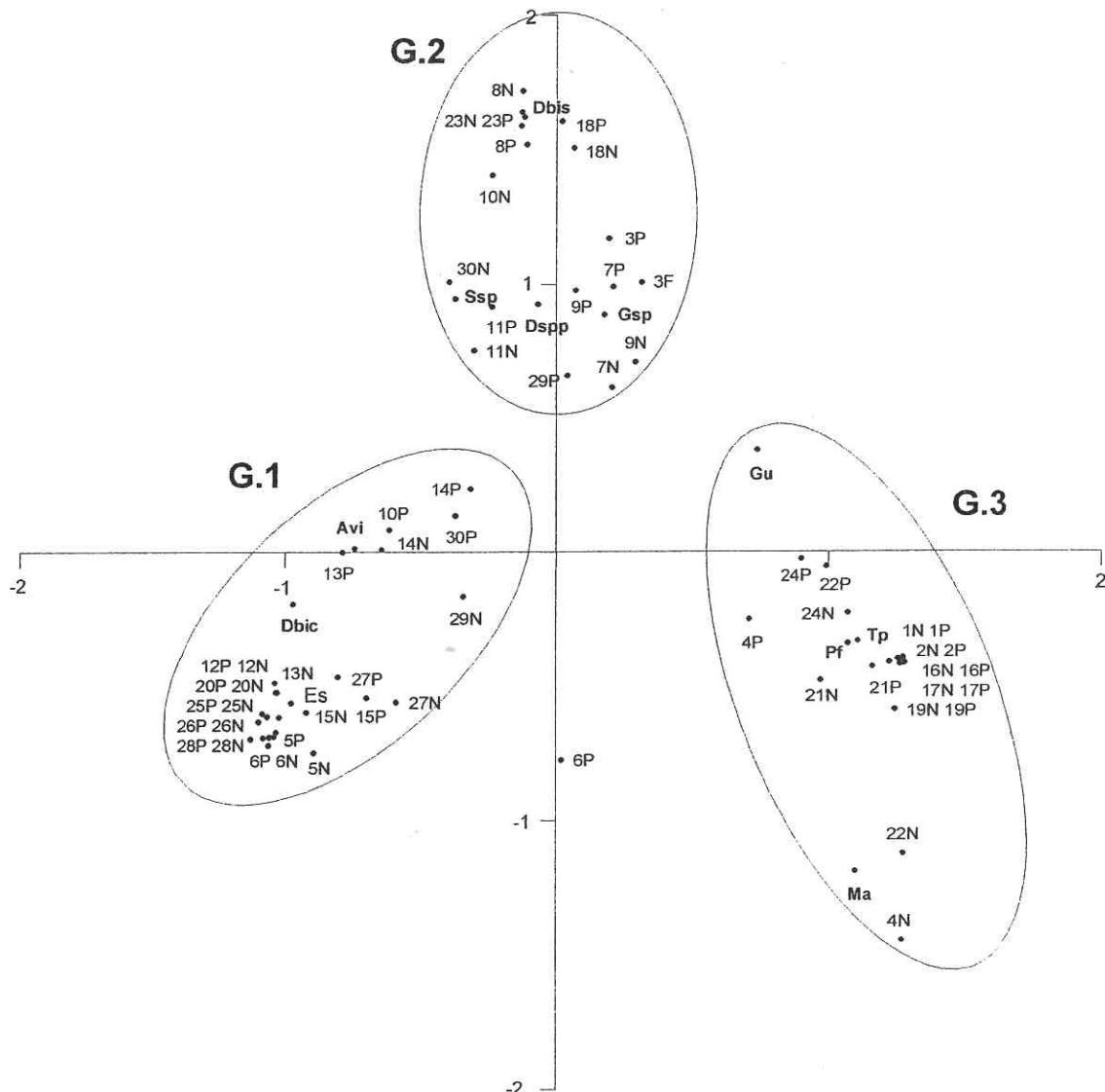
hereafter). Fig. 3 shows the ordination diagram (factor 1 x factor 2) from FCA for the 11 x 60 data matrix, i.e. the relative abundances of the cyclopoid species from the 15 wells in April and October 1999, using either the net-sampler or the pump. All the species, including the rarer ones (<0.05 % of total abundance) were taken into account. To allow the relationships between groups of similar samples to be better appreciated, factorial discriminant analyses (FDA hereafter) were performed on the data.

**Figure 2.** Patterns of relative abundances for the most common cyclopoid species collected in the 15 stations using the tested sampling methods. (a): phreatobiological net-sampler (b): centrifugal pump.  
Dspp: *Diacyclops* spp. ; Dbis: *Diacyclops bisetosus* ; Pf: *Paracyclops fimbriatus* ; Tp: *Tropocyclops prasinus* ; Es: *Eucyclops serrulatus* ; Ma: *Macrocylops albidus*.



Three main groups of samples (G.1, G.2, G.3) could be clearly discriminated along the F1/F2 axes (Table 2,  $p<0.001$  for Wilk's test). Strong similarities were observed within these groups for the samples collected with the net-sampler and the pump in a given well, on the same date: on the ordination diagram, in most cases they both belong to the same biological group and thus exhibit similar patterns of relative abundances. Altogether, 26 of the 30 pairs of samples (collected with the net-sampler / the pump) constitute closely matched-pairs in the same group. The only exceptions were the samples 6, 10, 29 and 30, i.e. wells 6, 10 in April 1999 and wells 14, 15 in October 1999 respectively.

FDA were then performed on a re-arranged data set so as to test the hypothesis that the sampling methods influenced the relative abundance patterns. The samples collected using the net-sampler and the pump were separated and the discrimination between these two groups was tested. The very low discriminant power (0.1) allowed us to reject the hypothesis that the observed biological structures in the samples could be induced by the sampling method used (Wilks' test,  $W = 5.96$ ,  $\alpha$  probability = 0.546).



**Figure 3.** Results of a factorial correspondence analysis for the cyclopoid species collected with the two tested methods in the 15 wells. All the species were considered. Contribution to total variance of the two first axes : 42 %. Samples with letter N : collected with the net-sampler, samples with letter P : collected with the pump.

Sample numbers 1-15 : April 1999, 16-30 : October 1999.

Dspp: *Diacyclops* spp. ; Dbis: *Diacyclops bisetosus* ; Dbic: *Diacyclops bicuspis* ; Pf: *Paracyclops fimbriatus* ; Tp: *Tropocyclops prasinus* ; Es: *Eucyclops serrulatus* ; Ma: *Macrocylops albidus* ; Gsp: *Graeteriella* sp. ; Gu: *Graeteriella unisetigera* ; Ssp: *Speocyclops* sp. ; Avi: *Acanthocyclops viridis*.

**Table 2.** Matching of the samples and results from FDA for cyclopoid species collected using the two tested devices. Discriminant power, Wilk's test and  $\alpha$  (type I-error) probability.

% of paired samples (net-sampler / pump)	86.7	
Discriminant analysis :	F1 axis	F2 axis
Discriminant power	0.99	0.89
W (Wilks' coefficient)	590.4	116.1
$\alpha$ probability	< 0.001	< 0.001

### 3. Abundance patterns for macrocrustaceans

#### 3.1 Relative abundance patterns

Fig. 4 shows the relative abundance patterns for macrocrustaceans (amphipods / isopods) collected in the wells in April and October 1999. The patterns were on the whole very similar for the samples collected with the phreatobiological net and with the pump. Samples from wells 1, 2, 4, 10, 12, 15 exhibited strong resemblance (maximum differences in species relative abundances < 10 %) while samples from wells 6, 14, 3, 11 were characterized by global similarities (maximum differences 15-30 %). The samples with markedly different patterns were collected in wells 7, 8, 9, 13 and were characterized by very low abundances (well 7, 8, 9: mean abundance per sample < 5 / well 13: mean abundance per sample < 10).

#### 3.2 Multivariate analysis

The data were investigated with FCA (Fig. 5) for the 9 species collected in the wells with either the net-sampler or the pump. All the species were considered including the rarer ones. FDA were then performed in order to separate groups of samples in relation to their patterns of relative abundances. As macrocrustaceans exhibited lower abundances in the samples than microcrustaceans (cyclopoids: n (mean individual number per sample) = 1538 / amphipods+isopods: n = 31.5), the sampling devices were compared for:

- 1) all the samples, i.e. 53 samples;
- 2) samples with abundance  $\geq 5$ , i.e. 45 samples;
- 3) samples with abundance  $\geq 15$ , i.e. 32 samples.

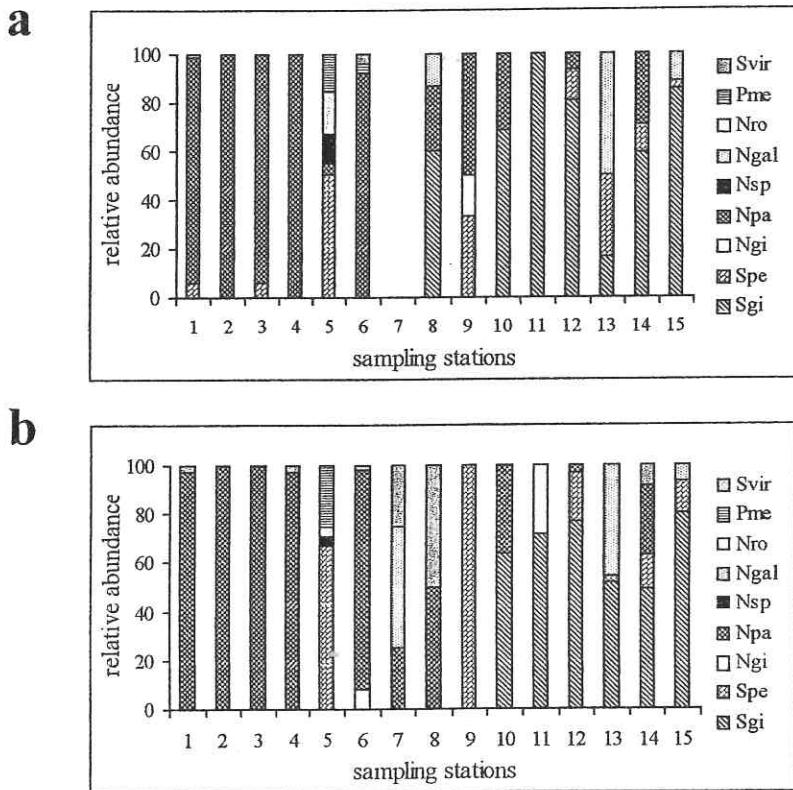
The results are provided in table 3.

**Fig. 4.** Patterns of relative abundance for the most common amphipod + isopod species collected in the 15 stations using the tested sampling methods. Sample 7a empty (no specimens collected).

a: phreatobiological net-sampler

b: centrifugal pump.

Species: Svir: *Stenasellus virei*, Pme: *Proasellus meridianus*, Nro: *Niphargus robustus*, Ngal: *Niphargus gallicus*, Nsp: *Niphargus* n. sp., Npa: *Niphargus pachypus*, Ngi: *Niphargus gineti*, Spe: *Salentinella petitii*, Sgi: *Salentinella gineti*.



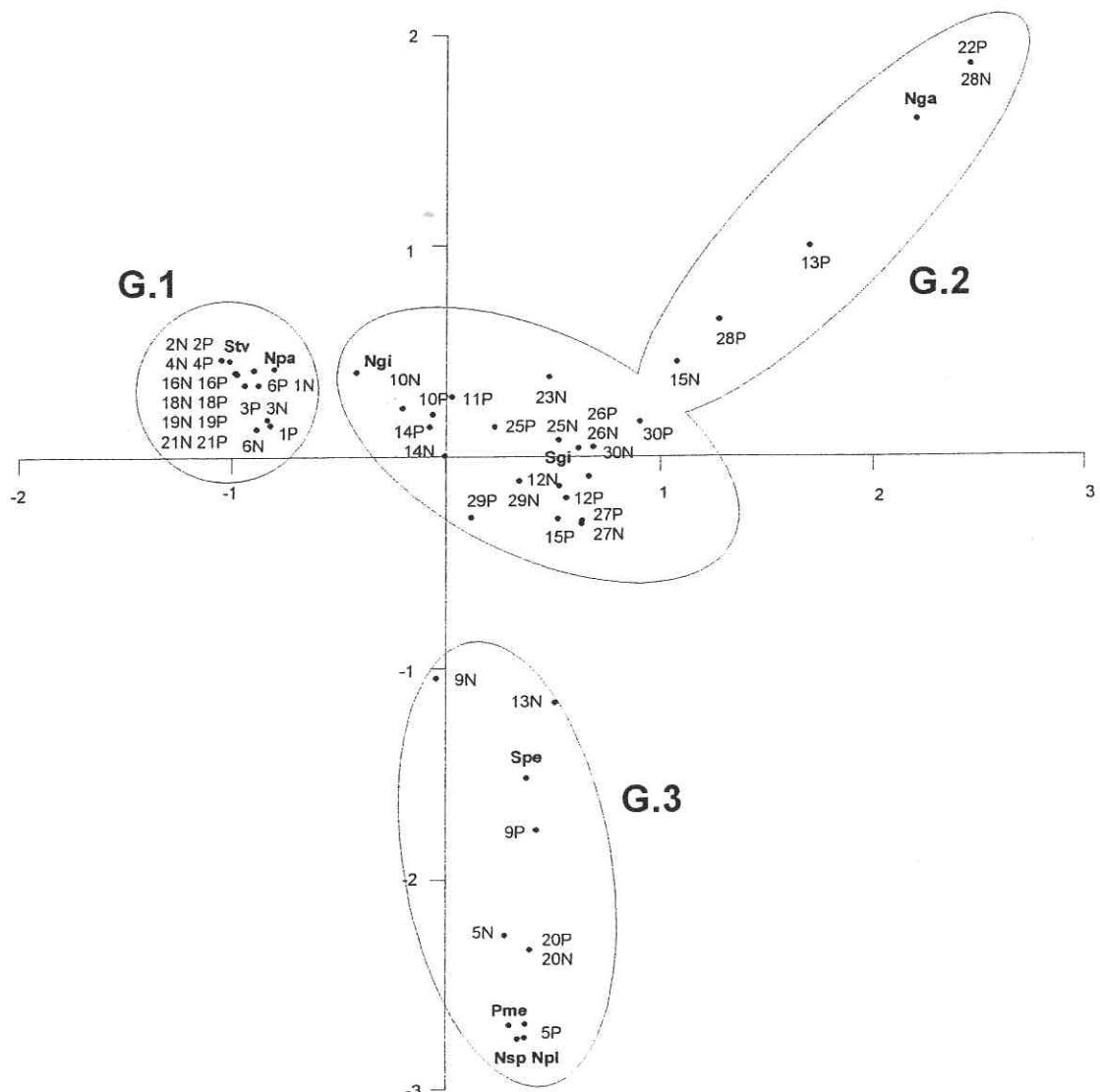
Three groups of samples (G.1, G.2, G.3) could be clearly distinguished ( $p < 0.001$  for all  $n / n \geq 5 / n \geq 15$ , Wilks' test). A strong trend to coupling was observed within these groups for the samples collected with the net-sampler and with the pump in a given well, on the same date. The number of paired samples (collected with the net-sampler and with the pump, exhibiting global similarity) increased when samples with increasing abundance were considered: 86.8 % (all n) / 93.3 % (n  $\geq 5$ ) / 96.9 % (n  $\geq 15$ ).

To confirm that the sampling methods did not induce particular patterns, FDA was performed on the data set after separating the samples collected using the two methods. No discrimination of the samples could be observed in relation to the method used (discriminant power 0.07), so the hypothesis that the sampling devices influenced the abundance patterns could be rejected ( $W = 3.77$ ,  $\alpha$  probability for Wilks' test = 0.76).

**Table 3.** Matching of the samples and discriminant analysis for amphipod / isopod species collected using the two tested devices. Discriminant power, Wilk's test and  $\alpha$  (type I-error) probability.  
n: abundance of invertebrates per sample.

% of paired samples (net-sampler / pump)	for all n		for $n \geq 5$		for $n \geq 15$	
	86	90	90	92.6		
Discriminant analysis :	F1 axis	F2 axis	F1 axis	F2 axis	F1 axis	F2 axis
Discriminant power	0.92	0.87	0.94	0.90	0.95	0.90
W (Wilks' coefficient)	208.3	169.9	190.6	85.8	129.7	56.4
$\alpha$ probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

**Figure 5.** Results of a factorial correspondence analysis for the amphipod species collected with the two tested methods in the 15 wells. All the species were considered. Contribution to total variance of the two first axes : 44.5 %. Samples with letter N : collected with the net-sampler, samples with letter P : collected with the pump. Sample numbers 1-15 : April 1999, 16-30 : October 1999. Svir: *Stenasellus virei* ; Pme: *Proasellus meridianus* ; Nro: *Niphargus robustus* ; Ngal: *Niphargus gallicus* ; Nsp: *Niphargus* n. sp. ; Npa: *Niphargus pachypus* ; Ngi: *Niphargus gineti* ; Spe: *Salentinella petiti* ; Sgi: *Salentinella gineti*.



## DISCUSSION

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The two sampling devices tested were used at contrasting stations and periods, and thus provided powerful comparative data concerning diverse situations usually encountered in the field: wells with different characteristics, piezometric levels, water extraction regimes, hydrogeological settings (cf. Table 1). Unlike the pump, the phreatobiological net is usually considered as a qualitative sampling method (MALARD et al. 1997). Actually, as the volume of strained water can be estimated, this sampling technique may rather be considered semi-quantitative. Indeed, the approximate density of invertebrates collected can be evaluated using:

$$D_x = \frac{i}{\pi \cdot r^2 \cdot h \cdot n}$$

Where:

$d$  = density of collected invertebrates in well  $x$  ( $m^{-3}$ )

$i$  = number of individuals collected with the net-sampler in well  $x$

$r$  = radius of the net

$h$  = height of the filtered water column

$n$  = number of up-and-down movements of the device.

However, in this study we did not focus on the quantitative versus qualitative aspects of groundwater sampling were not considered. Our criterion of comparison was the ability to describe one particular aspect of the phreatical assemblages, the patterns of relative abundance for the interstitial crustacean fauna.

### 1. Global efficiency of the tested devices.

On the whole, the community structure for microcrustaceans / macrocrustaceans in the Ariège alluvial aquifer was described in the same way with the two methods tested. Very similar results were obtained using either the phreatobiological net or the pump for all the taxa considered, whatever their size (small / large species), ecological preferences (stygoxen / stygophile / stygobite species) or locomotory behavior (swimming species / creeping or walking species). Although it is assumed to be taxon-dependant (BOU 1974, BOUTIN & BOULANOUAR 1983), the net-sampler did not induced any

discrimination in the collected fauna in comparison with the pump. This observation may be attributed to the way this device was used in the stations: the sampler was first lowered down to the bottom of the well, then shaken several times in contact with the sediment to stir the water and dislodge the walking or creeping invertebrates. Afterwards, it was alternatively pulled up and lowered through the whole volume of the well, including against the linings where some taxa might be present.

It is still possible that some animals may avoid capture. Active, swimming cyclopoid species may escape the net or the pipe, staying in the water column or finding shelter in crevices. For those sheltered in crevices, the comparative efficiency of the two devices should differ, as they may not be captured with the net, whereas they may be sucked out by the pump. This was clearly not observed in this study, probably because the pumping rates used (about  $14 \text{ m}^3 \cdot \text{h}^{-1}$ ) induced restricted effective sampling areas in these large wells: the suction of the pump may not be strong enough to remove invertebrates from the surrounding zones of the aquifer (MALARD et al. 1997). In these wells, the efficiency could then be considered similar for the two methods tested.

## **2. Effects of abundance.**

In order to allow comparisons, mean density for the collected taxa was evaluated with the pump: about 1000 individuals per  $\text{m}^3$  were found in this aquifer. Microcrustaceans exhibited much higher abundances than macrocrustaceans (cyclopoids,  $d \sim 960 \text{ ind. m}^{-3}$ ; amphipods+isopods,  $d \sim 13 \text{ ind. m}^{-3}$ ). Such densities cannot be considered exceptional, as authors reported a similar range from aquifers with similar pumping devices (MALARD et al. 1994, 1996).

However, the number of collected invertebrates was low in some samples and potentially raised the problem of statistical significance in the results, a key problem in ecology. The match between the relative patterns of abundance provided by the two methods was better, the greater the abundance in the samples: for cyclopoid species, wells 7 / 14 with marked differences for the sampling devices exhibited the lowest densities from all the wells. Sample 6P isolated on the FCA ordination diagram also had very low abundance (6P:  $n = 65$ ; mean for well 6 in April 1999  $n = 45.5$ ; mean for all the wells  $n = 1538$ ). The same trends were observed for amphipod / isopod taxa: better matches were always found between the two methods when the size of the samples increased (percentage of matching sample for the net-sampler and the pump for all  $n$ : 86 %; for  $n \geq 5$ : 93.3 %; for  $n \geq 15$ : 96.9 %). The wells with significant differences (i.e. 7, 8, 9, 13) were characterized by the lowest amphipod densities (7, 8, 9:  $d < 5$  per

sample; 13:  $d = 6.5$  per sample). The greatest care must therefore be taken in interpreting the small-sized samples, or those collected in sparsely populated aquifers.

Yet, these considerations can be applied in general whatever the sampling method used to collect the fauna and thus they are relevant to the two sampling devices tested. In the same way, all the species including the rarer ones in terms of abundance or occurrence (e.g. cyclopoid species *Graeteriella* sp., *A. viridis*, *Speocyclops* sp. with abundance < 0.05 % of total abundance) were considered in this study. While the two methods provided different results when a sole specimen of a species was found in a given well, the global efficiency in sampling the fauna could be considered similar for the phreatobiological sampler and for the pump, even for rare species.

### 3. Choice of a sampling method.

It is obvious that a combination of various techniques will always provide the most complete results when working on large wells (BOUTIN & BOULANOUAR 1983, BOULAL 1988), so it should be recommended whenever possible. This may be of particular importance when working in aquifers where no biological data is previously available or where all the taxa, including the rarer ones, have to be considered. Yet, in most cases a single, adapted sampling device will provide efficient results (VERVIER 1990, MATHIEU et al. 1991).

As the sampling efficiency can be considered to be the same for the two methods tested, other comparative criteria can also be taken into consideration (table 4).

**Table 4.** Comparison of the tested devices according to the sampling criteria.

Sampling criterion	net-sampler	pump
damaged animals	none	macrocrustacean
faunal and physicochemical sampling	–	+
faunal sampling	qualitative <sup>(1)</sup>	quantitative
sampling in deep aquifers	+	+
use (ease of use / sampling duration)	+	–
convenience (weight / size / maintenance)	+	–
cost	low	high

(1) see discussion

The ability to collect invertebrates without damaging them is of primary interest for biological sampling devices. As frequently reported by authors (MALARD et al. 1997), we observed that the centrifugal pump often damaged the macroinvertebrates to such an extent they were sometimes unrecognizable. Microcrustaceans, on the contrary, did not seem affected by pumping. The phreatobiological net-sampler provided invertebrates in very good condition, whatever their size or taxa. This was of special interest for large, fragile *Niphargus* species that tend to lose appendices or bristles that are frequently required for identification.

Another important feature is the ability to simultaneously measure biological and physico-chemical parameters in aquifers. Unlike the centrifugal pump, the net-sampler should not be used for both biological and physico-chemical sampling. Though it may be possible to collect ground water using adapted containers (such as those designed for water sampling in lakes or oceans), the efficiency of such methods may be greatly restricted by 1) the size of the wells, especially when sampling small piezometers; 2) the limited volume of the sample and 3) the inability to extract ground water from the surrounding zones of the aquifer, i.e. the water and the animals collected may be representative of local physico-chemical well characteristics.

Finally, the technical and practical constraints of the devices tested should also be taken into consideration. The dimensions of the device, the ease of use, convenience, duration, cost... are of immediate interest in field studies where many factors may affect the sampling conditions. The advantageous features of the phreatobiological net-samplers are their extreme ease of use and convenience. As no generating unit and discharge pipes are needed, the equipment is very light and sampling can be performed very quickly. In the same way, the maintenance and cost of such devices are extremely low by comparison with pumping devices. Nevertheless, a main characteristic of net-samplers is that the sampling of invertebrates from the surrounding zones of the aquifer is not possible. The results obtained should then be carefully considered, as the collected species may be representative of local, phreatic assemblages. In particular, well colonization by “accidental”, non-adapted epigean species may occur. In this aquifer, however, previous results do not support this hypothesis, at least for amphipod species (DUMAS et al., submitted).

Taking all these considerations into account, the phreatobiological net-sampler may thus be a valuable alternative to traditional pumping devices in aquifers, providing the diameter of the wells are wide enough to allow the operation of a net.

### **Acknowledgements**

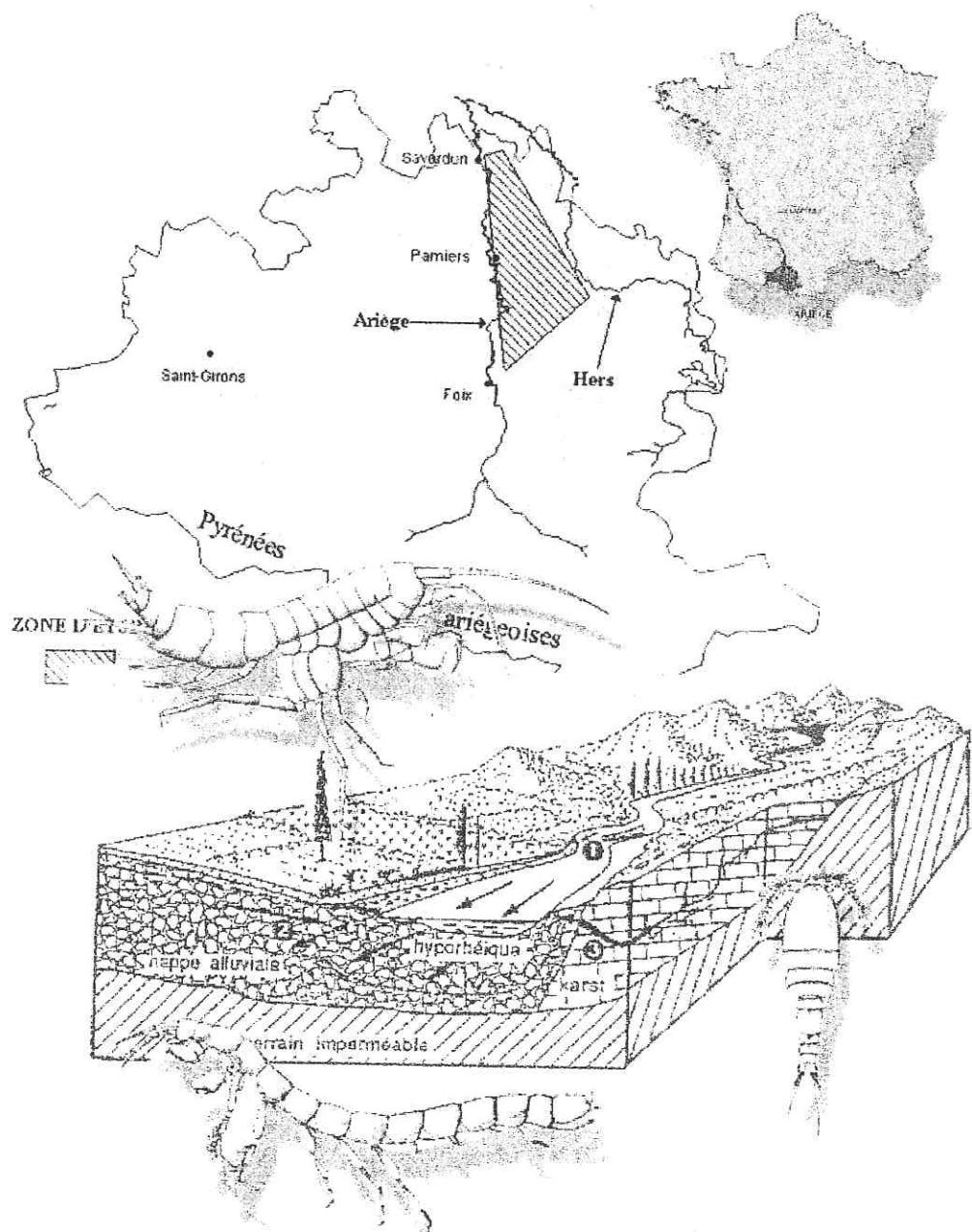
This work was supported by the French Adour-Garonne Water Agency and the Ariège regional council. We would like to give special thanks to F. LESCHER-MOUTOUÉ for cyclopoid identification and to C. BOU for his help on the field and amphipod identification. We are grateful to Pr. J. GIBERT for useful comments on the manuscript. This is a contribution to the programme of the Laboratoire Souterrain de Moulis, CNRS -UMS T1816 (France).

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## - CHAPITRE 2 -





## **Faunal distribution in an agriculturally-impacted alluvial aquifer.**

### **Abstract**

A study was undertaken to investigate the possible impacts of agricultural pollutants (mainly nitrate fertilizers) on the interstitial communities of the Ariège alluvial aquifer (French Pyrénées). Fifteen wells were sampled over two contrasting seasons for zoological and physicochemical analysis. Physicochemical parameters of the aquifer showed slight variations, while temporally stable, diverse crustacean assemblages were found in the wells. Agriculturally-induced pollution was clearly detected in ground waters with strong among-site nitrate variations, but weak correlations were found between environmental and biological data; community parameters (abundance, taxonomic richness, dominance) did not differ between polluted and non-polluted sites. At the scale of this aquifer, the field results suggest that nitrates do not influence distribution patterns and cannot be considered as a major threat for interstitial crustaceans.

## **INTRODUCTION**

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For many years, the occurrence of groundwater pollution and related impact on hypogean organisms have been studied in groundwater ecosystems (see review in NOTENBOOM et al. 1994). Shallow groundwater zones (e.g. alluvial aquifers) in particular, are known to be very sensitive to contamination, because pollutants enter these zones directly before reaching deeper layers. The impact on invertebrate communities has been extensively studied, and can be related to many abiotic and biotic factors (e.g. structure and hydrodynamics of aquifers, nature of pollutants, individual and non-individual responses of organisms to contamination) (GIBERT 1990, MAYER et al. 1993, MALARD et al. 1994 b, NOTENBOOM et al. 1995, BOUTIN et al. 1995).

Pollution in alluvial aquifers is well documented in Europe and USA, especially for Fe-Mn, agrochemical or sewage contamination (e.g. SINTON 1984, BOUTIN 1984, NOTENBOOM et al. 1994, PLENET et al. 1996, MALARD et al. 1998, BOULAL et al. 1997). Among the effects on invertebrate communities, changes in abundance and/or diversity indices are frequently established (CULVER et al. 1992, NOTENBOOM et al. 1994, THORNE & WILLIAMS 1997). Changes in the ratio epigean/hypogean species have also been documented (BOULANOUAR 1986, MALARD et al. 1996, BOULAL et al. 1997). This wide range of faunal responses to perturbation underlines how complex the relationships between fauna and environmental factors can be (MÖSSLACHER 1998). Thus, the use of invertebrates in groundwater monitoring has already proved to be an effective tool for understanding the mechanisms of contamination in various hydrogeological settings (PLENET et al. 1992, MALARD et al. 1994 a).

Crustaceans (mainly copepods, amphipods and isopods) constitute a main component of groundwater assemblages (LESCHER-MOUTOUÉ 1973, 1986, JUBERTHIE & DECOU 1994, ROUCH 1995, STRAYER et al. 1997) and often exhibit high abundance and taxonomic richness in interstitial habitats. Changes in groundwater cyclopoid assemblages have been regularly found in cases of anthropic (mainly industrial) disturbance (PLENET et al. 1996, MALARD et al. 1998), but little data exist on community responses to large-scale, agriculturally induced perturbations. Long-term studies on sewage-polluted aquifers in Morocco (BOUTIN 1984, BOULANOUAR 1986, BOULAL et al. 1997) showed that the diversity and abundance of amphipod and isopod stygobite species significantly decreased with groundwater contamination, whereas global taxonomic richness and abundance of interstitial fauna did not.

The effects of generalized fertilization (in particular nitrates) on groundwater crustacean populations has received scant attention. If low nitrate toxicity was found *in vitro* for macrocrustacean amphipod species (FAKHER EL ABIARI et al. 1998), impact on interstitial crustaceans in natural conditions is not known.

To test the hypothesis that agricultural pollution affected groundwater communities, a study was carried out on the crustacean distribution in an alluvial aquifer where agricultural practices affected groundwater quality. Phreatic cyclopoids, amphipods and isopods were sampled from 15 wells scattered throughout the Ariège river aquifer (French Pyrénées) and the physico-chemical characteristics of ground waters were investigated simultaneously, to answer the following questions:

- 1) Do community parameters (abundance, diversity expressed as taxonomic richness, dominance) differ between polluted and non-polluted sites ?
- 2) At the scale of the entire aquifer, is there evidence of relationships between crustacean distribution patterns and groundwater quality ?
- 3) Can agriculturally-induced pollutants, in particular nitrates, be considered as a major threat for interstitial crustacean assemblages ?

## MATERIAL & METHODS

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### 1. Study area

The Ariège aquifer is located in the Southwest of France, close to the northern border of the French Pyrénées. Mainly stretching to the east of the Ariège river, the continental plain is filled with quaternary detritic deposits (gravels, sand and clays). The substratum is composed of Oligo-Miocene, horizontal limestone layers alternating with clay and sand (CUGNY 1980).

Between the two main rivers (Ariège and Hers), the sedimentary layers constitute four embedded alluvial terraces with W-E grading topography. Water resources are mainly contained in the two lower, continuous layers (the low plain and the low terrace). Higher levels are composed of zones of altered detritic deposits scattered over the plain containing small, isolated aquifers that were not considered in this study.

The aquifer is drained by the Ariège and Hers rivers, with a general flux from south to north. It is essentially fed by runoff rainwater. The study of the present paper focuses on the central part of the plain, delimited by the cities of Varilhes in the south and Saverdun in the north.

The Ariège alluvial plain ranges over a 250 km<sup>2</sup> area and has low population densities, the main urban settlements never exceeding 20 000 inhabitants. Industrial activities are scattered over the whole area, but the emphasis is on agriculture in the plain: generalized cereal production (maize) with intensive fertilization has led to groundwater contamination; in particular local nitrate concentrations often reach 100-150 mg/l. Heavy metal or pesticide contamination is not a serious threat in the area (Ariège Chamber of Agriculture, personal communication).

### 2. Sampling procedures

Sampling was carried out in 15 wells scattered over the alluvial plain, in agricultural and suburban areas. The wells were agricultural or domestic wells, 1-2 m in diameter, properly closed wherever possible to obviate any strong epigean influence. Depth ranged from 8 to 15 m and piezometric levels from -0.50 to about -8 m. According to the wells, water extraction was for cereal irrigation, home everyday consumption, or livestock needs.

Sampling took place in October 1998 (low water period) and April 1999 (high water period), to cover one complete hydrological season. As the use of complementary devices is recommended for phreatobiological sampling (BOULAL 1988), fauna was collected at each site using a phreatobiological net (CVETKOV 1968, BOU 1974) with 90 µm-mesh size, drawn up 10 times through the entire water column, and a centrifugal, petrol-powered FLYGT pump, immersed down to the bottom of the wells. After preliminary tests, the volume of water extracted was fixed at 2 m<sup>3</sup> to evaluate statistically significant animal densities. The samples were strained through a 90 µm-mesh plankton net and immediately preserved in the field in 70 % ethanol.

## **2. Faunal sorting and identification**

In the laboratory, Bengal rose was added to stain the specimens in order to distinguish them easily. The animals were sorted using Dolfuss chambers under a stereomicroscope. For samples with very high animal densities, sub-sampling was used. Afterwards, all specimens were identified and counted using a Nchet 300 microscope. Isopods and amphipods were all identified to species level.

Some cyclopoid species were not easily recognizable without dissection so, in this context of an ecological study, *Diacyclops languidoides*, *D. clandestinus* and the very rare *D. languidus* were identified at the genus level and were assigned to *Diacyclops* spp. Some harpacticoid and ostracods were collected but could not be identified, so these rare specimens were not considered in this study.

## **3. Assessment of groundwater contamination**

Environmental factors (temperature, conductivity, pH, dissolved oxygen, redox potential) were measured in the field with the appropriate Merck-type apparatuses. An additional 500 cm<sup>3</sup> sample of water was taken at each site for later chemical analyses. Calcium, magnesium, chlorides, sulfates, phosphates, nitrates, ammonium, oxydability (BOD), total alkalinity (TA), and Ca/Mg hardness (TH) were analyzed according to standard methods (RODIER 1966, PINTA 1971, AMINOT 1974).

As sampled wells differed in groundwater quality, a simple integrated estimate of the pollution status of each site was calculated following THORNE & WILLIAMS (1997) so that sites could be readily compared. This was achieved by ranking the sites for each of the 5 main pollution-related parameters, using the mean values: the highest concentrations for NO<sub>3</sub>, Cl, NH<sub>4</sub>, SO<sub>4</sub> and the lowest concentration for O<sub>2</sub>.

received the highest ranking. After summing, an average rank (“pollution index”, PI hereafter) was then obtained for each site.

#### **4. Statistical tests**

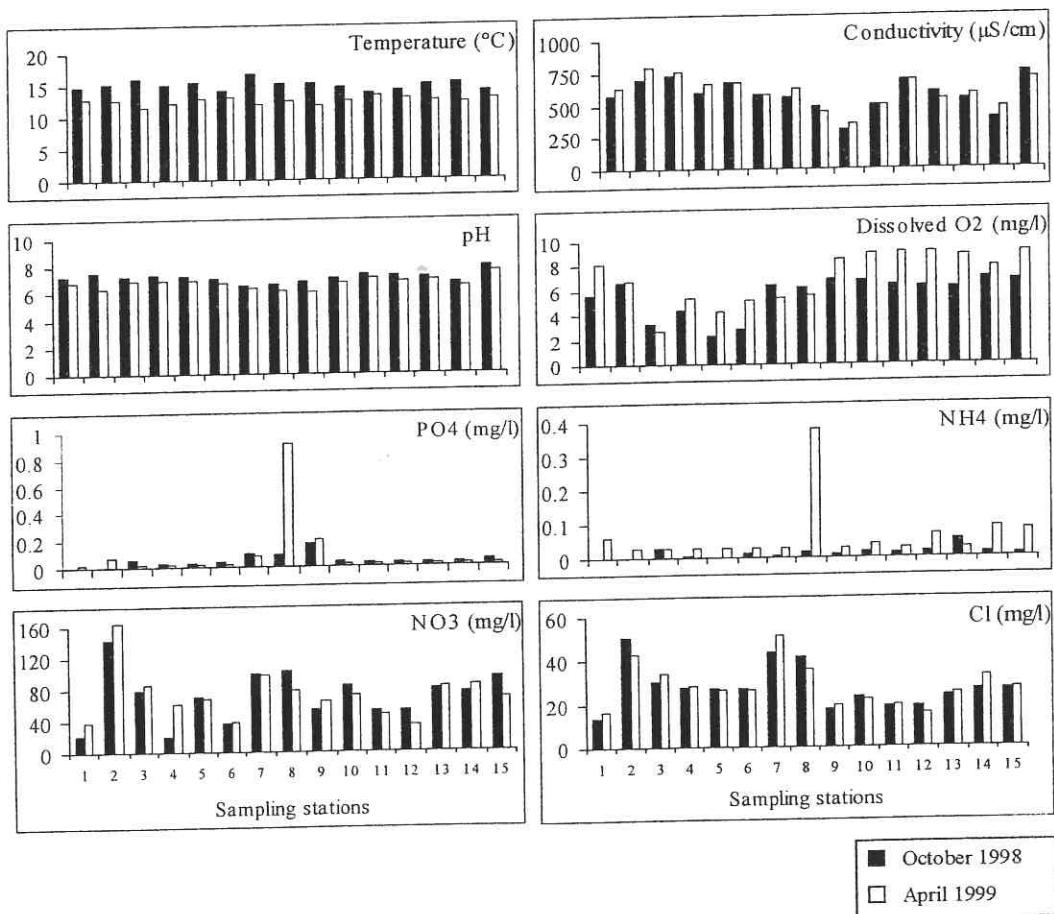
The following tests: Wilcoxon matched-pairs signed-rank test (SIEGEL 1956), correlation coefficient test (SNEDECOR & COCHRAN 1957), Pearson’s correlation moment, multivariate analysis (principal components analysis, PCA hereafter) were performed with a STATITCF computer statistical package in order to check for significant differences between sites, and to investigate relationships between cyclopoid assemblages and environmental variables.

## RESULTS

### 1. PHYSICO-CHEMICAL CHARACTERISTICS

#### 1.1. Groundwater quality

In the area studied, groundwater was mainly alkaline, well mineralized and well oxygenated (cf. Fig. 1). Most factors (temperature, conductivity, dissolved oxygen, pH, nitrates and chlorides) exhibited among-site variations; only ammonium and phosphates showed relatively similar values except for wells P7, P8, P9. A global temporal stability was found for physico-chemical variables as similar patterns were observed over the sampling period for most environmental variables.



**Figure 1.** Main physico-chemical characteristics of ground waters in the 15 sampling stations of the Ariège alluvial aquifer in October 1998 and April 1999.

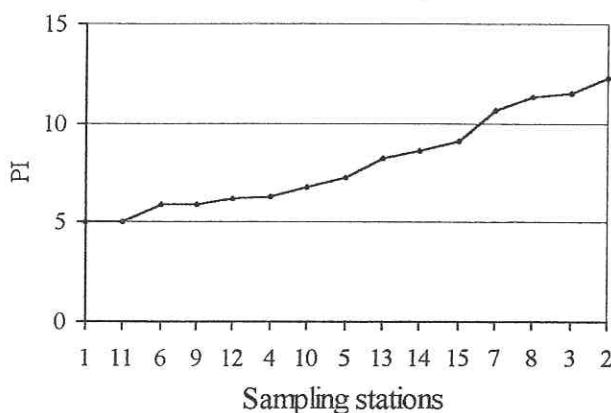
Oxygen in the wells exhibited average values (5-8 mg/l) except for P3, P5, P6, and concentrations in these sites (3-4 mg/l) never fell to anoxic or even hypoxic levels. Seasonal effects appeared as O<sub>2</sub> concentrations were higher for the second sampling period (Wilcoxon matched-pairs signed-rank test, n=15, p<0.005); a statistically significant increase in ammonium between October and April was also recorded (Wilcoxon, n=12, p<0.005).

Ammonium and phosphates remained low except in farmyard wells P7 to P9 with phosphates exceeding background concentrations whatever the sampling period. P8 also showed a strong ammonium input in April, probably resulting from liquid manure contamination (observed on the site).

Nitrate concentrations showed the highest variability among sites and nearly always exceeded the standard limits for drinking water (50 mg/l) with a mean of 71 mg/l. The maximum was found in well P2 (>160 mg/l) while the minimum was in well P1 (20 mg/l) (Table 1).

To distinguish polluted and non-polluted sites, the wells were ranked using the pollution index (PI), theoretically comprised between 1 and 15 (Fig. 2). Three groups corresponding to a global groundwater quality assessment could be separated along a *relative* pollution gradient:

- Group A (no or very slight pollution), with  $1 \leq PI \leq 5$ : wells P1, P11;
- Group B (moderate pollution), with  $6 \leq PI \leq 10$ : P6, P9, P12, P4, P10, P5, P13, P14, P15;
- Group C (heavy pollution), with  $11 \leq PI \leq 15$ : wells P7, P8, P3, P2.



**Figure 2.** Classification of the 15 sampling sites of the Ariège alluvial aquifer along a pollution gradient, using the mean values (October 1998 and April 1999 data) of the 5 main pollution-related parameters : NO<sub>3</sub>, Cl, NH<sub>4</sub>, SO<sub>4</sub>, O<sub>2</sub>.  
Pollution index (PI) is theoretically comprised between 1 and 15 (1 = no pollution, 15 = heavy pollution).

Only 2 stations could be considered “reference” sites with low contamination levels (stations 1, 11). The majority of the sites belonged to the second group and exhibited intermediate pollution levels.

The ordination of the stations based upon groundwater quality did not show any definite spatial patterns, as groups of stations seemed to be scattered over the study area regardless of station location.

Correlation between environmental variables (Table 1) was high for nitrates, chlorides, magnesium and sulfates ( $r_{NO_3/Mg}=0.90$ ,  $r_{NO_3/Cl}=0.81$ ,  $r_{SO_4/NO_3}=0.78$ ,  $p<0.01$ ) while it was generally low for other variables. Nitrates, ammonium and phosphates exhibited great ranges of variation, mainly due to peaks in station 8 during the second survey. Except these latter, environmental variables were on the whole characterized by low temporal variability over the study period.

**Table 1.** Main pollution-related variables of ground waters in the 15 sampling stations (mean values and range of values) of the Ariège alluvial aquifer in October 1998 and April 1999, with coefficients of correlation between variables ( $r$ ). Levels of significance : \* $p<0.05$  and \*\* $p<0.01$ .

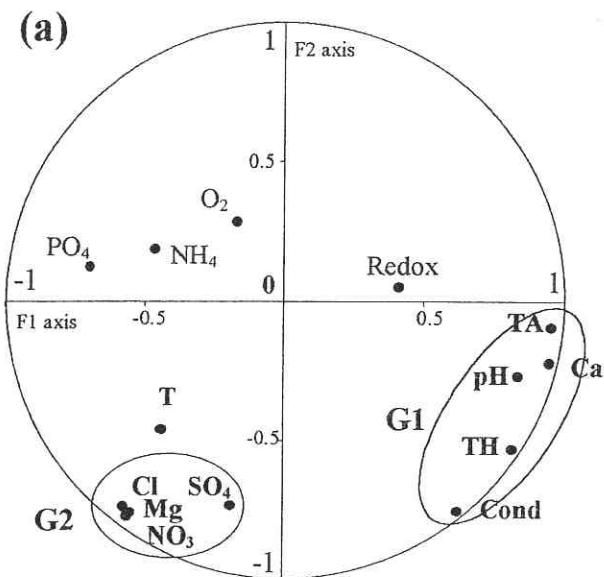
Variable (units)	mean	(range)	oxyge n	Cl	NO <sub>3</sub>	SO <sub>4</sub>	NH <sub>4</sub>	PO <sub>4</sub>	BOD	Mg
Oxygen (mg/l)	6.26	(2.3 - 9)	1	-0.31	0.05	0.22	0.08	-0.01	-0.18	0.01
Cl (mg/l)	27.73	(13 - 51.2)		1	0.81**	0.49	0.19	0.33	-0.01	0.85**
NO <sub>3</sub> (mg/l)	71.37	(20 - 163.2)			1	0.78**	0.14	0.25	-0.41	0.90**
SO <sub>4</sub> (mg/l)	23.67	(12 - 51.2)				1	-0.16	-0.01	-0.20	0.73*
NH <sub>4</sub> (μg/l)	42.60	(1 - 380)					1	0.85**	-0.15	0.09
PO <sub>4</sub> (μg/l)	71.33	(16 - 908)						1	-0.14	0.24
BOD (mg/l)	2.25	(1 - 10.9)							1	-0.19
Mg (mg/l)	8.34	(3.5 – 30.2)								1

## 1.2. Multivariate analysis

Ordination of variables was performed by PCA (Fig. 3a, 14 variables x 15 sites). The first two factorial axes revealed a strong structure underlying the data as they accounted for 65.8 % of total variance. The first factorial axis (F1) could be mainly related to total alkalinity (TA), calcium, pH, Ca/Mg total hardness (TH), phosphates, and conductivity (correlation between F1/TA=0.95, F1/Ca=0.95, F1/pH=0.83, F1/TH=0.81, F1/PO4=0.70, F1/cond=0.62). The second axis (F2) was related to chlorides, sulfates, magnesium, nitrates and conductivity ( $r_{F2/Mg}=0.77$ ,  $r_{F2/NO_3}=0.76$ ,  $r_{F2/Cl}=0.74$ ,  $r_{F2/SO_4}=0.73$ ,  $r_{F2/Cond}=0.75$ ). Oxygen, redox potential, temperature, and ammonium remained close to the centre of the diagram and thus have low contribution to axis determination (correlations <0.5).

Two main groups could then be clearly discriminated along the F1 and F2 axes:

- Group G1, with natural mineralization variables mainly related to axis F1 (total alkalinity, pH, calcium, Ca-Mg hardness, conductivity)
- Group G2, with pollution potentially related variables mainly related to axis F2 (nitrate, chloride, sulfates, magnesium, conductivity).

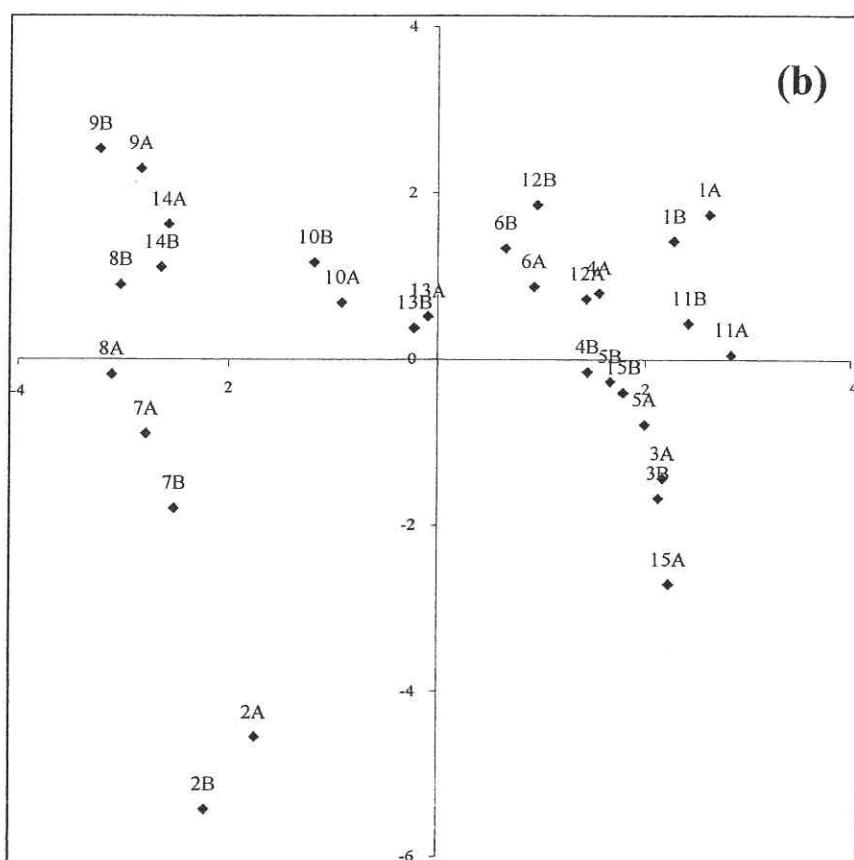


**Figure 3.** Results of a principal components analysis ordination diagram (factor 1 x factor 2) for the physico-chemical data, using the mean values (October 1998 and April 1999 data).

Contribution to total variance of the two first axes : 65.8 %. (a): Variable plot (b): station plot

Variables: temperature, dissolved oxygen (O<sub>2</sub>), conductivity (Cond), redox potential (redox), pH, total hardness (TH), alkalinity (TA), chlorides, calcium, magnesium, phosphates, nitrates, sulfates, ammonium.

Stations: 1-15 station number A-B: sample from October 1998 – April 1999.



Samples from station 2 are plotted close to the negative extremity of the F2 axis and stand out against the other stations (Fig. 3b). Their position confirms the very low water quality attributed to station 2 using the pollution index. On the whole, the relative positions of the samples along F2 confirm the P.I. classification: the more negative scores the stations have, the more impacted they are. The same trends can be found along F1, with a natural mineralization gradient from the right part to the left part of the axis.

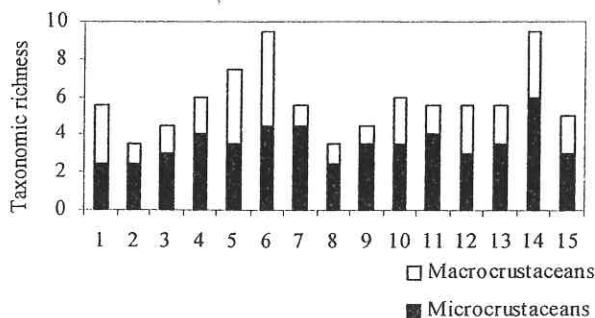
## 2. FAUNAL CHARACTERISTICS

### 2.1. Global composition of the fauna

In this area, biological aspects of groundwater had not been studied until now. Thirteen cyclopoid species were collected: widely distributed, stygoxene taxa (*P. fimbriatus*, *T. prasinus*, *E. serrulatus*) associated with hypogean genera or species (*Graeteriella*, *Speocyclops* or *Diacyclops* spp.). Amphipods were seven exclusively groundwater-dwelling *Niphargus* and *Salentinella* species plus one *Ingolfiella* species, while isopods were poorly represented by two species with one stygobite (*Stenasellus virei*) (Table 2).

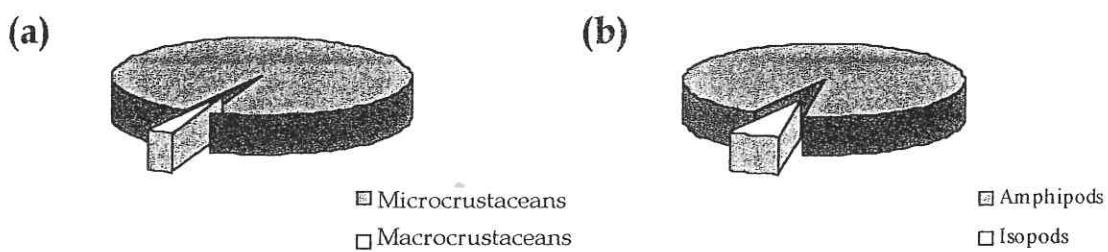
<b>Cyclopoida</b>	<i>Graeteriella unisetigera</i> *	<i>Niphargus</i> n.sp.*
<i>Macrocylops albidus</i>	<i>Graeteriella</i> sp.*	<i>Ingolfiella thibaudi</i> *
<i>Eucyclops serrulatus</i>	<i>Speocyclops</i> sp.*	
<i>Tropocyclops prasinus</i>		
<i>Paracyclops fimbriatus</i>	<b>Amphipoda</b>	<b>Isopoda</b>
<i>Acanthocyclops viridis</i>	<i>Salentinella gineti</i> *	<i>Stenasellus virei</i> *
<i>Diacyclops bicuspидatus</i>	<i>Salentinella petiti</i> *	<i>Proasellus meridianus</i>
<i>Diacyclops bisetosus</i>	<i>Niphargus pachypus</i> *	
<i>Diacyclops languidus</i>	<i>Niphargus gineti</i> *	
<i>Diacyclops clandestinus</i> *	<i>Niphargus robustus</i> *	
<i>Diacyclops languidoides</i> *	<i>Niphargus gallicus</i> *	

**Table 2.** Taxonomic composition of the microcrustacean and macrocrustacean assemblages from the stations in October 1998 and April 1999 – Ariège alluvial aquifer. Stygobite species are marked with an asterisk (\*).



**Figure 4.** Taxonomic richness in the 15 stations in October 1998 and April 1999 (mean values) for microcrustaceans and macrocrustaceans.

Taxonomic richness in the wells ranged from 3 to 12 species and was in general higher for microcrustacean species as epigean species were collected in addition with hypogean ones (Fig. 4). In contrast, epigean macrocrustacean species (*Proasellus meridianus*) were found only in two stations (stations 5 and 6). Invertebrate abundance was much greater for microcrustaceans than for macrocrustaceans (Fig. 5).



**Figure 5.** (a) Relative abundance of microcrustaceans and macrocrustaceans in the samples (October 1998 / April 1999 mean values). (b) Relative abundances of Amphipods and Isopods within the macrocrustaceans group (October 1998 / April 1999 mean values).

## 2.2. Microcrustacean assemblages

The cyclopoid fauna was dominated by species of *Diacyclops*, more specifically by *D. bisetosus* (33% of total abundance) and *Diacyclops* spp. (41%) which together were found at 14 of the 15 study sites (Table 3). *T. prasinus* (13% of total abundance) was also widely distributed (12 of the 15 sites). Some usually rarer species were also present in lower abundance (*G. unisetigera*, *Graeteriella* sp.) over a few sites (2-4 sites).

Cyclopoid abundance range was 500-1300 individuals per m<sup>3</sup>, the latter density in April 1999. No significant differences were found in species distribution patterns (Wilcoxon matched-pairs signed-rank test, n=15) or taxonomic richness (n=13) between the sampling periods. Few stygobite species were

collected over the area, whereas the majority of cyclopoid were epigean taxa, i.e. species typically found in surface waters (fig. 6a).

**Table 3.** Composition of the groundwater cyclopoid community in the 15 stations in October 1998 and April 1999. Mean number of individuals collected for each species or group of species. Abundance %: percentage of total abundance of each species. Occurrence %: percentage of stations in which the species or group of species was found. Stygobite species are marked with an asterisk (\*).

Cyclopoid species	Total number	Abundance %	Occurrence %
<i>Macrocylops albidus</i>	639	1.16	25
<i>Eucyclops serrulatus</i>	4038	7.31	56.25
<i>Tropocyclops prasinus</i>	7223	13.07	75
<i>Paracyclops fimbriatus</i>	1831	3.31	62.5
<i>Acanthocyclops viridis</i>	1	0.002	6.25
<i>Diacyclops bicuspis</i>	40	0.07	18.75
<i>Diacyclops bisetosus</i>	18762	33.95	87.5
<i>Diacyclops</i> spp.*	22614	40.92	87.5
<i>Speocyclops</i> sp.*	79	0.14	12.5
<i>Graeteriella unisetigera</i> *	29	0.05	25
<i>Graeteriella</i> sp.*	5	0.01	18.75

### 2.3. Macrocrustacean assemblages

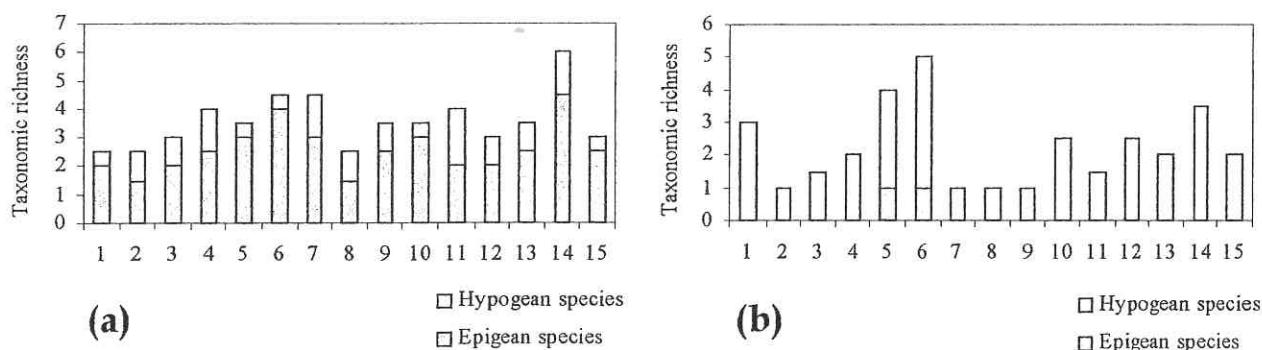
Ten species (7 amphipods, 3 isopods) were found in the samples, regularly occurring in karstic systems and porous aquifers from southern France (RUFFO 1986, COINEAU 1963, 1968, GINET 1996, BOU unpubl.). Amphipods clearly dominated the sampled fauna (seven species accounting for 95% of total abundance including one new, undescribed *Niphargus* species) and were present in all the sites except in wells P7, P8 and P9 for the first sampling period (cf. Table 4). Assemblages were specifically dominated by widely-distributed *Niphargus pachypus* (58% of total abundance, found at 13 out of the 16 study sites) and *Salentinella gineti* (30% of total abundance, 6 out of the 16 sites); some rarer species also exhibited large distribution patterns (the amphipod *S. petiti*, 3% of total abundance, found in 10 wells, and the isopod *Stenasellus virei*, 4%, in 7 wells). Except one single species, all macrocrustaceans collected were stygobite species (Fig. 6b).

Total abundance did not exhibit significant differences between the sampling periods (Wilcoxon matched-pairs signed-rank test, n=14) while a slight increase was noticed for taxonomic richness in April (Wilcoxon matched-pairs signed-rank test, n=11, p<0.05), essentially due to amphipod collected in wells P7, P8 and P9.

**Table 4.** Composition of the groundwater macrocrustacean community in the 15 stations in October 1998 and April 1999. Mean number of individuals collected for each species or group of species. Abundance %: percentage of total abundance of each species. Occurrence %: percentage of stations in which the species or group of species was found. Stygobite species are marked with an asterisk (\*).

Species	Total number	Abundance %	Occurrence %
<b>Amphipoda</b>			
<i>Salentinella gineti</i> *	547	29.97	37.5
<i>Salentinella petiti</i> *	58	3.18	62.5
<i>Niphargus gineti</i> *	33	1.81	25
<i>Niphargus pachypus</i> *	1059	58.03	81.25
<i>Niphargus robustus</i> *	9	0.49	12.5
<i>Niphargus</i> n. sp. *	17	0.93	6.25
<i>Niphargus gallicus</i> *	14	0.77	18.75
<i>Ingolfiella thibaudi</i> *	1	0.05	6.25
<b>Isopoda</b>			
<i>Proasellus meridianus</i>	43	2.36	12.5
<i>Stenasellus virei</i> *	44	2.41	43.75

**Figure 6.** Taxonomic richness of epigean and hypogean species in the 15 station in October 1998 and April 1999 (mean values). “hypogean” species: exclusively groundwater-dwelling species (stygobite). “epigean” species: all the others. (a) Microcrustacean taxa. (b) Macrocrustacean taxa.



## 2. 4. Community structure and environmental factors

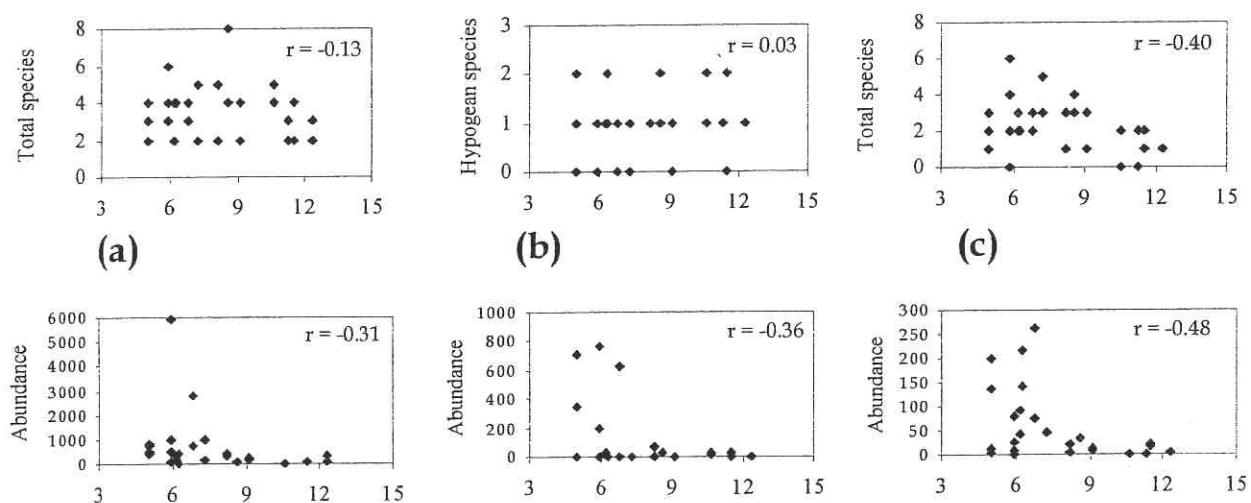
Low correlations were found between physico-chemical factors and biological descriptors of the interstitial communities (abundance, diversity expressed as taxonomic richness) for both micro- and macrocrustacean taxa (cf. Table 5). No differences were detected when either total or hypogean cyclopoid species were considered.

**Table 5.** Correlations between faunal and physico-chemical environmental variables for the groundwater crustacean communities in the 15 sampling stations of the Ariège alluvial aquifer in October 1998 and April 1999. Total species: sum of epigean and hypogean species. Levels of significance : \*\* $p<0.01$ .

	Microcrustaceans				Macrocrustaceans	
	Total species		Hypogean species		Total species	
	Abundance	Taxonomic richness	Abundance	Taxonomic richness	Abundance	Taxonomic richness
O <sub>2</sub>	-0.005	-0.014	-0.034	0.163	-0.013	-0.311
pH	-0.499	-0.249	-0.455	-0.226	0.221	0.190
Conductivity	-0.397	-0.319	-0.308	-0.034	-0.043	-0.009
Temperature	0.104	0.077	0.134	0.165	-0.236	-0.145
NO <sub>3</sub>	0.144	-0.168	0.163	0.062	-0.516	-0.496
NH <sub>4</sub>	0.923**	-0.343	0.943**	-0.072	-0.156	-0.222
SO <sub>4</sub>	-0.124	-0.315	-0.126	-0.057	-0.409	-0.456
Cl	0.216	0.033	0.268	0.193	-0.428	-0.373
PO <sub>4</sub>	0.961**	-0.322	0.939**	0.033	-0.347	-0.459
Pollution index	0.292	-0.197	0.338	0.044	-0.524	-0.472

The exceptions were for ammonium and phosphates concentrations, as high positive correlations were found between cyclopoid abundance and PO<sub>4</sub>/NH<sub>4</sub> concentrations for both total and hypogean species ( $r>0.90$ ,  $p<0.01$ ).

**Figure 7.** Correlation between taxonomic richness, abundance and pollution index for (a) total cyclopoid species. (b) hypogean cyclopoid species. (c) macrocrustacean species.



Assemblages in heavily polluted wells (group C) did not show different abundance/diversity indices when compared to wells from group A or B. Non-significant correlations existed in general between

global pollution index and taxonomic richness or abundance, whatever the species were considered (total / hypogean). Yet, the results suggest a potential influence of groundwater contamination upon amphipod/isopod taxa ( $r$  scores:  $-0.40$  /  $-0.48$  for macrocrustacean taxonomic richness / abundance) (Fig. 7).

As cyclopoid were collected in much greater abundance in well 8 in comparison with other stations (number of cyclopoid collected: 32 675 ; mean for stations except P8: 1727), the data were also investigated excluding P8. No major changes were observed.

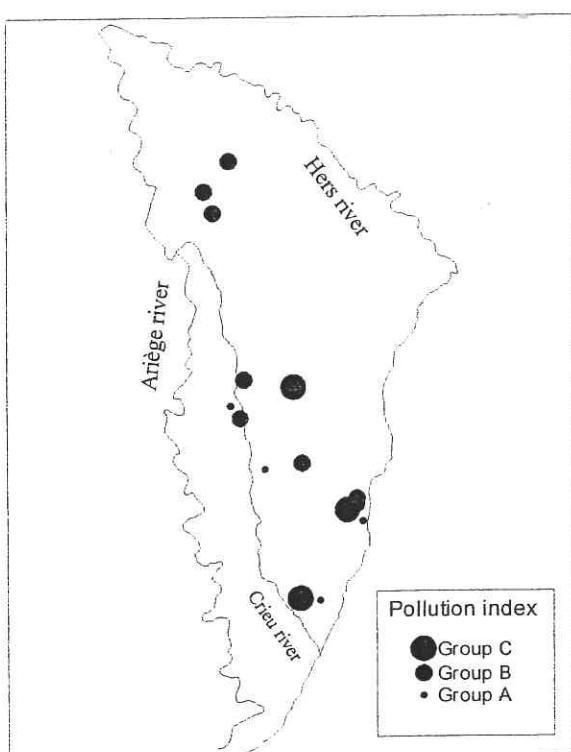
## DISCUSSION

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### 1. Groundwater quality in the Ariège aquifer

The Ariège alluvial aquifer had well oxygenated, agriculturally impacted ground waters. Evidence of groundwater perturbation arises from simple, discrete chemical analysis, but PCA discrimination of environmental variables allows the distinction between natural and anthropic processes. Due to the high oxygen levels, high nitrate concentrations were found in most sampled wells, while under reductive conditions their concentrations should be lower (KOROM 1992).

As a matter of fact, a progressive and constant increase in nitrate concentrations has been observed for twenty years in the area, as a result of diverse agricultural practices, in particular high fertilizer inputs quickly reaching the shallow ground waters (SEGUNIN 1980). Long-term surveys, associated with information campaigns were then initiated by the Ariège Chamber of Agriculture to reduce groundwater contamination (e.g. the “Ferti-neuf” Operation, in 1993). Nitrate levels therefore constitute an evidence of groundwater perturbation in the Ariège aquifer.



**Groundwater quality in the Ariège aquifer.**  
pollution index for the sampled stations.

In the area, nitrate contamination did not show any geography, geology or hydrogeology-related patterns (as observed in SEGUNIN 1980). Ancient scattered local inputs (fertilization) reaching the saturated zone through physical, chemical and biological processes may then be responsible for the present distribution.

Chlorides exceeding 20 mg/l in freshwater are generally associated with urban/industrial pollution (NISBET & VERNEAUX 1970). The position of chlorides on the ordination diagram shows strong correlation with nitrate concentration, thus chloride presence in the area may be related to groundwater pollution. In the same way, sulfates in aquifers generally result from local geological factors, although

industrial/urban pollution or sulfur oxidation can be responsible for the presence of sulfates in ground waters (NISBET & VERNEAUX 1970, BAKALOWICZ 1974). For the study area, previous work on the characteristics of the Ariège aquifer does not support the hypothesis of geological origin for sulfates (CUGNY 1980). Moreover, the strong association with nitrate and chloride concentrations on the ordination diagram (Fig. 3) argues in favor of an anthropic origin for the sulfates in the Ariège aquifer. The same phenomenon occurs for magnesium, that may result from liming and/or magnesium-fertilizing over the study area. Evidence of at least two different contamination origins and/or processes may also be deduced from the ordination diagram that clearly separates  $\text{NH}_4/\text{PO}_4$  from the  $\text{NO}_3/\text{Cl}/\text{SO}_4/\text{Mg}$  association.

These preliminary results should nevertheless be carefully considered, because of the limited temporal coverage of this study and the number of stations sampled. Extensive literature (see NOTENBOOM et al. 1994) on groundwater chemistry sets the emphasis on the complexity and diversity of contamination processes in aquifers. These complex interactions and the own particular characteristics of each sampled well (e.g. water extraction, vicinity of pollution source, sediments) may also explain the different chemistry values between close stations. For example, wells P1 and P2 are located about 800 m from each other but correspond to the extremities of the pollution gradient. Further work will be required to determine, on a larger scale, the real extent and nature of contamination in this aquifer.

## **2. Crustacean assemblages and groundwater quality**

Temporally stable, diverse interstitial crustacean assemblages were found in the sampled wells over the two-seasons sampling period as community parameters (abundance, diversity, dominance) showed only very slight variations between October (low water period) and April (high water period).

### **2.1 Dissolved oxygen.**

Oxygen concentration did not clearly influence either cyclopoid or amphipod / isopod assemblages because weak correlations existed between  $\text{O}_2$  and fauna abundance or diversity, whatever species were considered. In the same way, no difference were recorded when total or stygobite species were investigated. Relationships between animal abundance / biomass and oxygen concentration in interstitial biotopes have been thoroughly studied by many authors (see review in MALARD & HERVANT 1998).

While a decrease in invertebrate abundance can generally be associated with oxygen depletion in aquifers (WILHM & MCCLINTOCK 1978, DANIELOPOL 1991, MÖSSLACHER 1998), many studies stress

how ecological and historical factors constraining hyporheic communities can make interpretation difficult (STRAYER et al. 1997). Yet, we could observe that a slightly higher global cyclopoid abundance occurred in the wells in April 1999, when oxygen levels were high. On the contrary, in October 1998 (low water/low oxygen period), the number of individuals sampled was lower. The same phenomenon was also recorded in an alluvial aquifer of the Danube Wetland (DANIELOPOL 1991, POSPISIL 1994) where crustaceans (cyclopoids, ostracods, amphipods, isopods and cladocerans) exhibited lower abundance in late summer/autumn than in winter/spring, in correlation with oxygen conditions in the sediments. Nevertheless, care must be taken when interpreting these observations because of the great differences in invertebrate abundance between samples.

In conclusion, no influence of dissolved oxygen on faunal diversity (expressed as taxonomic richness) was clearly recorded in the present study, using either hypogean or total (epigean + hypogean) crustacean species. This was probably due to the high oxygen levels measured, that cannot be considered as limiting for small, hyporheic and ecologically low-metabolic animals (MALARD & HERVANT 1998).

## 2.2. Agriculturally-induced contamination.

Crustacean assemblages did not appear to be directly influenced by the high fertilizer inputs over the area, as pollution-related variables that we expected to influence communities did not show any apparent relationships with community characteristics. The high correlations between cyclopoid abundance/diversity and ammonium or phosphates may only be related to local factors in well P8. These strong relationships arise in fact from the considerable influence of well P8 on the data set; indeed, P8 had the highest animal density and  $\text{PO}_4/\text{NH}_4$  levels far exceeded the background concentrations. The same analysis performed after excluding P8 revealed non significant correlations between fauna abundance and  $\text{PO}_4/\text{NH}_4$  concentration, therefore this correlation may be considered an artefact. This was also suggested as no similar correlations were observed between macrocrustaceans and phosphate or ammonium levels.

Considering the wide range of nitrate concentration observed over the study area (20-163.2 mg/l), nitrates would have been expected to have the highest impact on groundwater communities, but species were distributed over the sampled wells regardless of nitrate concentration. Differences in sensitivity or tolerance were not observed among the species studied. Global water quality of the sites, estimated with an integrated pollution index, did not influence neither the microcrustacean nor the macrocrustacean

assemblages, and no differences in community parameters were observed between polluted or non-polluted sites. Moreover, with respect to the present nitrates levels, no tolerance or resistance gradient appeared when considering epigean or stygobite taxa. Thus, crustacean distribution patterns at the scale of the Ariège aquifer were not directly related to groundwater quality. This statement is not rare in aquifers: although some authors have found significant correlations between fauna and physicochemical parameters such as dissolved oxygen, pollutant concentrations or nutrient supply (STROMMER & SMOCK 1989, NOTENBOOM et al. 1994, MALARD & HERVANT 1998), other studies have reported lacks of association (GODBOUT & HYNES 1982, NOTENBOOM et al. 1995, PLÉNET et al. 1995).

These field results clearly imply that diffuse, agriculturally-induced groundwater pollution, especially nitrate contamination, cannot be considered a major threat to present crustacean communities of the Ariège aquifer. They fit with previous studies on the comparative toxicity of common groundwater pollutants (FAKHER EL ABIARI et al. 1998), in which acute nitrate toxicity for stygobite *Metacrangonyx* and *Typhlocirolana* species was found only at very high levels ( $>200$  mg/l). Toxicity of ammonium, nitrites and phosphates was in every case much higher than that of nitrates.

These observations do not definitely exclude potential effects on sensitive interstitial species. Progressive exclusion of non-tolerant species may have already occurred over the area and may still occur in the future if nitrate levels keep on increasing.

### 2.3 Sampling frequency.

The problem of limited sampling periods is a perennial question in ecology, as temporal variations generally occur for most environmental and biological parameters. While focus on short-term variations in the aquifer requires frequent sampling, more widely spaced sampling can be applied if global trends in physico-chemical or biological data are investigated, regardless of smaller-scale temporal variations.

The main objective of this study being to check for differences in general assemblages between polluted and non-polluted sites, sampling performed twice in the year, at the beginning and at the end of the hydrological season, allowed us to cover one temporal unit in the aquifer. We are aware that significant variations in water levels and environmental characteristics also occur over both shorter and longer periods in aquifers, so this scale has to be considered intermediate. As the observed environmental and faunal patterns showed very strong similarities, our results suggest an overall environmental and biological stability in this aquifer over the period considered. However, further studies should monitor

long-term patterns in this area and also focus on small-scale factors in this aquifer to investigate possible local cyclopoid distribution patterns.

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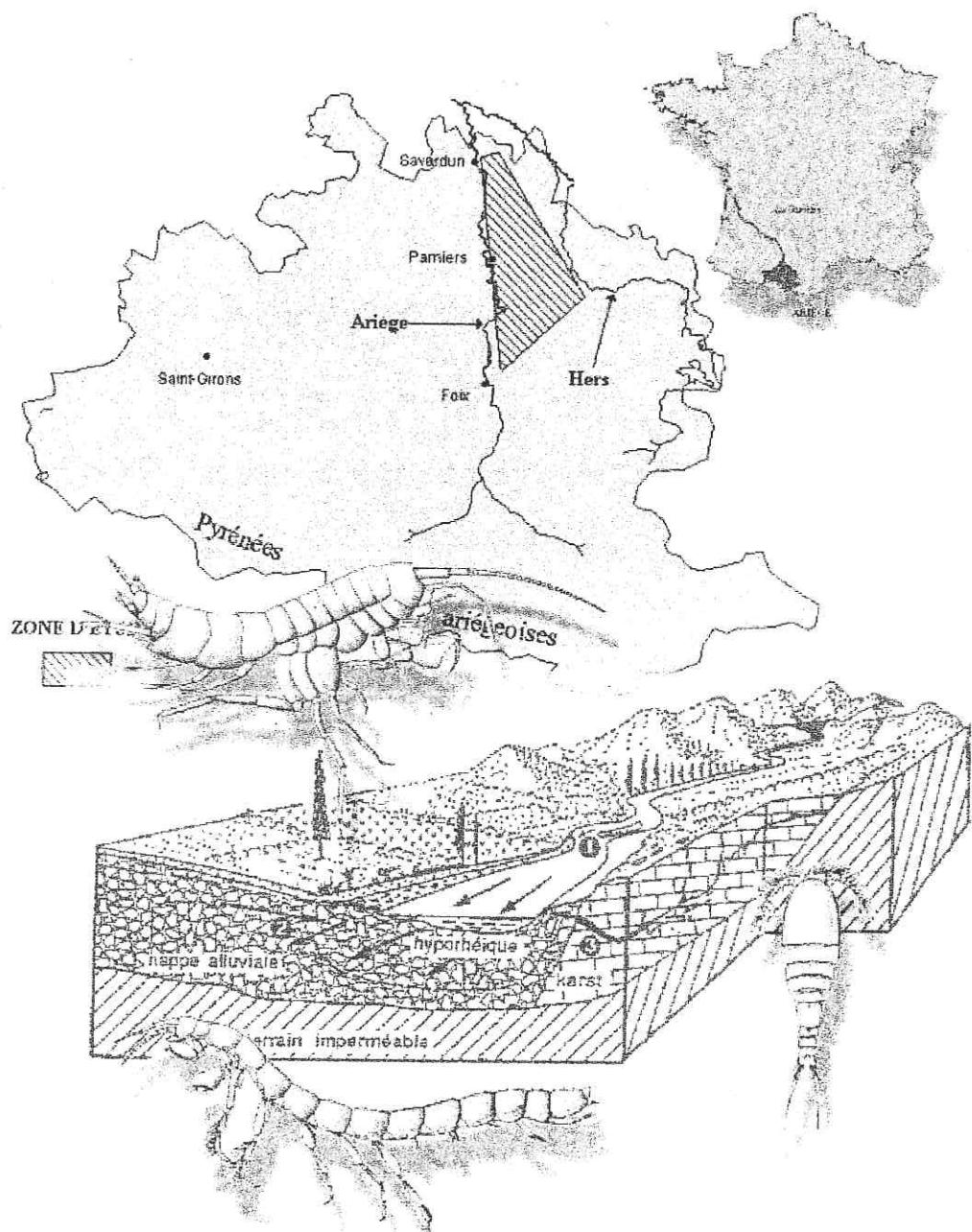
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## - CHAPITRE 3 -





## **Groundwater crustaceans as indicators of aquifer hydrodynamics and utilization.**

### **Abstract**

The spatial distribution of interstitial crustaceans was investigated in a French Pyrenean alluvial aquifer. Micro- and macrocrustaceans were sampled in wells scattered over a 250 km<sup>2</sup> area, to test the hypothesis that regional hydrogeological context of the stations and groundwater utilisation (domestic, agricultural) influenced the interstitial communities.

The assemblages were dominated by epigean and hypogean cyclopoid taxa associated to stygobite amphipod and isopod species. Low correlations existed between groundwater physico-chemical parameters and fauna distribution patterns, whatever processes (natural or anthropic) were considered. At the scale of the aquifer, groundwater quality did not influence the crustacean distribution. The spatial distribution of the fauna was clearly related to hydrogeology and hydrodynamics. Strong relationships were found between assemblage composition and hydrological context of the stations (wells from the alluvial plain or from the alluvial terrace) for both microcrustacean and macrocrustacean taxa. In addition, cyclopoid communities were also influenced by contrasted groundwater extraction regimes between agricultural and domestic wells. With respect to spatial and temporal scales and providing adequate sampling methods, the interstitial crustacean fauna may constitute indicators of aquifer hydrodynamics (sediment permeability, water-table depth) and groundwater extraction.

## **INTRODUCTION**

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The interstitial fauna of shallow ground waters has been intensively studied on various scales, from microscale (cm) to macroscale (km) (see DANIELOPOL 1989, WARD & PALMER 1994, WARD et al. 1998). Although the local distribution of invertebrates is well documented, ecological studies on the scale of a whole aquifer still remain rare, mainly because extensive sampling of the interstitial fauna is difficult (MARMONIER et al. 1992, DANIELOPOL 1991, MAUCLAIRE 1999). However, previous data indicate that the ecological distribution of hypogean invertebrates should be investigated at a range of scales to understand how subterranean organisms live in relation to groundwater ecosystem structures (DANIELOPOL 1989, WARD & PALMER 1994, MÖSSLACHER 1998).

Among interstitial-dwelling invertebrates, crustaceans have developed efficient morphological, behavioural or physiological adaptations allowing the colonization of the subterranean biotopes (VANDEL 1964, GINET & DECOU 1977, CAMACHO et al. 1992, DANIELOPOL et al. 1994, HERVANT et al. 1996, 1998). Thus, they are commonly found in ground waters.

Yet, although extensive literature exists on crustacean assemblages in ground waters, until recently the majority of the studies dealt with karstic systems or shallow ground waters in relation with river channels (hyporheic zones). The data obtained showed that faunal distribution patterns could be frequently related to the aquifer structure and dynamics (CREUZÉ DES CHÂTELLIERS 1991, MARMONIER et al. 1992, MÖSSLACHER 1998) and to groundwater perturbations, if any (SINTON 1984, NOTENBOOM et al. 1994, BOUTIN et al 1995, BOULAL et al. 1997).

Crustacean assemblages may then represent natural indicators of ground waters quality and/or dynamics. Unfortunately, only little data are available on crustacean distributions at large scale (aquifer-scale), mainly because faunal sampling is hard in aquifers zones not accessible with traditional sampling devices (Bou-Rouch pump, core-samplers) (MALARD et al. 1997, MAUCLAIRE 1999).

The distribution of micro and macrocrustaceans was thus investigated at the scale of a whole alluvial aquifer, the Ariège aquifer (French Pyrénées). Based on recent developments in sampling the fauna of deep water-table aquifers (MALARD et al. 1997), the phreatic amphipods and isopods were collected from 15 wells scattered over the whole floodplain during three surveys between October 1998 and October 1999.

The relationships between faunal distribution patterns and environmental factors were investigated at the scale of the aquifer to test whether the crustacean assemblages could be considered indicators of groundwater characteristics.

The question was **can the crustacean phreatic assemblages be related :**

- a) to the groundwater physicochemical parameters ?, and / or
- b) to the aquifer hydrodynamics (transmissivity, water-table depth, groundwater extraction) ?

### **Study area**

The Ariège floodplain is a continental plain of 250 km<sup>2</sup> located in the north of the French Pyrénées. Stretching over 50 km from north to south, the topography is smooth, grading uniformly west-east. Maximum width (10 km) of the plain occurs in the "Basse Boulbonne" area between the Ariège river and its main tributary, the Hers river.

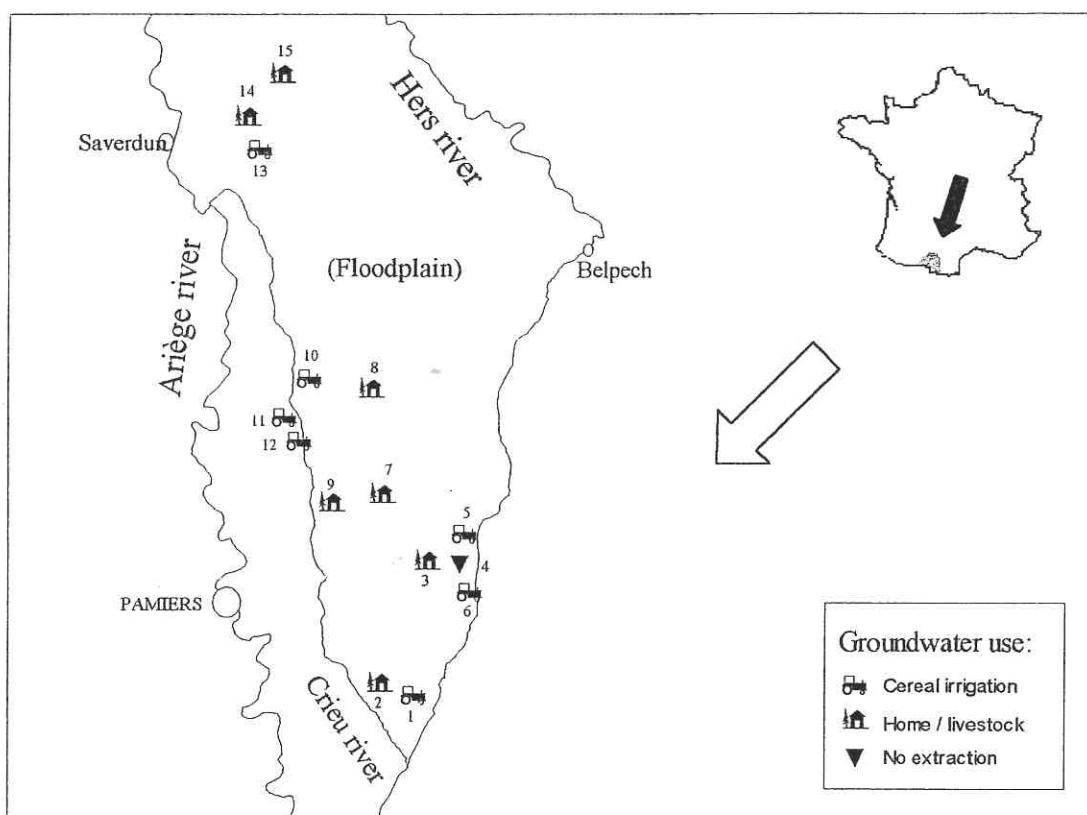
The sedimentary system of the plain is formed by superposed layers, constituting embedded alluvial terraces. Older, higher levels are composed of detritic layers characterized by strong, physicochemical alteration and scattered zones with superficial "grep" deposits (ferruginous concentrations). As they only include small, fragmented aquifers, these layers were not considered in this study. Water resources are mainly contained in the alluvial plain and the lower terrace, characterized by marked difference in hydrodynamics and hydrogeology (CUGNY 1980). As ground water extraction was needed for domestic, agricultural or industrial purposes, water-supply facilities were established in the last decades. They consist mainly in production wells, scattered over the whole floodplain and typically located close to the farms.

## MATERIAL & METHODS

### 1. Sampling sites

Sampling was carried out in stations P1 to P15, i.e. fifteen agricultural or domestic wells, scattered over the study area. The average piezometric level was about 4.50 m below the soil surface (range 0.60 - 8 m). Well diameters were 1-1.50 m with a depth range of 5-11 m. Well status differed from site to site ; water extraction was essentially for irrigation, domestic use or livestock (Figure 1).

**Figure 1.** Study area: the floodplain of the Ariège river. Main rivers (Ariège, Hers, Crieu). Location of the sampled stations and groundwater utilization.



Wells could then be separated into three categories in relation to general use:

- 1) abandoned wells, water extraction having stopped for 2-3 years (P4) ;
- 2) wells for cereal irrigation (P1, P5, P6, P10, P11 to P13) ;
- 3) wells for domestic/livestock use (P2, P3, P7 to P9, P14 and P15).

The main characteristics of the wells are summarized in Table 1.

**Table 1.** Sampled stations of the Ariège alluvial aquifer (French Pyrénées). Wells main characteristics, water-table, geological location and general utilization. Piezometric levels were not monitored in October 1998.

Station (n°)	Diameter (m)	Depth (m)	Piezometric level (m)		Sedimentary system	Groundwater extraction status
			04-1999	10-1999		
1	1.00	10.00	3.02	2.49	First alluvial terrace	Cereal irrigation
2	1.00	10.00	6.09	6.36	First alluvial terrace	Home consumption
3	1.00	4.50	2.35	2.68	First alluvial terrace	Home consumption
4	1.00	9.50	1.9	2.28	First alluvial terrace	Abandoned
5	1.00	9.50	1.45	1.8	First alluvial terrace	Cereal irrigation
6	1.20	8.00	0.46	0.67	First alluvial terrace	Cereal irrigation
7	1.00	5.00	2.6	3.21	First alluvial terrace	Home consumption
8	1.00	7.50	3.17	3.52	First alluvial terrace	Home consumption
9	1.16	9.00	6.15	6.56	Alluvial plain	Home consumption
10	1.20	8.50	5.7	8.47	Alluvial plain	Cereal irrigation
11	1.50	9.00	5.76	6.42	Alluvial plain	Cereal irrigation
12	1.50	9.00	7.83	8.44	Alluvial plain	Cereal irrigation
13	1.20	10.00	5.1	5.8	Alluvial plain	Cereal irrigation
14	1.10	5.00	3.95	4.28	Alluvial plain	Home consumption
15	1.20	9.50	6.3	8.68	Alluvial plain	Home consumption

## 2. Faunal sampling

Sampling took place at three contrasting seasons between 1998 and 1999. Sampling period was divided into three successive stages: from 01 to 16 October 1998 (low-water period), from 13 to 16 April 1999 (high-water period) and from 11 to 14 October 1999 (low-water period). Fauna was collected from each well using complementary devices (BOULAL 1988): a phreatobiological net (CVETKOV 1968, BOU 1974) with 90 µm mesh-size, drawn up 10 times through the entire water column, and a centrifugal FLYGT pump operated by a petrol engine, immersed down to the bottom of the well. After preliminary tests, the volume of water pumped was fixed at 2 m<sup>3</sup> to collect statistically significant animal densities. This water was strained through a 90 µm-mesh plankton net (cf. details in DUMAS & FONTANINI 2000).

After fixation in the field with 70 % ethanol, the interstitial fauna (microcrustaceans, i.e. cyclopoids and macrocrustaceans, i.e. amphipods and isopods) was sorted in the laboratory using Dolfuss chambers, then identified and counted.

### **3. Physicochemical parameters**

Environmental factors (temperature, conductivity, pH, dissolved oxygen, redox potential) were measured in the field simultaneously with faunal sampling in a flow-through chamber with a WTW thermo-conductimeter, a WTW redoxmeter and a WTW oxymeter. Calcium, magnesium, chlorides, sulfates, phosphates, nitrates, ammonium, biochemical oxygen demand (BOD), total alkalinity (TA) and Ca/Mg hardness (TH) were analyzed in the laboratory according to standard methods (RODIER 1966).

### **4. Data analysis**

Taxonomic richness, total and relative abundances for all and common species were used to assess microcrustacean / macrocrustacean community structure. Factorial correspondence analyses were performed on the relative abundances of the collected taxa. Rank-species dominance curves were plotted following the method of LAMBSHEAD et al. (1983) to assess general patterns of dominance in the samples. Relationships between physico-chemical and biological data were investigated using principal components analysis, while the impact of wells location and groundwater extraction regimes was tested with factorial discriminant analysis.

## RESULTS

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### 1. Fauna general characteristics

Ten macrocrustacean species (8 amphipods, 2 isopods) and thirteen cyclopoid species (cf. chapter 2 of the present study) were found in the samples over the sampling periods. Nine of the ten amphipod/isopod taxa were stygobite species that occur regularly in karstic systems and porous aquifers from southern France (RUFFO 1986, COINEAU 1968, GINET 1996). In contrast, only five cyclopoid taxa were exclusive groundwater-dwelling, whereas the others constituted widely-distributed surface species occasionally encountered in ground waters (LESCHER-MOUTOUÉ 1973, 1986, POSPISIL & DANIELOPOL 2000).

#### Cyclopoida

<i>Macrocylops albidus</i>	JURINE 1820	<i>Diacyclops bicuspis</i>	CLAUS 1857
<i>Eucyclops serrulatus</i>	FISCHER 1851	<i>Diacyclops bisetosus</i>	REHBERG 1880
<i>Tropocyclops prasinus</i>	FISCHER 1860	<i>Graeteriella unisetigera</i> *	GRAETER 1910
<i>Paracyclops fimbriatus</i>	FISCHER 1853	<i>Graeteriella</i> sp. *	
<i>Acanthocyclops viridis</i>	JURINE 1820	<i>Speocyclops</i> sp. *	
<i>Diacyclops languidus</i>	SARS 1863		
<i>Diacyclops languidoides</i> *	LILLJEBORG 1901		
<i>Diacyclops clandestinus</i> *	KIEFER 1926		
		Assigned to <i>Diacyclops</i> spp.*	

#### Amphipoda

<i>Salentinella gineti</i> *	BALAZUC 1957
<i>Salentinella petiti</i> *	COINEAU 1963
<i>Niphargus gineti</i> *	BOU 1965
<i>Niphargus pachypus</i> *	SCHELLENBERG 1933
<i>Niphargus robustus</i> *	CHEVREUX 1901
<i>Niphargus gallicus</i> *	SCHELLENBERG 1935
<i>Niphargus</i> n. sp. *	
<i>Ingolfiella thibaudi</i> *	COINEAU 1968

#### Isopoda

<i>Proasellus meridianus</i>	RACOVITZA 1919
<i>Stenasellus virei</i> *	DOLFFUSS 1897

**Table 2.** List of the crustacean taxa collected in the stations P1 to P15. Very rare harpacticoid and ostracods were not considered. Stygobite species are marked with an asterisk (\*).

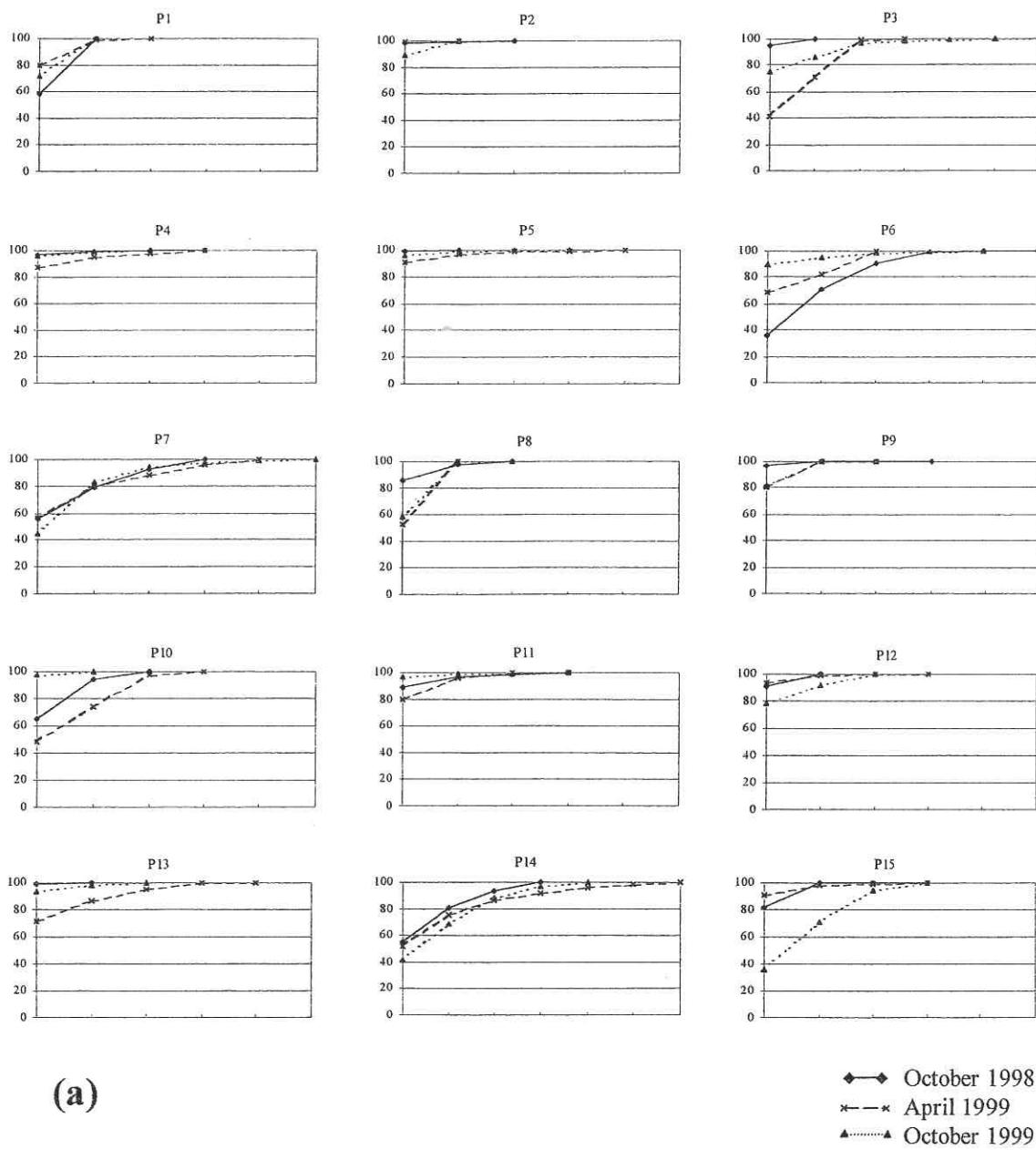
## 2. Dominance patterns

Strong dominance of a few species was usually observed within the samples, and was in general slight higher amphipod/isopod than for cyclopoid assemblages. The extreme situation was best illustrated for wells P2, P4, P5, P9, P11 and P12 where one cyclopoid species accounted for more than 90% of total abundance (Fig. 2a). On the contrary, few wells (P3, P6, P7, P10, P14) showed more balanced assemblages, but except for P7 and P14 this generally occurred at one single period.

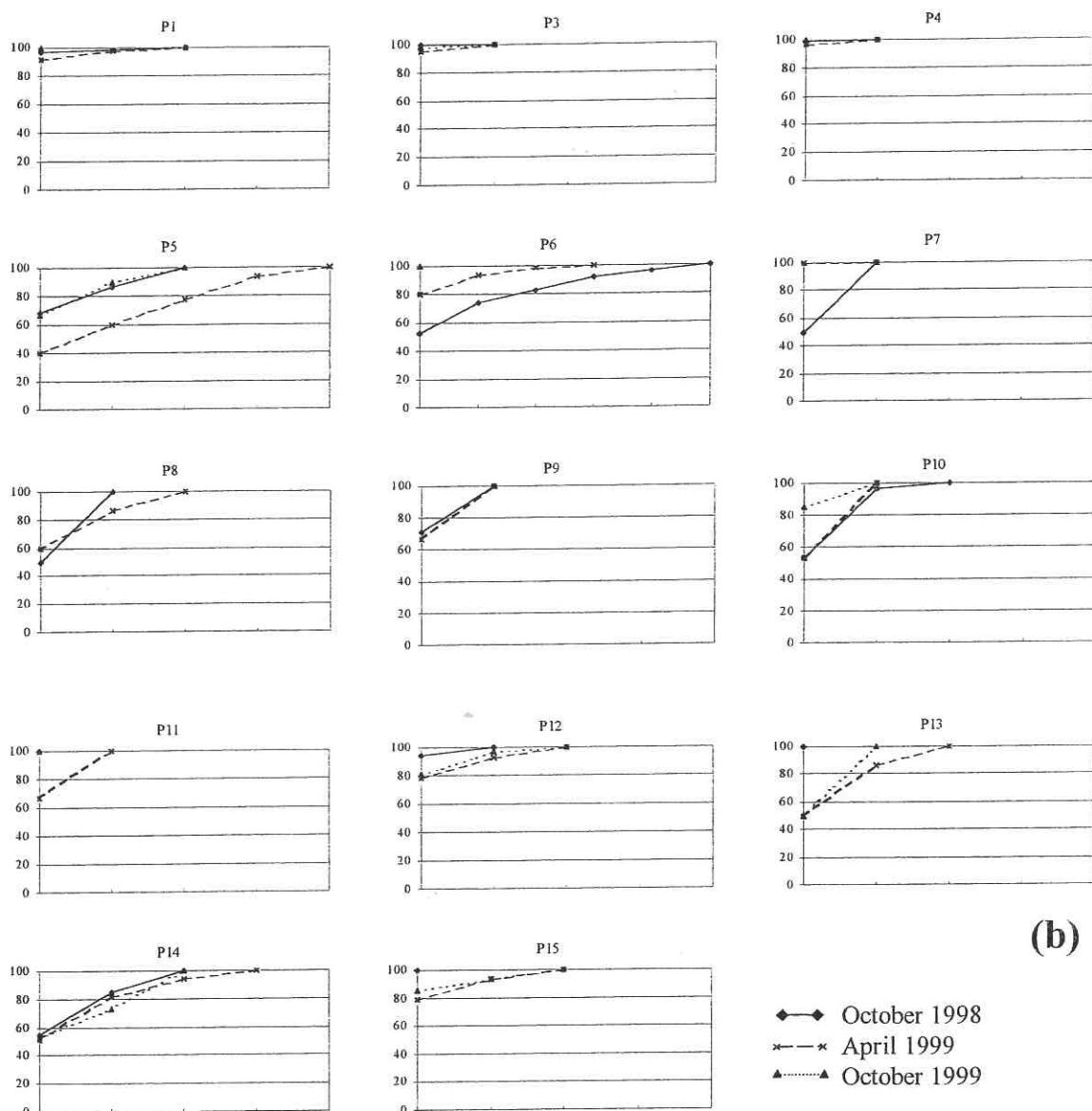
**Figure 2.** RSA-curves (cumulative percentages of ranked relative abundances) for the collected species in the 15 stations of the Ariège aquifer (all species were considered). X-axis : number of species. Y-axis : relative abundances.

(a): microcrustacean taxa (b): macrocrustacean taxa.

RSA-curves were not plotted for macrocrustacean assemblages in station P2 as only one species was collected per period.



On the whole, dominance patterns did not demonstrate great temporal variation at most sites, except in samples with low invertebrate abundances (e.g. P3 for cyclopoid species, P7 to P9 for amphipod species, Fig. 2b). When changes in community organisation happened, they could not be directly related to physico-chemical modifications of ground waters.

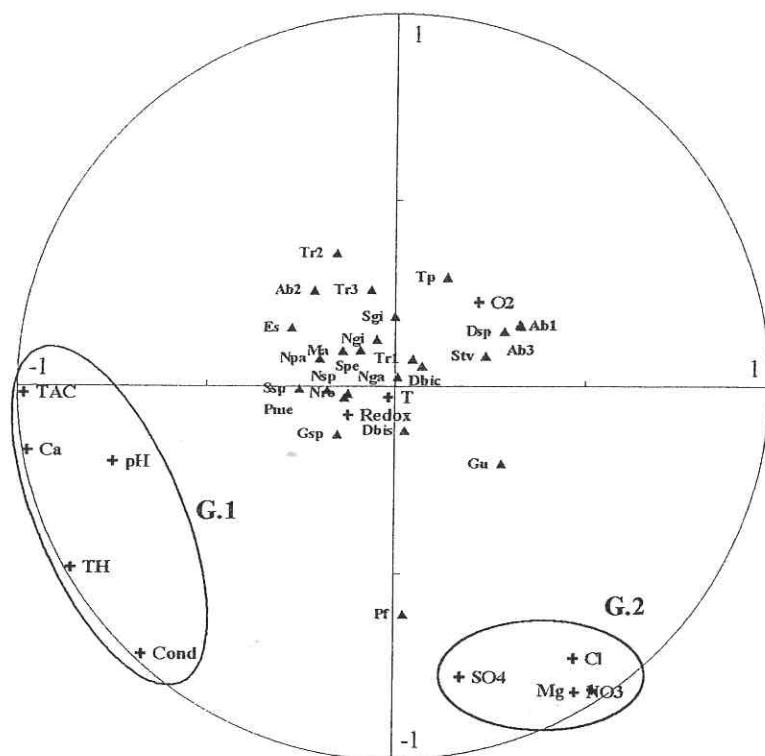


## 2. Physicochemical parameters and crustacean assemblages

In the area studied, groundwater physicochemical parameters exhibited strong global similarities between the sampling periods, associated with spatial (among-site) variations (data were partially presented in DUMAS & LESCHER-MOUTOUÉ 2000).

Principal Components Analysis (PCA hereafter) was performed on physicochemical data, excluding biological data that were introduced as additional variables. 12 physico-chemical variables (ammonium and phosphates excluded, as great deviations were observed in variance due to punctual contamination in stations P8 / P9) and 25 biological variables (relative abundances for each species, total abundance and taxonomic richness for microcrustaceans, macrocrustaceans taken separately and together) were considered.

The resulting factorial axes were then based upon physicochemical similarity/dissimilarity between stations, faunal variables being secondary on the ordination diagram to check for relationships between crustacean assemblages and groundwater physical and chemical parameters (Fig. 3).



**Figure 3.** PCA ordination plot (F1x F2) for physicochemical and biological data.

+ Physicochemical variables

Temperature (T), dissolved oxygen ( $O_2$ ), conductivity (Cond), redox potential (redox), pH, total hardness (TH), alkalinity (TA), chlorides, calcium, magnesium, nitrates, sulfates.

▲ Biological variables

Sgi: *Salentinella gineti*, Spe: *S. petiti*, Ngi: *Niphargus gineti*, Nga: *N. gallicus*, Npa: *N. pachypus*, Nro: *N. robustus*, Nsp: *Niphargus* sp., Pme: *Proasellus meridianus*, Stv: *Stenasellus virei*, Dbic: *Diacyclops bicuspis*, Dsp: *Diacyclops* spp, Dbis: *D. bisetosus*, Pf: *Paracyclops fimbriatus*, Tp: *Tropocyclops prasinus*, Es: *Eucyclops serrulatus*, Ma: *Macrocylops albidus*, Gu: *Graeteriella unisetigera*, Gsp: *Graeteriella* sp., Ssp: *Speocyclops* sp.

Ab1, Ab2, Ab3 / Tr1, Tr2, Tr3: total abundance / taxonomic richness for microcrustacean, macrocrustacean and total (microcrustacean + macrocrustacean) taxa.

As previously observed (Chapter 2 of the present study), the discrimination of groundwater quality-related parameters was strong along the factorial F1/F2 axes (contribution to total variance: F1+F2 = 64.9%).

The same groups of environmental variables was still found over the three surveys:

Group 1: natural mineralization variables mainly related to F1 ;

Group 2: pollution-related variables from anthropic (agricultural) origin, mainly related to F2.

All the faunal variables, either diversity or abundance descriptive measures (taxonomic richness, species relative abundances, total abundance) were aggregated together close to the centre of gravity on the ordination plot. Correlation with F1 or F2 axis were low in every case ( $\leq 0.3$ , except for the cyclopoid species *Paracyclops fimbriatus*) and suggested that biological and physico-chemical variables could be considered almost independent.

### 3. Spatial distribution patterns

Faunal distributions were investigated using factorial analyses on the relative abundances of the collected taxa. After preliminary tests, the initial data matrix (i.e. 23 taxa x 45 stations) was reduced in order to separate microcrustaceans from macrocrustaceans. Factorial correspondence analyses (FCA hereafter) were first performed on all the collected taxa, then a second analysis was performed excluding rare taxa.

Relationships between distribution patterns and aquifer structure/groundwater extraction were tested using factorial discriminant analyses (FDA hereafter) according to the characteristics of the sampled stations (Table 1). Discrimination was tested for the following groups:

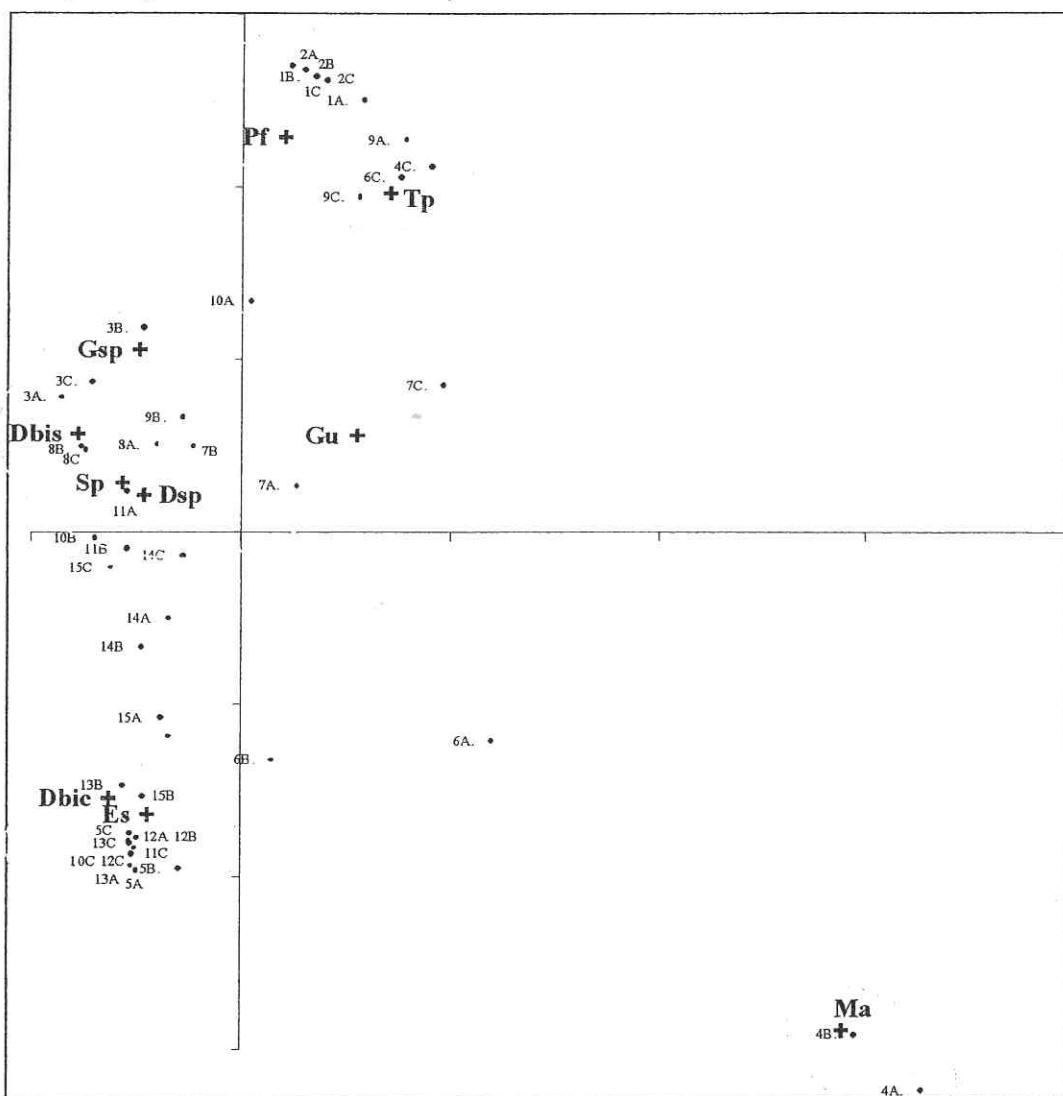
**Table 3.** Groups of stations and discriminant criteria for factorial discriminant analyses.

Stations	Discriminant criteria
1, 2, 3, 4, 5, 6, 7, 8, 9	Alluvial terrace.
10, 11, 12, 13, 14, 15	Alluvial plain.
1, 5, 6, 10, 11, 12, 13	Groundwater for irrigation.
2, 3, 7, 8, 9, 14, 15	Groundwater for domestic/livestock use
4	No groundwater extraction.

### 3.1 Cyclopoid distribution patterns: factorial analysis.

The ordination plots were strongly similar when either all the species or only the common ones were considered, with percentages of variance explained by the two first axes between 40 and 50% (all species, F1+F2 40.5%, common species: F1+F2 47.5%). Four main groups could be distinguished based upon species dominance (Fig. 4) :

**Figure 4.** FCA (station plot) for the cyclopoid taxa in the 15 stations (Ariège aquifer). Horizontal axis: F1, vertical axis: F2. 1-15: number of sampled station. A, B, C: sampling surveys (October 1998, April 1999, October 1999). Dbic: *Diacyclops bicuspidatus*, Dsp: *Diacyclops*.spp, Dbis: *D. bisetosus*, Pf: *Paracyclops fimbriatus*, Tp: *Tropocyclops prasinus*, Es: *Eucyclops serrulatus*, Ma: *Macrocylops albidus*, Gu: *Graeteriella unisetigera*, Gsp: *Graeteriella* sp., Ssp: *Speocyclops* sp. All the species (contribution F1+F2: 40.5%).



The first group was composed of two samples from the station P4, with *Macrocylops albidus* dominating the assemblages. The second was constituted basically by samples from stations P1, P2 for

all the periods and samples from wells P4, P6 and P9 in October 1998/1999 ; dominant taxa were *Tropocyclops prasinus* and *Paracyclops fimbriatus*. Samples from stations 5, 12, 13 and 14 essentially were associated with *Eucyclops serrulatus* and *Diacyclops bicuspidatus*, while the last group was mainly for stations 3, 7, 8 with *Graeteriella*, *Speocyclops* and *Diacyclops* species. Interestingly, these latter species were all groundwater-dwelling species (stygobite and stygophile).

### 3.2 Cyclopoid distribution: discriminant analysis.

FDA revealed significant discrimination of faunal assemblages based upon both hydrogeological processes (stations from the geological plain / terrace, cf. Table 4a) and groundwater utilization (Table 4b). Discriminant power ranged from 0.3 to about 0.7 with associated probabilities (Wilk's test) between 0.001 and 0.05.

**Table 4.** Discriminant analyses for cyclopoid taxa in the Ariège aquifer.

(a): FDA based upon geological location of the stations (alluvial plain versus alluvial terrace)

(b): FDA based upon groundwater extraction regimes of the stations (no extraction / moderate, all the year / strong, seasonal).

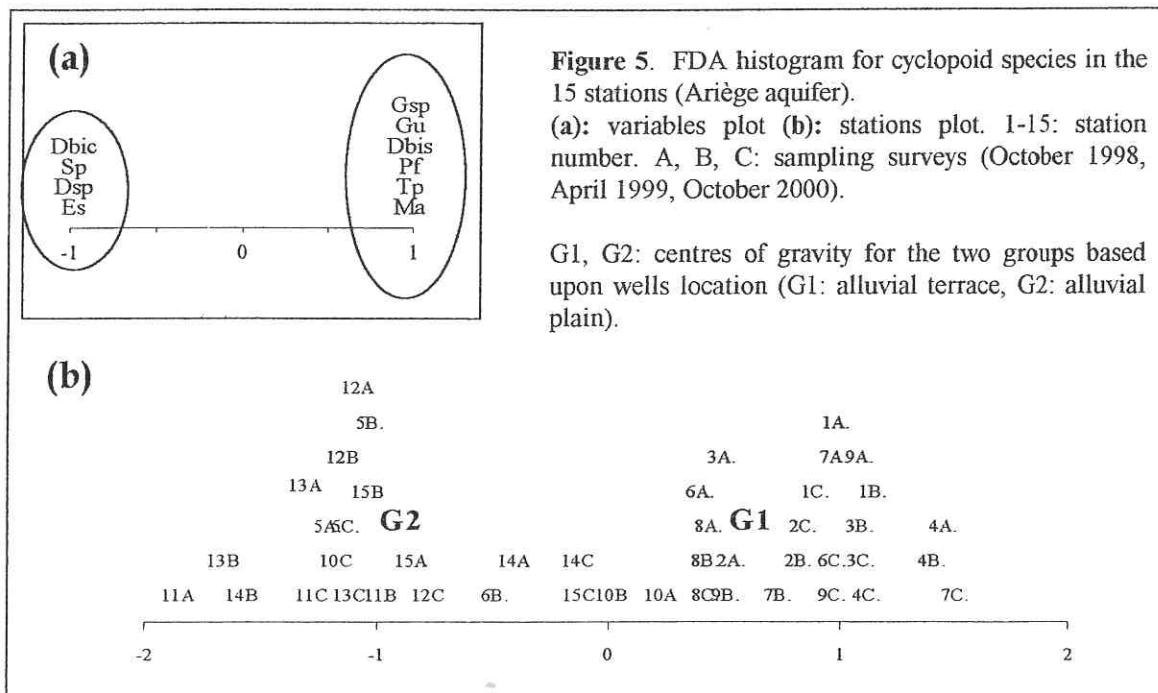
Number of groups for FDA, discriminant power (correlation with factorial axes), Wilk's coefficient and associated levels of significance (type I-error probability).

	(a) Sedimentary system (alluvial plain / alluvial terrace)		(b) Wells status (ground water extraction)	
	All the species n=2	Common species	All the species n=3	Common species
Number of groups				
% well-ranked	82.2 %	82.2 %	77.8 %	71.1 %
Discriminant power				
F1 axis:	0.57	0.46	0.68	0.67
F2 axis:	-	-	0.48	0.312
W (Wilk's coefficient)				
F1 axis:	31.92	24.46	67.33	59.29
F2 axis:	-	-	24.56	15.07
Levels of significance				
F1 axis:	0.05	< 0.001	< 0.001	< 0.001
F2 axis:	-	-	< 0.01	< 0.05

### Hydrogeological context

Cyclopoid assemblages differed between samples in relation with sites location (Fig. 5a, 5b). On the whole, assemblage composition exhibited well-marked difference between G.1 and G.2 (percentage of well-ranked samples from FDA: 82.2% for both total or common species).

Assemblages from group G.1 (stations located in the terrace) were characterized by *Macrocylops albidus*, *Paracyclops fimbriatus*, *Tropocyclops prasinus*, *Diacyclops bisetosus* and the stygobite species *Graeteriella unisetigera*, *Graeteriella sp.* In contrast, wells from G.2 (plain) harboured *Eucyclops serrulatus*, *Diacyclops bicuspis* and the hypogean *Diacyclops spp.*, *Speocyclops sp.*



### Groundwater utilization

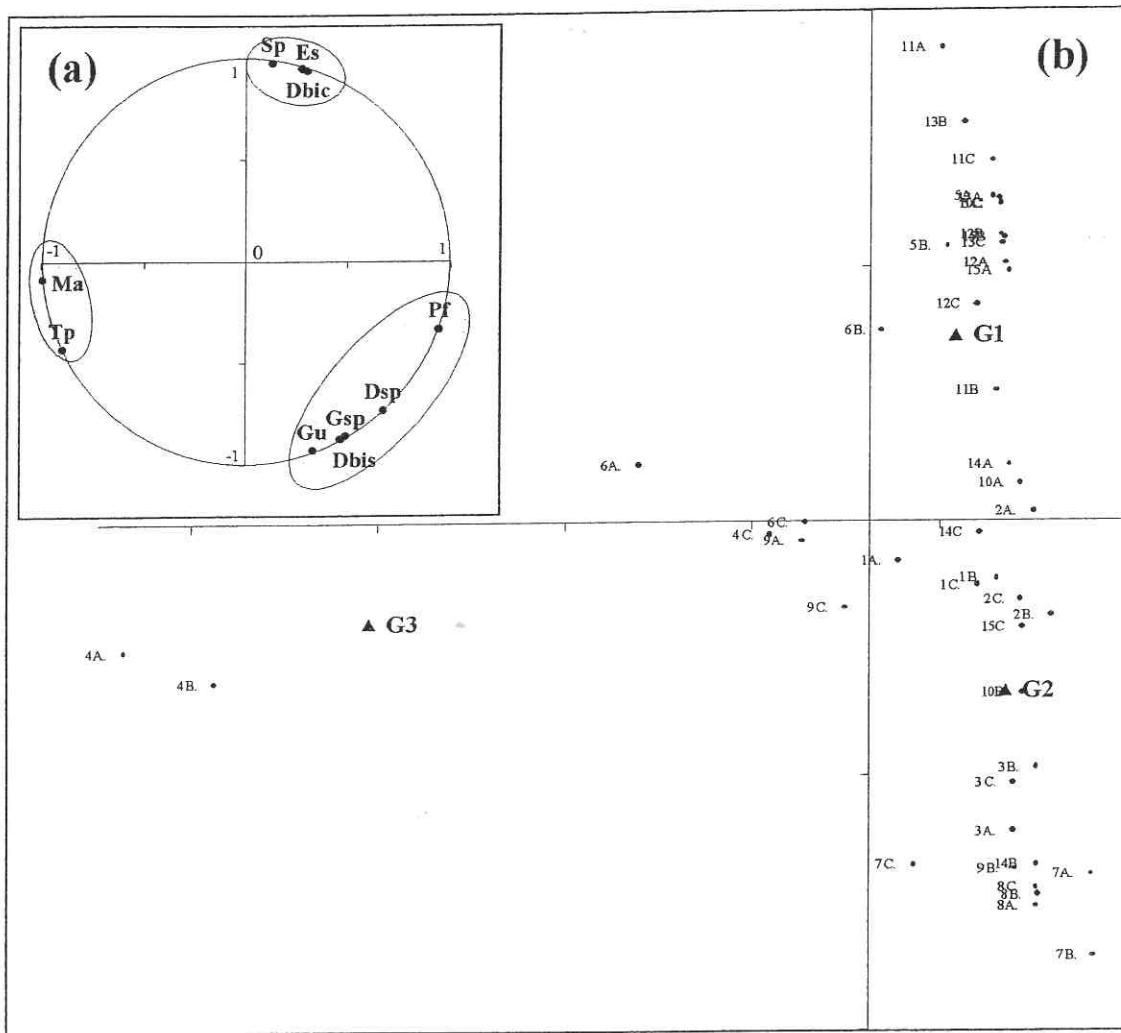
When investigating the potential impact of groundwater extraction, the station P4 had an extreme position on the FDA ordination plot (Fig. 6b) and therefore could be suspected of biasing the results. However, the same analysis performed excluding P4 showed the same trends (FDA, discriminant power 0.34, p<0.05 for Wilk's test, well-ranked samples 71.4%).

Three groups of species were separated along F1/F2, in relation with wells status (Fig. 6b):

- Epigean *Macrocylops albidus*, *Tropocyclops prasinus*, generally found in the samples from station P4 (abandoned well) ;
- Epigean and hypogean *Speocyclops sp.*, *Eucyclops serrulatus* and *Diacyclops bicuspis* from wells used for cereal irrigation ;
- *Graeteriella*, *Diacyclops* and *Paracyclops* species, found mainly in stations with water supply for domestic purposes.

Except for cyclopoids from the genus *Speocyclops*, all the groundwater-dwelling species were aggregated in the lower right quarter of the variable plot (Fig. 6a) and were then associated to G.2 samples, i.e. *Graeteriella unisetigera*, *Graeteriella* sp. and *Diacyclops* spp.

**Figure 6.** FDA for the cyclopoid taxa in the 15 stations (Ariège aquifer). (a) variable (b) station plots. Horizontal axis: F1, vertical axis: F2. 1-15: number of sampled station. A, B, C: sampling surveys (October 1998, April 1999, October 1999). G1, G2, G3: centres of gravity for the three groups of wells based upon groundwater extraction regimes (G.1: irrigation, G.2: domestic use, G.3: abandoned).



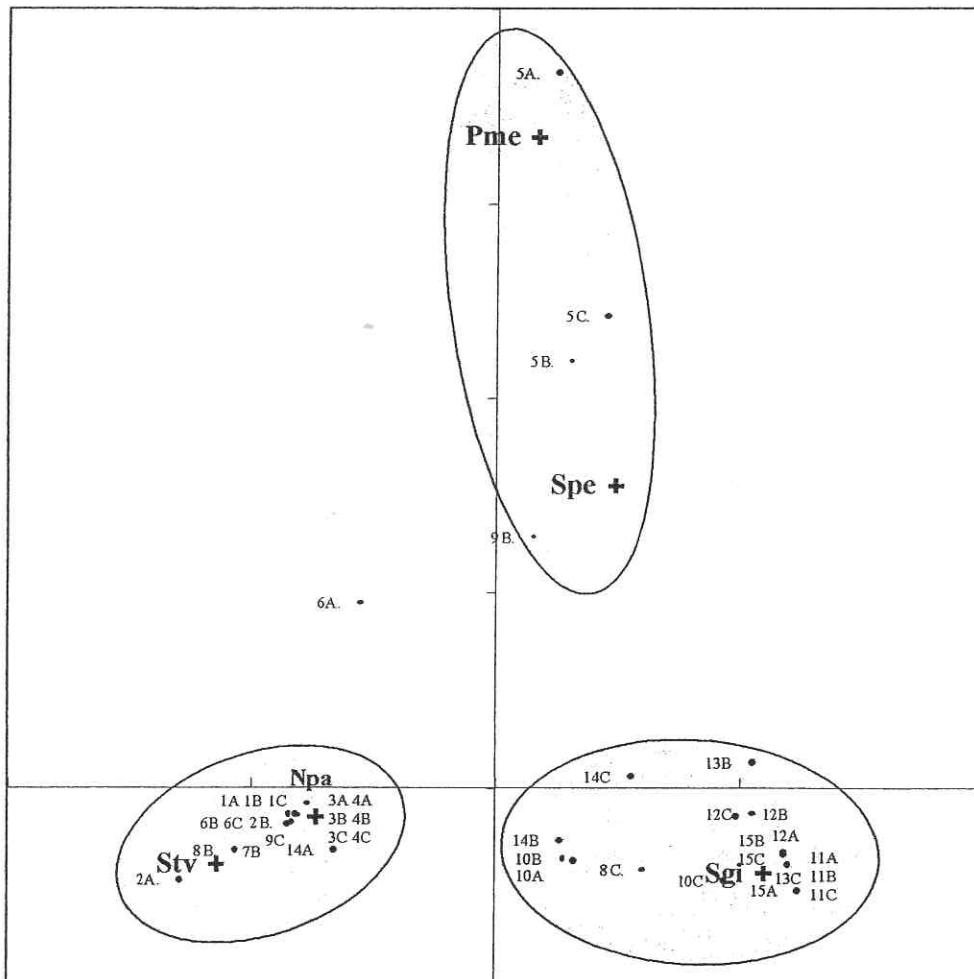
### 3.3 Amphipod / isopod distributions : factorial analysis. Patterns

As for cyclopoids, the ordination plots were similar when either all the species or only the common ones were considered. Yet, total variability was better explained taking only the common taxa into account (variability explained by the first two axes: all taxa, 45.6% / common taxa, 63.6%).

Based upon the coordinates of the samples, three homogeneous, well biologically-discriminated groups were thus obtained (Fig. 7):

- Stations P1 to P4, P6, P7 for all the surveys and P8, P9 for one. Assemblages in these wells were specifically dominated by *Niphargus pachypus* / *Stenasellus virei* association.
- Stations P10 to P15 (all the surveys) and P8 (one survey), dominant taxa being *Salentinella gineti*.
- Station P5 with one sample from P9. In this group, *Salentinella petiti* and *Proasellus meridianus* were together found in well P5, whereas P9 was characterized by *S. petiti* only.

**Figure 7.** FCA (station plot) for the common amphipod/isopod taxa in the 15 stations (Ariège aquifer). Horizontal axis: F1, vertical axis: F2. 1-15: number of sampled station. A, B, C: sampling surveys (October 1998, April 1999, October 1999). Sgi: *Salentinella gineti*, Spe: *S. petiti*, Npa: *N. pachypus*, Pme: *Proasellus meridianus*, Stv: *Stenasellus virei*.



These results suggested different macrocrustacean assemblages between stations from the alluvial plain (i.e. stations P1 to P9) and from the alluvial terrace (P10 to P15). P5 was the exception as samples 5A, 5B and 5C constituted an isolated group closely related to F2 axis, whereas the other samples were discriminated along F1.

### 3.4. Amphipod/isopod distribution: discriminant analysis

FDA confirmed this pattern, with high, significant discrimination between stations from different location over the floodplain (Table 5a). In contrast, no significant results were found in relation to groundwater utilization (Table 5b). The same trends were observed when either all or only the common species were considered.

**Table 5.** Discriminant analyses for macrocrustacean taxa in the Ariège aquifer.

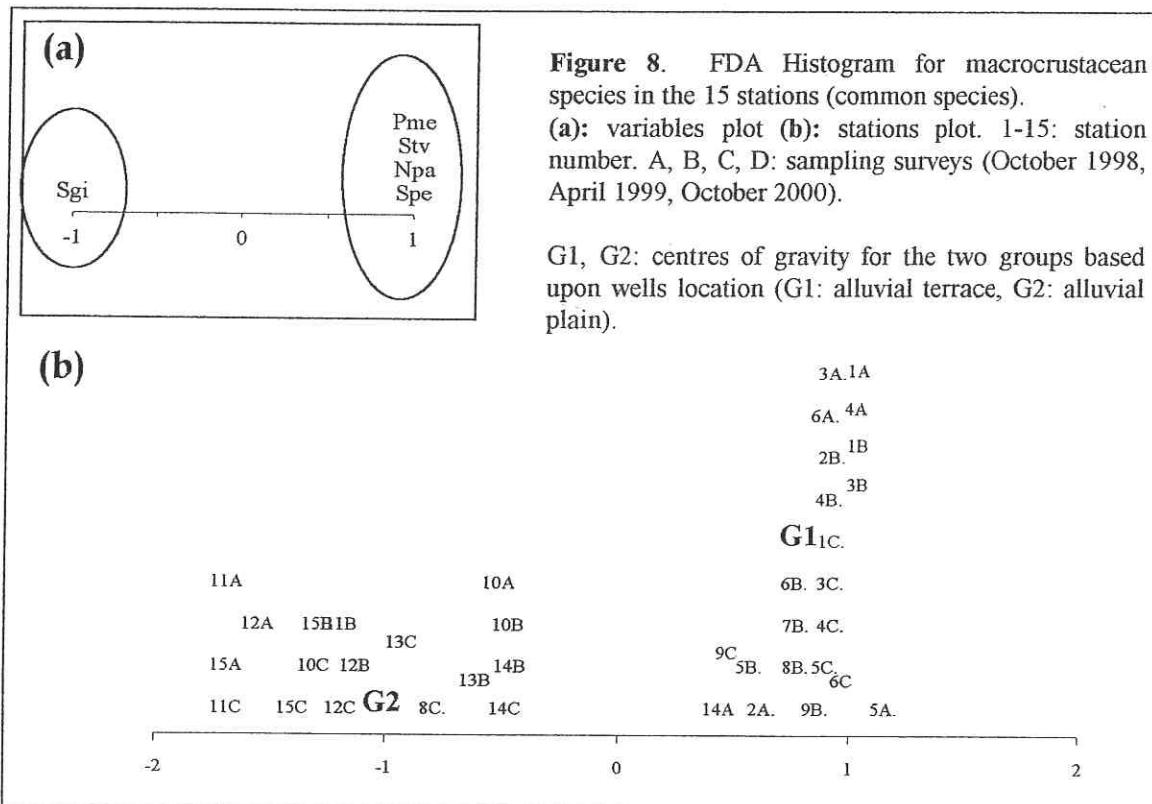
(a): FDA based upon geological location of the stations (alluvial plain versus alluvial terrace)

(b): FDA based upon groundwater extraction regimes of the stations (no extraction / moderate, all year / strong, seasonal).

Number of groups for FDA, percentage of well-ranked stations, discriminant power (correlation with factorial axes), Wilk's coefficient and associated levels of significance (type I-error probability).

	(a) Sedimentary system (alluvial plain / alluvial terrace)		(b) Wells status (ground water extraction)	
	All the species n=2	Common species	All the species n=3	Common species
Number of groups				
% well-ranked	82.1 %	94.9 %	48.7 %	64.1 %
Discriminant power				
F1 axis:	0.72	0.78	0.68	0.25
F2 axis:	-	-	0.69	0.14
W (Wilk's coefficient)				
F1 axis:	41.6	52.96	14.79	14.6
F2 axis:	-	-	5.67	5.08
Levels of significance				
F1 axis:	< 0.001	< 0.001	0.68	0.14
F2 axis:	-	-	0.69	0.28

A strong discrimination was observed between the two groups of stations from FDA (Fig. 8a, 8b, percentage of well-ranked samples: 94.9 %). Marked difference occurred between the macrocrustacean communities from stations 1-9 (*Salentinella petiti*, *Niphargus pachypus*, *Stenasellus virei*, *Proasellus meridianus*) and 10-15 (*Salentinella gineti*).



**Figure 8.** FDA Histogram for macrocrustacean species in the 15 stations (common species).

(a): variables plot (b): stations plot. 1-15: station number. A, B, C, D: sampling surveys (October 1998, April 1999, October 2000).

G1, G2: centres of gravity for the two groups based upon wells location (G1: alluvial terrace, G2: alluvial plain).

3A, 1A

6A, 4A

2B, 1B

4B, 3B

**G1**

1C.

6B, 3C.

7B, 4C.

9C, 5B,

8B, 5C,

6C

0

1

2

## DISCUSSION

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### 1. Crustacean assemblages in the wells

The presence of invertebrate species in wells or piezometers may result from two main processes: 1) colonization of the well from the surrounding zones of the aquifer where populations may be present, in relation to aquifer characteristics and species adaptation, and 2) accidental arrivals from surface runoff waters and/or infiltration (BOULAL et al. 1997) of species that cannot be considered representative of groundwater assemblages. The species collected in a given well may then be either adapted, hypogean species (stygobite/stygophile) or non-adapted, epigean species (stygoxene).

The macrocrustacean species harboured in the wells from the Ariège alluvial aquifer were in majority (9 of the 10 collected species) efficient, exclusively groundwater-dwelling animals (stygobites). In contrast, cyclopoid assemblages were specifically and numerically dominated by epigean species, although stygobite *Diacyclops* spp. sometimes formed dense populations in some stations.

For macrocrustaceans, the only stygoxene species was the isopod *Proasellus meridianus*, found only in two wells (P5, P6) in the whole study area. The choice of mostly properly closed, raised wells probably allowed us to avoid any strong epigean influence (colonization of the wells by surface species): *Proasellus meridianus* was found in the two unprotected, flush on the ground wells P5 and P6 located in zones where heavy flooding was observed. In the same way, the importance of water table depth was highlighted as P5 and P6 where *Proasellus* was found were characterized by the shallowest piezometric levels (P5 -1.50 m, P6 -0.50 m, whereas over the area the maximum was about 8 m, with an average of 4.50 m below the soil in the studied zone): studies indicate that taxonomic richness and faunal abundance in sediments display global negative patterns with increasing depth, especially for non-adapted, epigean taxa (MARMONIER et al. 1993, MARIDET et al. 1996). The stygoxene taxon *Proasellus meridianus* may therefore be considered atypical of the Ariège alluvial aquifer fauna.

### 2. Epigean / hypogean taxa in ground waters

The faunal assemblages from the Ariège alluvial aquifer were thus characterized by macrocrustacean taxa dominated by hypogean species, associated with microcrustacean taxa (generally) dominated by epigean species. This situation was commonly found in the literature.

Generally in ground waters the species of amphipods and isopods are mainly hypogean and in many cases the numbers of hypogean species exceed those of epigean ones in the same area. For instance, epigean taxa of macrocrustacean (*Gammarus* sp., *Asellus* sp.) are often associated with amphipod stygobite species (*Niphargus* sp., *Salentinella* sp.) in aquifers from the Rhône basin but mainly in the upper part of the aquifers linked to rivers, in the hyporheic zone close to the channel (MARMONIER et al. 1992, CREUZÉ DES CHÂTELLIERS et al. 1992). The alluvial aquifer of the Rhône river thus harbours 15 stygobite amphipod (GINET 1983, J. GIBERT, personal communication). Similarly, 7 exclusively hypogean amphipod / isopod species and no epigean species were collected in the hyporheic zones of two streams from the French Pyrénées (ROUCH 1988, 1995).

In contrast, groundwater cyclopoid assemblages appear frequently composed of hypogean and epigean species, these latter with higher taxonomic diversity.

MAUCLAIRE (1999) reported 9 epigean and 6 stygobite species from three alluvial aquifers from the Rhône basin. 14 epigean and 12 hypogean species were recorded in a Danube wetland (POSPISIL & DANIELOPOL 2000). In the same way, 7 stygobite / 7 epigean and 3 stygobite / 4 epigean species were collected in the hyporheic zones of two Pyrenean streams (ROUCH 1988, 1995).

Further data would be needed in order to attempt to explain this situation ; it may be the result of species traits (body size, diet, locomotion) and/or greater habitat affinities allowing an easier colonisation of interstitial biotopes by epigean microcrustacean taxa rather than by epigean macrocrustaceans.

### 3. Diversity / dominance patterns

Compared with other aquifers, the Ariège assemblages exhibited moderately high taxonomic diversity. In the area, 13 cyclopoid, 7 amphipod and 3 isopod species were collected. Data from the literature for cyclopoids ranged from four species in two piezometers in a small, Pyrenean alluvial aquifer (ROUCH 1995), seven species in a large alluvial aquifer in the Danube region (MÖSSLACHER 1998) to 23 species within a 0.8 km<sup>2</sup> area in a Danube wetland (POSPISIL & DANIELOPOL 2000) or 31 species at 14 sites scattered over the United States (STRAYER & REID 1999).

In the same way, for Amphipods 3 Niphargidae species were collected in 8 piezometers from 3 alluvial aquifers of the Rhône river basin (MAUCLAIRE 1999) while 11 amphipod / isopod taxa were collected in interstitial waters of a backwater of the Rhône river (DOLE-OLIVIER 1998) and up to 15 species in the whole alluvial systems of the Rhône river basin.

Many authors also reported high diversity in shallow hyporheic zones, that can be considered as transitional zones (ecotones) and harbour epigean and hypogean species (GIBERT et al. 1997, MAUCLAIRE 1999). For instance, 15 cyclopoid, 4 amphipod and 3 isopod species were reported from a shallow hyporheic system of a small Pyrenean river (ROUCH 1988).

On the whole, our result thus suggest an intermediate crustacean diversity for the Ariège aquifer at both local and regional scales (i.e.  $\alpha$ - diversity and  $\gamma$ -diversity *sensu* HEYWOOD & WATSON 1995).

Considering the dominance patterns in the area, characterized by one dominant species and several rarer ones for either microcrustacean or macrocrustacean taxa, the same situation was found in the literature for groundwater ostracod (DANIELOPOL et al. 1997), harpacticoid (ROUCH 1995) and cyclopoid species (DUMAS & LESCHER-MOUTOUÉ 2000). It could be the result of competitive interactions between species in a stable environment, of environmental disturbance such as groundwater contamination (SHAW et al. 1983, LAMBSHEAD et al. 1983) or, according to DANIELOPOL et al. (1999), the result of various life history strategies in order to avoid the over-exploitation of dispersed energy resources in these constraining biotopes. Dominance patterns will be discussed with more details in chapter 4 of this study.

#### **4. Faunal assemblages and groundwater physicochemical characteristics**

The biological variables describing the crustacean assemblages in the sampled wells appeared to be independent of the environmental variables. This was true whatever community descriptors (diversity, abundance, dominance indices) and physicochemical processes (contamination resulting from agricultural-induced pollution, natural mineralization processes linked to hydrodynamics and hydrogeology) were considered.

These results imply that, with respect to study scales, groundwater physicochemical parameters did not play a major role in the spatial distribution of crustacean taxa at the scale of this aquifer.

#### **5. Spatial distribution patterns of crustaceans in the Ariège aquifer**

Among the factors influencing interstitial groundwater invertebrates, sediment structure and hydrodynamics of the aquifer are generally assumed to play an important role (CREUZÉ DES CHÂTELLIERS 1991, MÖSSLACHER 1998).

Cyclopoids, amphipods and isopods, like all interstitial species whatever their strategic ecological pathway, do not randomly colonize the subterranean habitat: sediment characteristics and hydrodynamics act as constraining factors for interstitial species (GIBERT et al. 1994, STRAYER et al.

1997) and determine horizontal and vertical distribution patterns (MARIDET et al. 1996). *In fine*, species ecological preferences correspond to a global, biological integration of all the considered factors.

#### *Location of the stations and hydrogeological processes*

In the Ariège floodplain, previous studies on geology and hydrodynamics established marked functional differences between parts of the aquifer area (CUGNY 1980, SEGUIN 1980, Ariège Chamber of Agriculture and Adour-Garonne Water Agency, personal communications): the low plain and the low terrace could be clearly distinguished upon groundwater dynamics and sediment characteristics. The low plain corresponds to recent (Würm) alluvial deposits with moderate transmissivity ( $10^{-3} \text{ m}^2.\text{s}^{-1}$ ). The Ariège and Crieu rivers flow in this area, whose east/west average extension is 4-5 km. Sediment thickness ranges from 5 to about 16 m.

The low terrace, standing 2-3 m above the plain, is composed of more ancient (Riss) deposits exhibiting much lower hydrodynamical properties (transmissivity  $10^{-4} \text{ m}^2.\text{s}^{-1}$ ).

Characterizing wells using faunal composition gave a match with these hydrogeological structures: the interstitial communities in the alluvial plain were different from those in the alluvial terrace. Discrimination was significant between stations P1 to P9 from the central area of the floodplain (low terrace area with moderately permeable sediments, restricted groundwater circulation and shallow (1-4 m) water tables) and stations P10 to P15 situated in the low plain, characterized by more transmissive sediments with higher permeability/groundwater circulation, deeper water-tables (6-12 m). This was observed for both microcrustacean and macrocrustacean assemblages, whatever species (all / common) were considered ; yet, the strongest relationships were between amphipod/isopod species and the station hydrogeological context.

#### *Wells status and groundwater extraction*

In this aquifer, three main types of production wells can generally be found in relation to water utilisation and extraction regimes:

- Wells for agricultural irrigation, typically located close to cereal (maize) fields, with strong, seasonal groundwater extraction (July to September) ;
- Wells for domestic and/or livestock use, close to farms, characterized by moderate, all-year-round groundwater extraction ;
- Abandoned wells.

The results demonstrated that water extraction regimes in production wells could influence the interstitial fauna, as the community structure differed between abandoned, irrigation or domestic wells, at least for cyclopoids. This may be due to physical changes in sediment structure caused by intensive pumping. In case of production wells, for instance, this process is known to create high negative hydraulic gradients in the vicinity of the wells that increases water exchanges between surface and groundwater (VANEK 1995). Modifications in hydrodynamics locally occur that may affect clogging processes in sediment and faunal distribution. Small interstitial stygobite species (*Graeteriella*, *Diacyclops* species) appeared to be more sensible to water extraction than large, epigean *Macrocylops* or *Eucyclops* species. On the contrary, wells utilisation did not seem to affect the macrocrustacean fauna (type I-errors for FDA, F1: 0.68, F2: 0.69).

In conclusion, at large scale (floodplain scale), interstitial crustaceans may constitute powerful natural indicators of the aquifer hydrodynamics in relation with natural or anthropic factors: sediment characteristics (permeability), water-table depth, groundwater extraction volumes and frequency. Their efficiency was based on an assessment of dominance within the phreatical assemblages: while simple abundance/diversity measurements (number of individuals per species, number of species) could not be significantly associated with environmental parameters, relative abundance patterns performed well and provided valuable information.

These results confirm that species occurrence in the wells is not due to random processes. While some individuals may be representative of local characteristics (well contamination, accidental colonization etc.), the phreatobiological communities on the whole can be considered representative of the aquifer fauna, at least under certain conditions :

- 1) wells protected from strong surface influence ;
- 2) adequate sampling methods (MALARD et al. 1997, DUMAS & FONTANINI 2000) ;
- 3) wells with adequate characteristics: in addition with water extraction, well diameter may also influence the sampling efficiency. Preliminary tests in the same area on large (2-3 m in diameter) wells designed for drinking water supply with permanent, intensive groundwater extraction (range 30–100 m<sup>3</sup>/h) provided a very poor fauna in comparison for all taxa considered.

Taken these considerations into account, agricultural/domestic wells then provide a valuable access to the interstitial fauna from aquifer zones where sampling is difficult (located laterally from the river channels or deeper than usually accessible with traditional sampling devices such as Bou-Rouch pump or core-samplers).

## 6. Ecological patterns and study scales

This paper also confirm ecological preferences for the collected species: DANIELOPOL et al. (1999) found preferential occurrence of *S. gineti* in the deeper sediment layers from the hyporheic zone of a Pyrenean stream, whereas *N. pachypus* was found within superficial layers and the large *N. gineti* preferred highly permeable zones. In the Ariège aquifer, at the scale of the floodplain, the large species *N. gineti* and *S. gineti* were similarly associated with more transmissive, deeper aquifer zones while amphipod *N. pachypus* and isopod *S. virei* characterized wells from low transmissive, shallow aquifer zones. The intermediate position of *S. petiti* suggests lower specialisation as this small species was found in the sampled wells regardless of sediment permeability or depth.

The data obtained also suggest that ecological studies in aquifers should be performed over various size scales: if regional distribution of macrocrustaceans appears closely related to hydrogeological characteristics of the floodplain, on smaller scales, other parameters (nutrients, sediment heterogeneity, biological interactions...) may play an important role and define more-detailed, local distributions.

The atypical macrocrustacean assemblage of well P5 or the lower discriminations found for cyclopoids underline the complexity of these relationships and may result from local factors. DANIELOPOL (1989) showed that both mesoscale (m) and microscale (cm) factors are appropriate for understanding the fine ecological preferences of groundwater animals in their “ patchy ” environment.

In this context, further studies should focus on small-scale distribution patterns in this aquifer, in order to describe environmental changes from the piezometric levels to the deep zones of aquifer, to elucidate the strategies of hypogean species in response to the environmental factors and to identify the resulting diversity / distribution patterns.

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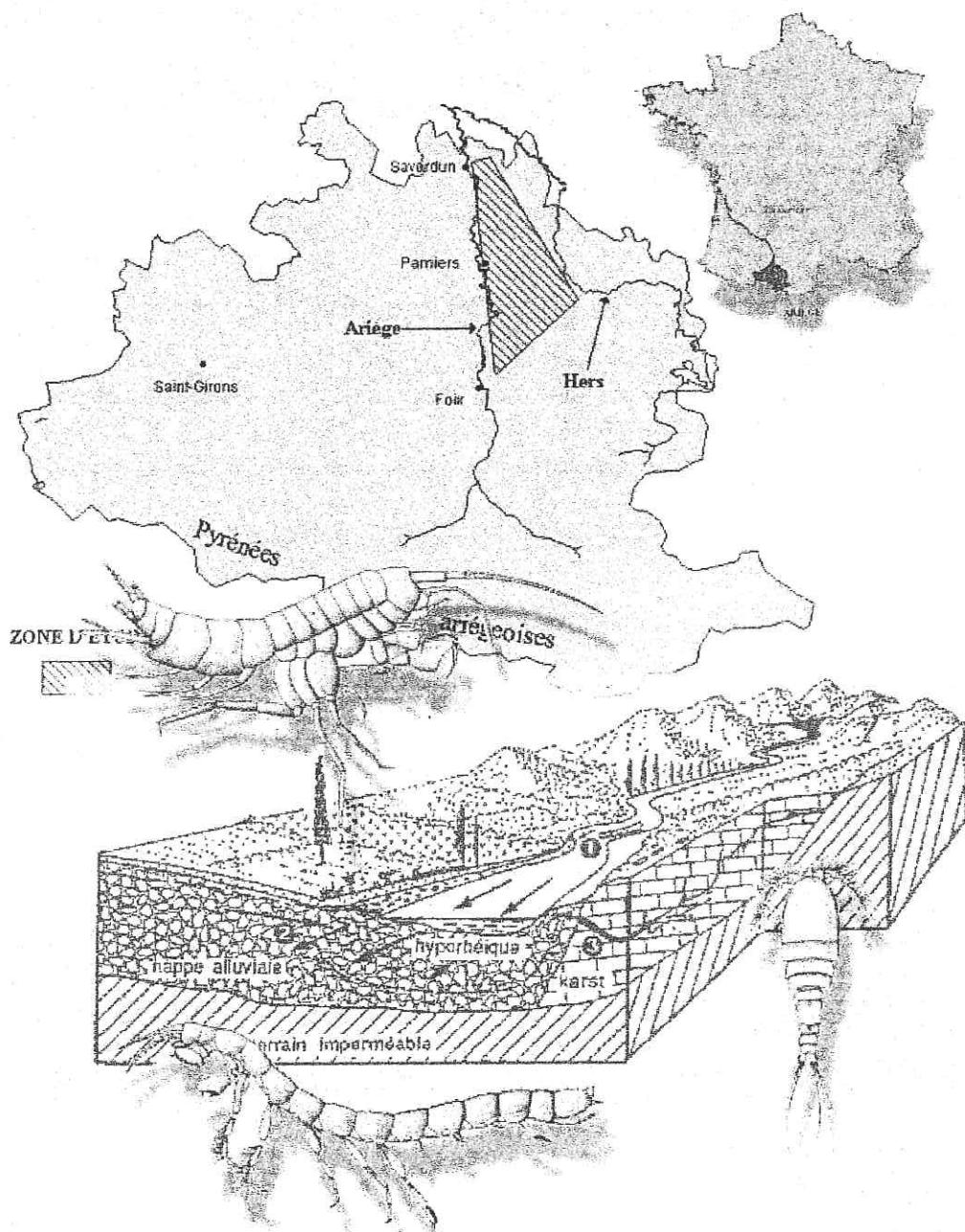
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## - CHAPITRE 4 -





## **Stability of interstitial crustacean communities and environmental variability in an alluvial aquifer.**

### **ABSTRACT**

The temporal stability of interstitial crustacean communities was investigated in relation with environmental variability in a semi-confined aquifer. We hypothesized that strong physico-chemical stability would enhance persistence and affect dominance patterns for groundwater fauna. Fifteen wells were sampled over a two-years period in order to assess the global stability of ground waters and to evaluate the temporal changes in species assemblages. The aquifer was characterized by limited connection with surface waters and a strong temporal stability for physical and chemical parameters. For each station, environmental patterns displayed only minor changes over the period, while among-sites differences were recorded. Interstitial crustaceans displayed rather diverse assemblages dominated by cyclopoid taxa. A high constancy was found for community composition and structure (i.e. taxonomic richness, diversity index) over the study periods. Dominance patterns generally displayed a strong dominance for one single species, whenever micro- or macrocrustacean assemblages were considered. When compared with literature data from surface waters, differences in faunal persistence were observed for interstitial fauna. With respect to local conditions and to the degree of connectivity between subterranean and surface systems, our results suggest an overall persistence for groundwater interstitial assemblages that is likely to be related to a marked environmental stability.

## INTRODUCTION

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The role of disturbance in ecosystems is a topical issue of both general and stream ecology (PICKETT & WHITE 1985, DEATH & WINTERBOURN 1994, RESH et al. 1988). Recently, this concept has gained prominence as a central organizing principle in community structure and dynamics, pushing aside the XX<sup>th</sup> old paradigm of systems at equilibrium in a constant environment (review in RESH et al. 1988, REICE et al. 1990).

The definition and measurement of environmental stability / disturbance have proved problematic (PICKETT et al. 1989, RYKIEL 1985, POFF 1992). Although the effects of a discrete disturbance may be quite simple to measure, assessing the global stability of a particular habitat or system is difficult. Recent work include magnitude, frequency as well as predictability of potential events in the definition of disturbance: “(disturbance is) any discrete event characterized by a frequency, intensity and severity outside a predictable range, that changes resources or physical environment” (RESH et al. 1988). Thus, the overall stability of a system can be assessed by measuring changes in its environmental variables (DEATH & WINTERBOURN 1994) with respect to the considered time-scales.

The role of environmental stability in regulating community structure and dynamics is of particular importance in freshwaters where disturbance are common (REICE et al. 1990, DEATH 1996, BOULTON et al. 1992). Community parameters are expected to be determined by both environmental changes and biological interactions, with respect to the spatial and temporal scales considered. In stream sediments for example, the distribution of the fauna is commonly linked to very short-term perturbations such as floods or spates (HYNES 1983, MARMONIER & DOLE 1986, MARMONIER & CREUZÉ DES CHATELLIERS 1991, BOULTON & STANLEY 1995), but impacts upon hyporheic fauna appear complex (PALMER 1992, WARD et al. 1998).

While many authors investigated the effects of similar discrete disturbance events on species density / diversity, very few studies deal with invertebrate community parameters (structure, dynamics) in relation with global habitat stability.

The results of recent studies on freshwater invertebrates communities over a 18-months period showed that the stable sites generally had more persistent communities than the unstable sites (DEATH & WINTERBOURN 1994). DEATH (1996), investigating the influence of environmental stability on the species abundances distribution, also found a gradient in distribution patterns: communities in unstable sites were characterized by one or two dominant taxa, those in habitats of intermediate stability had

relatively uniform species abundance patterns, and those in very stable conditions were again dominated by one or two taxa.

The aim of this study was to investigate the structure and dynamics of freshwater communities in an alluvial aquifer characterized by limited relationships between surface and ground waters. Samples were collected from wells during four surveys occurring over a two-years period, in order to assess the overall stability of the sites and to investigate its impacts on the interstitial assemblage composition, structure and dynamics.

As subterranean environments generally exhibit a lower variability in comparison with surface habitats, **we predicted our sites would be characterized by:**

- a) a strong, overall physicochemical temporal stability over the considered period, and
- b) globally stable, low-varying invertebrate communities dominated by one or two species (following DEATH 1996).

## MATERIALS AND METHODS

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### 1. Study sites.

All sites were located over a 250 km<sup>2</sup> floodplain in the central part of the French Pyrénées. The chosen sites were agricultural or domestic wells, with groundwater extraction related to domestic or agricultural needs (see Introduction, fig. 13).

The Ariège aquifer is composed of detritic deposits lying on horizontal limestone layers. Except for a small area in the southern part of the floodplain, water alimentation is essentially from run-off rainwater. Ground waters are globally drained by two main rivers that constitute West-East hydrodynamic limits (the Ariège and Hers rivers) and thus surface waters are considered having low influence in general on the aquifer (CUGNY 1980). The mean piezometric levels in the studied area were about 4.8 m below the soil, with maximum depth in the western part of the plain at about 8 m.

### 2. Assessment of site environmental stability.

To assess groundwater overall stability, thirteen physicochemical parameters were monitored at four occasions between 1998 and 1999, in order to cover two complete hydrological seasons. The surveys were at the beginning of October 1998, April 1999, October 1999 and April 2000. Water samples were collected using an immersed, petrol-powered centrifugal FLYGT pump connected to a PVC flexible discharge pipe and lowered to the bottom of the wells.

Temperature, conductivity, dissolved oxygen and redox potential were measured in the field with adapted MERCK-type apparatuses. In laboratory, the following parameters were measured: pH, total hardness, alkalinity, calcium, magnesium, sulfates, nitrates, chlorures, ammonium and phosphates. The details on sampling procedures have already been presented in a previous paper (DUMAS & FONTANINI 2000).

### 3. Sampling of invertebrates.

Invertebrates were sampled simultaneously with environmental parameters. They were collected using two complementary devices, following the recommendations of BOULAL (1988): a phreatobiological net-sampler and a 90 µm-mesh plankton net used to filter the groundwater extracted with the immersed pump. As they represented from far the majority of the taxa, only Crustaceans were

considered in this study, i.e. macrocrustacean (amphipods, isopods) and microcrustacean taxa (cyclopoids). The invertebrates were stained with Bengal pink, then sorted and counted using a stereomicroscope. For samples with very high densities, sub-sampling was used.

All the specimens were identified to species level, except for small, rare cyclopoid species that were identified to genus level for *Speocyclops* sp. and *Graeteriella* sp. In the same way, *Diacyclops languidoides*, *D. clandestinus* and the very rare *D. languidus* were assigned together to *Diacyclops* spp.

#### 4. Statistical methods.

Temporal variations in environmental parameters were investigated using multivariate analyses (Principal Component Analysis, PCA hereafter) and between-sites / between-dates non-parametric one-way analyses of variance (ANOVA hereafter) (SIEGEL 1956).

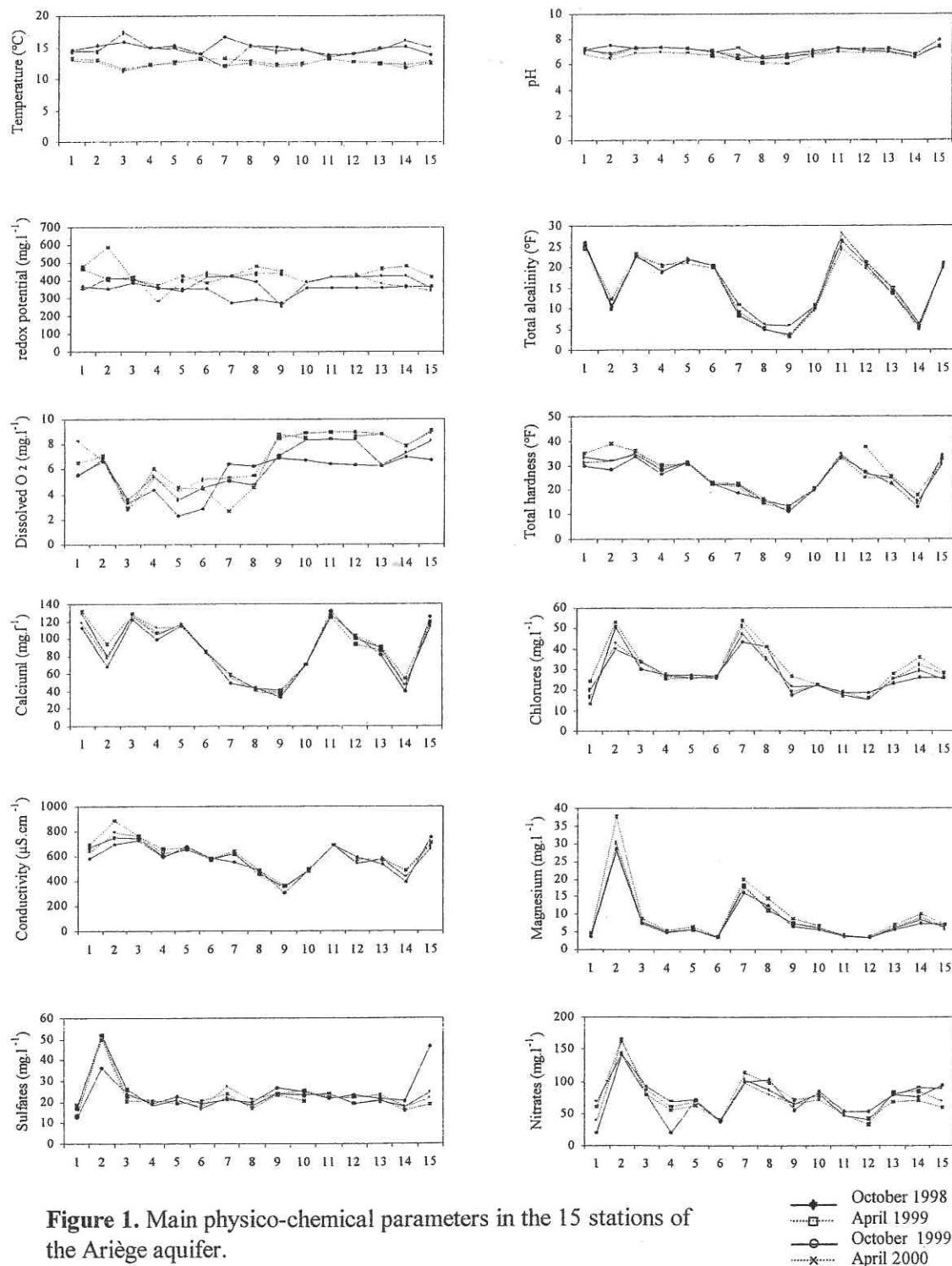
Community composition, structure and stability were assessed using factorial correspondence analysis and factorial discriminant analysis (FCA, FDA hereafter) on the species relative abundances in the samples. Dominance was assessed using rank-species abundance diagrams. Global persistence of the assemblages was described with Kendall coefficient of concordance (SIEGEL 1956). Abundance, mean taxonomic richness and diversity index (Shannon-Wiever index) were compared between periods using non-parametric one-way ANOVA and Kruskall-Wallis non-parametric tests.

Owing to the duration of the study, these results have to be considered with respect to the appropriate time-scale and refer to short-term aspects of community dynamics. In particular, “persistence” will be used in this paper to express the short-term temporal constancy of the invertebrate assemblages.

## RESULTS

### 1. PHYSICOCHEMICAL CHARACTERISTICS

#### 1.1 Spatial patterns



**Figure 1.** Main physico-chemical parameters in the 15 stations of the Ariège aquifer.

● October 1998  
 ■ April 1999  
 ○ October 1999  
 × April 2000

Data from the two first surveys were partially presented in DUMAS & LESCHER-MOUTOUÉ 2000. For the 4 surveys, between-site variations in water quality were marked for most parameters (cf. Fig. 1), in particular for calcium (range 33.2 - 132.2 mg.l<sup>-1</sup>), magnesium (3.4 – 37.6 mg.l<sup>-1</sup>), alkalinity (3 – 28 °F), chlorures (13.2 – 53.5 mg.l<sup>-1</sup>) and nitrates (19.6 – 165.7 mg.l<sup>-1</sup>). The greatest deviations occurred between groups of stations (7, 8, 9) and (11, 12).

All stations were characterized by well-oxygenated waters (mean 6.2 mg.l<sup>-1</sup>) whatever periods were considered ; the lowest concentrations were recorded for wells 3 to 8 but never fell to hypoxic conditions with the lowest levels at 2.3 mg.l<sup>-1</sup>. Stations 9 to 15 exhibited the better oxygenated ground waters with dissolved oxygen between 8 and 9 mg.l<sup>-1</sup> in April 1999 and April 2000.

## 2.2 Temporal patterns

### *Descriptive approach*

Except for temperature, redox potential and pH, groundwater physicochemical parameters were characterized by an overall stability between the four sampling surveys (Fig. 1). The similarity of the curves from October 1998 to April 2000 indicated no differences in water quality over the study period, whatever stations were considered. Conductivity, alkalinity, calcium and nitrates exhibited the strongest temporal stability (between-date Kruskall-Wallis one-way ANOVA, non significant differences,  $\alpha$ -probabilities (Type I-error) from 0.82 to 0.98, Table 1). Total hardness, magnesium, chlorures and sulfates were also characterized by minor, non-significant between-date variations (Kruskall-Wallis,  $\alpha$ -probabilities  $p_{Mg}=0.77$ ,  $p_{Cl}=0.65$ ,  $p_{SO_4}=0.35$ ).

Seasonal patterns were observed only for temperature (Mann-Whitney U test,  $U=26.5$ ,  $p<0.0001$ ) with groundwater ranging from 12.5°C (April) to 14.8°C (October), and for redox potential (Mann-Whitney,  $U=154$ ,  $p<0.0001$ ). No seasonal distribution could be found for dissolved oxygen in ground waters (Mann-Whitney,  $U=322$ , n.s.).

Between-period physico-chemical profiles were tested for each station using Kruskall-Wallis one-way ANOVA on the 14 environmental variables (Table 5). No differences were observed for water physicochemical parameters between the study surveys as all the stations were characterized by high  $\alpha$ -probabilities (between 0.832 and 0.998).

**Table 1.** Temporal variations of the groundwater main physico-chemical characteristics during the study period. Means, standard deviations between brackets, Kruskall-Wallis one-way analysis of variance.

	10 / 1998	04 / 1999	10 / 1999	04 / 2000	H	p
Temperature (mg.l <sup>-1</sup> )	14.9 (0.8)	12.4 (0.5)	14.6 (1.3)	12.5 (0.5)	39.31	<0.01
Redox potential (mV)	344.9 (34.8)	402.4 (47)	387.5 (46.4)	447.1 (54.9)	25.37	<0.01
Conductivity (μS.cm <sup>-1</sup> )	575.3 (122.9)	596.9 (121.7)	587 (117.8)	606.2 (133.3)	0.17	0.98
Oxygen (mg.l <sup>-1</sup> )	5.6 (1.6)	6.9 (2.1)	6.2 (1.7)	6.4 (2.3)	3.93	0.27
pH	7.1 (0.4)	6.7 (0.4)	7 (0.3)	7 (0.3)	9.15	0.027
Total Hardness (°F)	24.6 (7.6)	25.3 (7.5)	25.7 (7.3)	27.1 (8.4)	1.14	0.77
Total Alkalinity (°F)	15.6 (7.9)	15.1 (7.6)	16.2 (7.4)	15.2 (7.5)	0.34	0.95
Calcium (mg.l <sup>-1</sup> )	84.9 (33.2)	87 (32)	89.2 (31.6)	88.7 (31.6)	0.33	0.95
Magnesium (mg.l <sup>-1</sup> )	8.1 (6.6)	8.6 (7)	8.3 (6.6)	10.3 (9)	1.52	0.68
Chlorides (mg.l <sup>-1</sup> )	27.5 (10.4)	28 (9.9)	27.3 (8.6)	31.4 (11)	1.64	0.65
Sulfates (mg.l <sup>-1</sup> )	24 (8.1)	23.4 (8.4)	22.9 (8.6)	22 (8.3)	3.27	0.35
Nitrates (mg.l <sup>-1</sup> )	70.7 (32.6)	72 (31.7)	77.1 (26.4)	76 (32.2)	0.91	0.82
Ammonium (μg.l <sup>-1</sup> )	15.2 (13.5)	66 (89.3)	29.3 (30.8)	20 (0)	36.56	<0.01
Phosphates (μg.l <sup>-1</sup> )	57.4 (41.9)	96.4 (230.2)	58.4 (65.7)	61.8 (56.7)	10.54	0.01

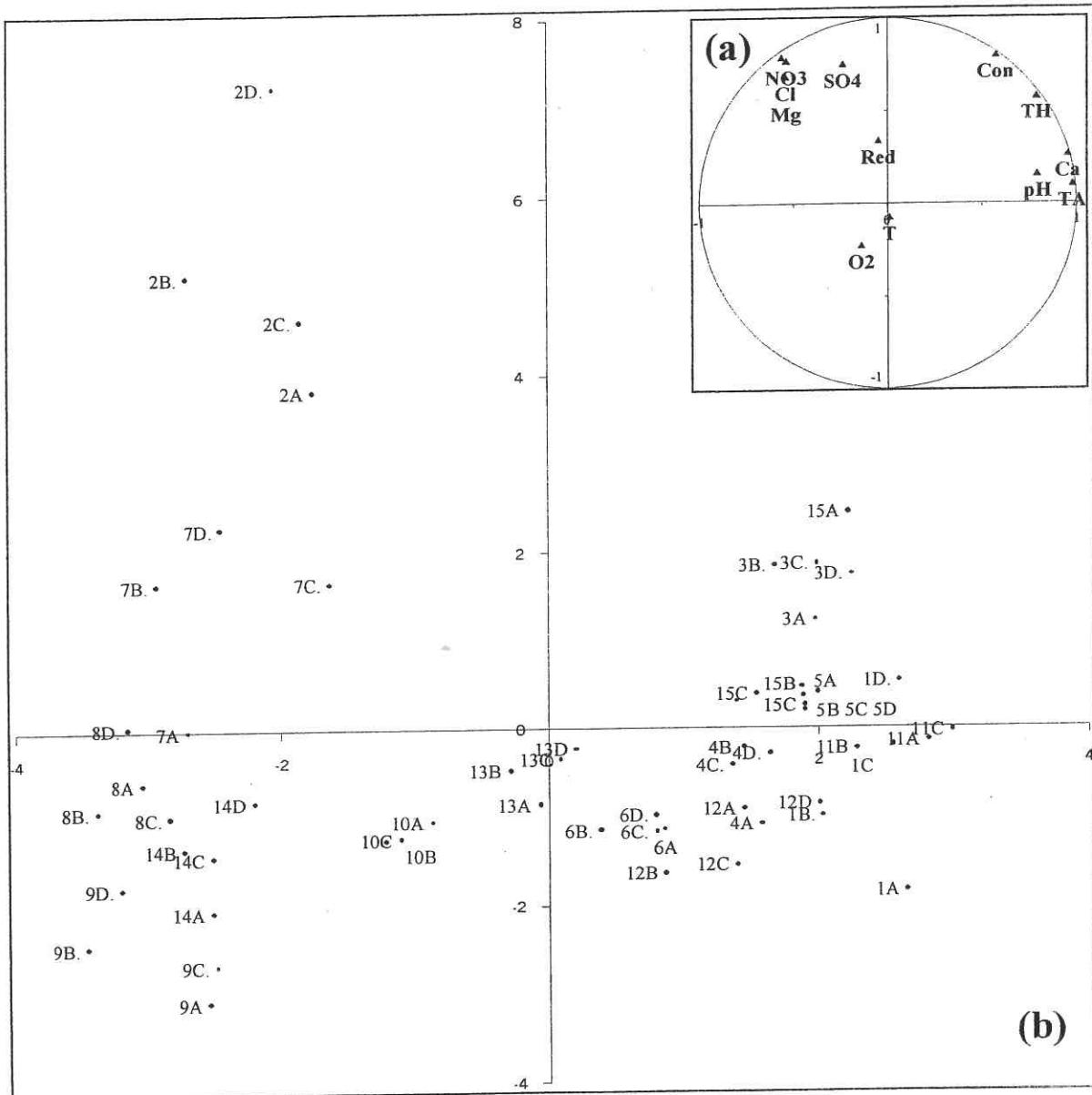
### Multivariate approach

The data were then subjected to PCA, in order to produce station temporal plots based upon physicochemical variability (Fig. 2). The initial data matrix was composed of 58 samples (15 stations x 4 periods, except station 10, 11 that were unavailable in April 2000) x 13 variables. The first two axes explained 66.1 % of total variance ; they were related to natural and anthropic chemical / hydrogeological processes (cf. chapter 2 of this study).

No major difference occurred between the four surveys as labels A, B, C, D were on the whole aggregated on the ordination plot for a given well. Samples were not ordinated along a seasonal gradient, as A, C and B, D did not constitute paired labels. Variability was mainly related to between-site variations resulting from temporally stable, individual physicochemical signatures for the 15 samples. Stations 3 to 6, 8, 10 to 14 were in particular characterized by the lowest within-group

variability and thus could be considered the most stables sites from physicochemistry. Minor temporal differences were noticed for wells 1, 12 and 15.

**Figure 2.** Principal components analysis ordination (factor 1 x factor 2) of the sampling stations by the groundwater physico-chemical data. F1 horizontal, F2 vertical. Contribution to total variance of the two first axes : 66.1 %. 1-15: sampling stations. A, B, C, D: study periods (10 / 1998, 04 / 1999, 10 / 1999, 04 / 2000).



The data set was then subjected to factorial discriminant analysis. Discrimination was tested between 15 groups of samples corresponding to samples A, B, C, D from each station so as to investigate the individual temporal variability of the sites.

High scores for discriminant power, Wilk's coefficients and probabilities indicated that stations had marked individual physico-chemical patterns associated with low among-period variability, i.e. exhibit marked temporal stability (Table 2).

**Table 2.** Discriminant analysis for groundwater physico-chemical parameters in the sampled stations. Discriminant power, Wilk's coefficients and associated probabilities for 15 groups of samples based upon station number.

	F1 axis	F2 axis
Discriminant power	0.99	0.98
Wilk's coefficient (W)	784.75	540.07
Levels of significance	p < 0.001	p < 0.001
Percentage of well-ranked samples	89.7 %	

## 2. FAUNAL CHARACTERISTICS

### 2.1 Assemblages composition

Assemblages were largely dominated by cyclopoid taxa over the study periods, these latter with strong variations in abundances between dates (Table 3, between-dates Kruskall-Wallis ANOVA, p<0.01) and between wells.

Cyclopoid communities were composed of an association of epigean (*Macrocylops albidus*, *Tropocyclops prasinus*, *Eucyclops serrulatus*, *Paracyclops fimbriatus*, *Acanthocyclops viridis*, *Diacyclops bicuspis*, *D. bisetosus*) and hypogean taxa (*Diacyclops*, *Speocyclops* and *Graeteriella* species), while macrocrustaceans were mainly characterized by groundwater-dwelling species all over the study period (*Stenasellus virei*, *Niphargus* and *Salentinella* species). Amphipod and isopod abundance varied between stations, but temporal patterns did not exhibit significant difference (Table 2, p = 0.93).

Diversity, expressed either as taxonomic richness or Shannon-Weaver index, did not vary significantly between the surveys for both microcrustacean and macrocrustacean taxa (Table 3, Kruskall-Wallis ANOVA, p (type I-error) between 0.16 and 0.34 for cyclopoids, amphipods and isopods).

**Table 3.** Temporal variations of groundwater faunal characteristics for the microcrustacean and meiocrustacean taxa. Kruskall-Wallis one-way analyses of variance between periods for taxonomic richness, Shannon-Weaver diversity index and total abundance in the 15 wells (means with standard deviations between brackets).

	10 / 1998	04 / 1999	10 / 1999	04 / 2000	H	p
<b>Microcrustaceans</b>						
Taxonomic richness	3.1 (1.2)	4 (1.5)	3.7 (1.5)	3.6 (1.1)	3.68	0.3
Diversity index (H)	0.72 (0.61)	1.01 (0.56)	0.85 (0.59)	0.73 (0.56)	3.34	0.34
Abundance	1089 (1824)	2595 (8321)	3557 (4523)	368 (442)	16.95	<0.01
<b>Meiocrustaceans</b>						
Taxonomic richness	1.8 1.6	2.67 1	1.87 1	2.07 1.3	5.17	0.16
Diversity index (H)	0.53 .068	0.92 0.52	0.59 0.54	0.66 0.63	4.68	0.20
Abundance	55.5 82.7	47.6 60.1	62.3 101.1	50.4 69.9	0.96	0.81

## 2.2 Dominance

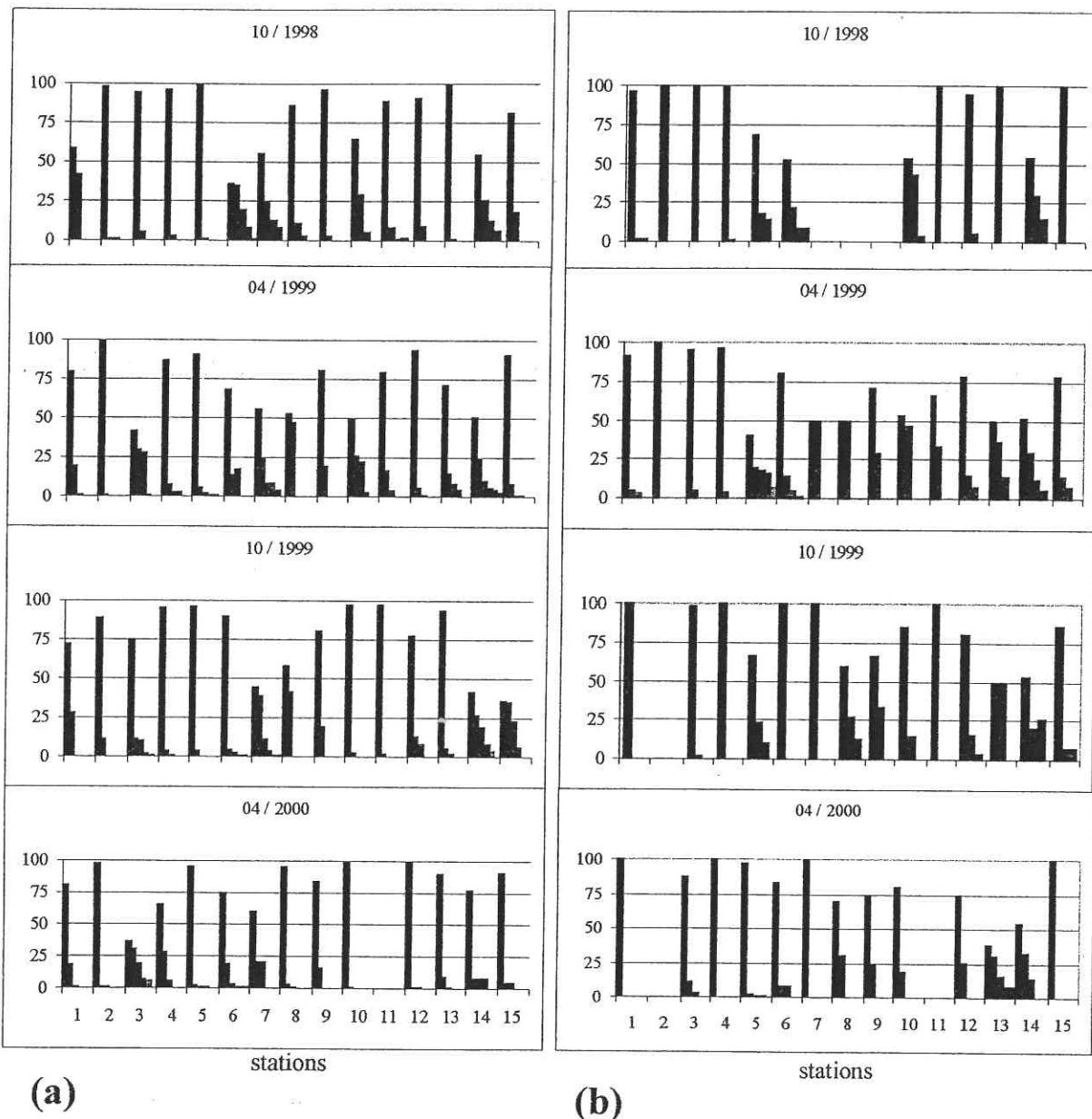
Strong dominance of few species was generally observed within the assemblages (Fig. 3) for both micro and macrocrustacean taxa.

Rank-species abundances diagrams were characterized by dominance patterns with one species frequently accounting for more than 75% of total abundance. This occurred in particular for cyclopoids in wells 2, 4, 5, 9, 12 and 13 with one species representing up to 99% of microcrustacean abundance. In the same way, macrocrustacean phreatic communities were largely dominated by a single species that regularly accounted for 80-100% of total abundance in wells 1 to 4, 12 and 15.

An overall, temporal stability was observed for dominance patterns between the surveys as minor difference occurred between rank-species diagrams, for a given station.

This situation was best illustrated for stations 2, 4, 5, 9, 11 to 13 characterized by one dominant cyclopoid species with fairly constant relative abundance between the four sampling dates, as for stations 1 to 4, 11, 12 and 15 when considering amphipod/isopod distributions. Some temporal variations still occurred in some wells, with changes from strong dominance to more balanced assemblages or vice-versa (e.g. wells 3, 6, 10, 13, 15).

**Figure 3.** Rank-species abundance diagrams for groundwater species in the 15 stations during the study periods. Ranked percentage of relative abundances in the samples for (a): cyclopoids (b): amphipods + isopods. All the taxa were considered. Well 11 was unavailable in April 2000.



### 3.3 Assemblage persistence for crustacean species

FCA were performed on faunal data in order to investigate the temporal variability of invertebrate community composition. After preliminary tests, the initial data matrix was modified by separating microcrustacean from meiocrustacean taxa, these latter having low abundances in comparison with cyclopoids. All the species, including the rare ones, were considered.

- Cyclopoid taxa (Fig. 4)

The first two axes explained 40.7% of total variance.

Hypogean, rare taxa (i.e. *Graeteriella* sp., *G. unisetigera*, *Diacyclops bisetosus*, *Diacyclops* spp., *Speocyclops* sp.) formed a dense group aggregated close to the centre of the ordination diagram, and thus had low contributions to axes determination (contributions < 0.01 for F1 axis, between 0.01 and 0.15 for F2 axis). F1 and F2 factorial scores were mainly related to epigean taxa (i.e. *M. albidus*, *P. fimbriatus*, *T. prasinus* and *E. serrulatus*) that were located close to the extremities of the two axes.

Station plots based upon cyclopoid composition exhibited difference in temporal variability between the 15 wells.

The majority of the stations, i.e. stations 1, 2, 5, 8, 12, 13 and 15, were characterized by an aggregation for A, B, C, D samples that indicated a global stability in assemblage structure during October 1998, April 1999, October 1999 and April 2000. Minor changes were observed for stations 4, 7 and 14 as three of the four samples constituted matched samples, laying near each other on the ordination diagram. Samples from wells 3, 9, 10 and 11 constituted paired plots and thus had similar assemblages at two dates while station 6 was characterized by scattered plots along the axes.

Discriminant analysis was performed on the species relative abundances, in order to evaluate the temporal variability of assemblages structure. The samples were separated upon station number and discrimination was tested between the 15 groups over the four surveys. Table 4 provides discriminant power, Wilk's coefficient and associated probabilities. They confirm the overall persistence for cyclopoid structure / composition.

**Table 4.** Discriminant analysis for cyclopoid relative abundances (common species) in the sampled stations. Discriminant power, Wilk's coefficients and associated probabilities for 15 groups of samples based upon station number.

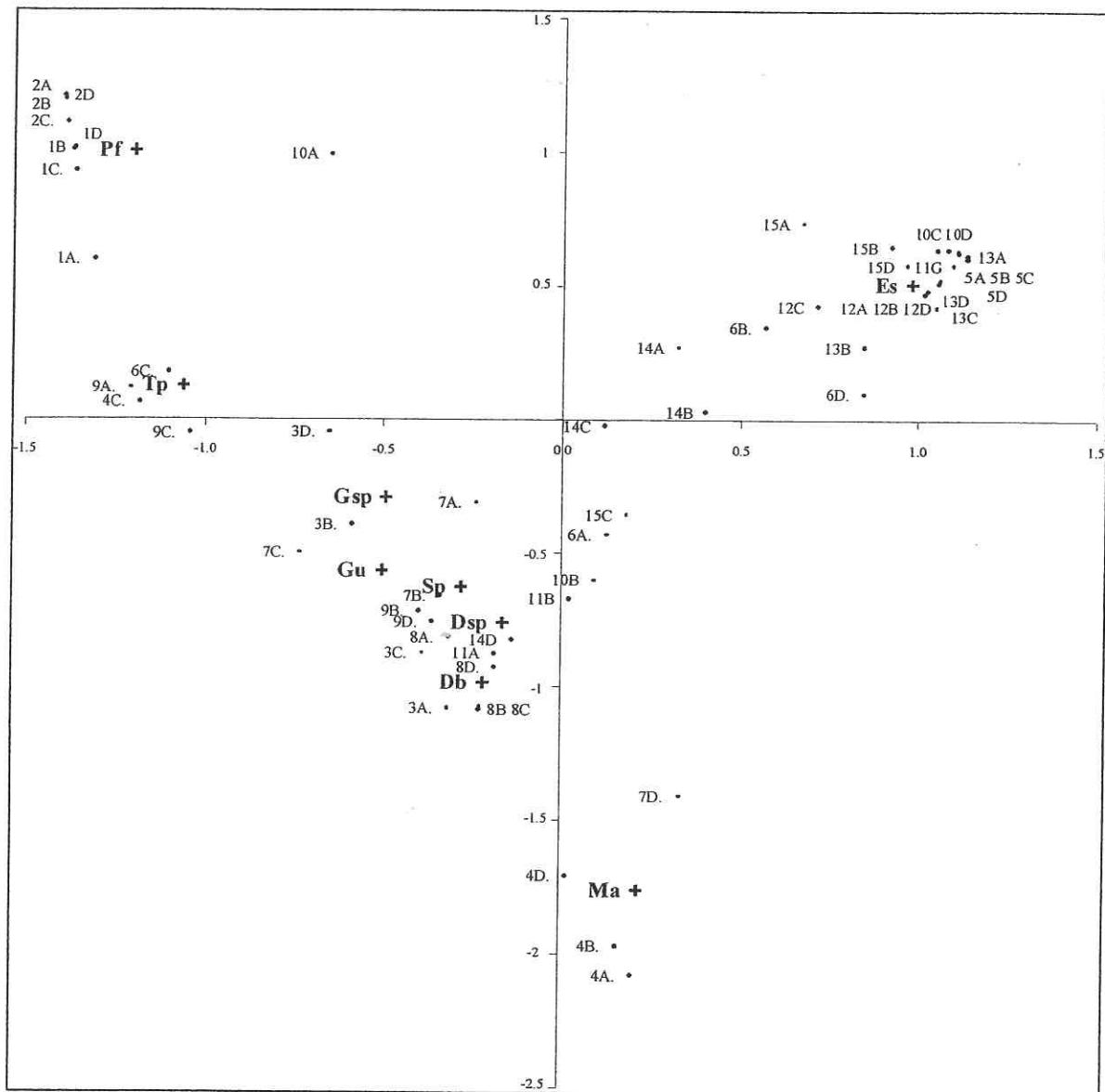
	F1 axis	F2 axis
Discriminant power	0.91	0.86
Wilk's coefficient (W)	361.42	247.38
Levels of significance	p < 0.001	p < 0.001
Percentage of well-ranked samples	57.6 %	

Kendall's coefficients of concordance for species rank distribution were generally high (cf. Table 5), and thus indicated no major changes in assemblages composition between dates for most stations.

**Figure 4.** Correspondence analysis ordination (factor 1 x factor 2) of the 15 sampling stations on the basis of their taxonomic composition (cyclopoid species). Contribution to total variance of the two first axes : 40.7 %.

1-15: sampling stations. A, B, C, D: study periods (10 / 1998, 04 / 1999, 10 / 1999, 04 / 2000).

Ma: *Macrocyclops albidus*, Es: *Eucyclops serrulatus*, Tp: *Tropocyclops prasinus*, Pf: *Paracyclops fimbriatus*, Db: *Diacyclops bisetosus*, Dsp: *Diacyclops* spp., Gu: *Graeteriella unisetigera*, Gsp: *G.* sp., Ssp: *Speocyclops* sp.



**Table 5.** Comparisons of the groundwater physico-chemical and faunal characteristics between the study periods for the 15 sampled wells.

H: between-period Kruskall-Wallis one-way analysis of variance for the 14 physico-chemical parameters. W: Kendall coefficient of concordance for microcrustacean and macrocrustacean taxa. p: levels of significance (missing values for assemblages with number of species  $\leq 3$ ).

	Physico-chemical characteristics		Faunal characteristics			
			Microcrustaceans		Meiocrustaceans	
	H	p	W	p	W	p
1	0.62	0.892	0.868	<.01	0.635	-
2	0.226	0.973	0.737	<.05	0	-
3	0.04	0.998	0.863	<.01	0.950	-
4	0.874	0.832	0.553	n.s.	0.929	-
5	0.004	0.999	0.518	n.s.	0.503	n.s.
6	0.138	0.987	0.689	<.01	0.890	<0.05
7	0.398	0.941	0.145	n.s.	0.111	-
8	0.042	0.998	0.635	0.05	0.457	-
9	0.357	0.949	0.849	<.01	0.333	-
10	0.049	0.997	0.532	n.s.	0.979	<0.01
11	0.009	0.995	0.808	<.05	1	-
12	0.158	0.984	0.621	<.05	1	-
13	0.073	0.995	0.925	<.01	0.351	n.s.
14	0.212	0.976	0.779	<.01	0.475	<0.05
15	0.047	0.997	0.48	n.s.	0.942	-

- *Amphipod taxa (Fig. 5)*

The first two axes explained 44.6 % of total variance.

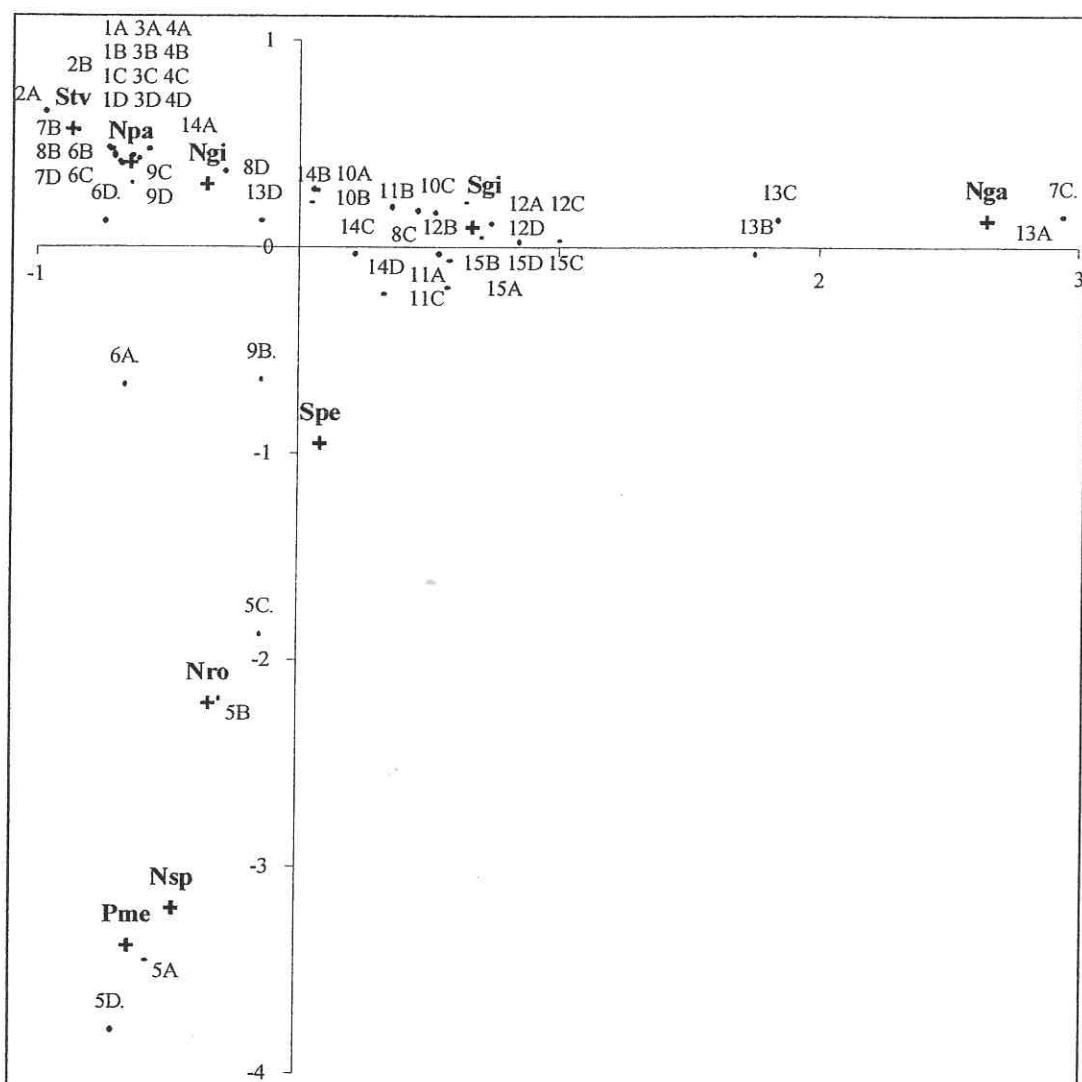
Except for those from station 5 that were stretched along F2, all the samples were mainly discriminated along the F1 axis and formed two dense groups. The first one was related to the species association *Niphargus pachypus* / *Stenasellus virei* / *Niphargus gineti*, while the other was mainly related to *Salentinella gineti* and *Niphargus gallicus*. F2 isolated the samples from station 5, characterized by the presence of *Proasellus meridianus* and some rare taxa (*Niphargus* sp., *Niphargus robustus*).

To investigate the role of rare species in the ordination of the stations, FCA was performed excluding the very rare *Niphargus gineti*, *N. gallicus*, *Niphargus* sp. and *N. robustus*.

On the whole, the same patterns were found when considering only the common species, as the relative position of the samples exhibited only slight variations (contribution to total variance for F1+F2: 63.6 %). As for cyclopoids, an aggregation of the samples A, B, C and D was observed for most stations and indicated that only minor changes in community structure occurred across the

sampling periods. This was best illustrated for the stations 1, 3, 4, 9 (first group), 10, 11, 12, 13, 14, 15 (group 2) and 5.

**Figure 5.** Correspondence analysis ordination (factor 1 x factor 2) of the 15 sampling stations on the basis of their taxonomic composition (amphipod + isopod species). Contribution to total variance of the two first axes : 44.6 %.  
 1-15: sampling stations. A, B, C, D: study periods (10 / 1998, 04 / 1999, 10 / 1999, 04 / 2000).  
 Stv: *Stenasellus virei*, Npa: *Niphargus pachypus*, Ngi: *Niphargus gineti*, Nga: *Niphargus gallicus*, Nro: *Niphargus robustus*, Nsp: *Niphargus* sp., Sgi: *Salentinella gineti*, Spe: *Salentinella petitii*, Pme: *Procasellus meridianus*.



## **DISCUSSION**

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### **1. Environmental stability in ground waters**

In the classical view of underground environments developed over the past decades, ground waters were considered non-varying, predictable systems characterized by high physical inertia (DELAMARE DEBOUTTEVILLE 1960, VANDEL 1964, MOHR & POULSON 1966, GINET & DECOU 1977, CULVER 1982). With the recent developments of groundwater ecology, this view has evolved. It is now recognized that all groundwater ecosystems are characterized by some amount of physical variation, with respect to their degree of isolation from surface systems: the closer to the surface, the more variable they are (GIBERT et al. 1994).

In interstitial ecosystems, environmental gradients in hydrodynamism and physicochemistry occur from surface to the deep parts of the aquifer. Variation, which is often of consequence in surface waters, usually is not very evident many meters below the surface (CASTANY 1985): DOLE-OLIVIER (1998), investigating the vertical patterns of physico-chemical variables in a backwater of the Rhône river, demonstrated that variations in environmental parameters such as temperature, specific conductance or dissolved oxygen were significantly damped with depth. Thus, if stability is relative in ground waters, it is usually much greater than in surface waters (GIBERT et al. 1994).

In the Ariège aquifer, the main groundwater physico-chemical parameters monitored (conductivity, dissolved oxygen, total hardness, alkalinity, chlorides, calcium, magnesium, nitrates, sulfates) were characterized by a strong temporal stability between October 1998 and April 2000. Variability was low and essentially due to among-site variation that resulted from local processes, in particular groundwater contamination due to agricultural practices or hydrogeology and hydrodynamics (SEGUIN 1980, CUGNY 1980, DUMAS & LESCHER-MOUTOUÉ 2000). Oxygen levels were in general high in this aquifer but were consistent with data from the literature (review in MALARD & HERVANT 1998), with variability mainly related to among-station variation. Seasonal patterns were observed for temperature, but ground waters had small ranges of variation: 2.2 °C between October and April (low-water / high water periods), whereas for the same seasons, mean variation was about 5 °C for surface rivers in the same area (cf. Table 4).

Temperature range usually constitutes a potential indicator of physical stability in freshwaters, even if other environmental parameters are required in order to assess the global stability of a system (DEATH

& WINTERBOURN 1994) : DEATH (1996), using a multivariate approach to evaluate the *global* environmental stability in ten streams in the southern Alps of New Zealand, found that the stable sites were characterized by lower annual temperature range (mean for five stable sites 3.4 °C) than the unstable ones (mean for five unstable sites 9 °C).

The very low variation in temperature observed in the studied area can be related to the relative isolation of the aquifer from surface influence (semi-confined aquifer): except in a restricted area in the southern part of the floodplain, alimentation is mainly from rainwater (CUGNY 1980) and drainage occurs from ground waters to the main rivers, the Ariège river and Hers river.

This was confirmed by the high mineralization found at all the stations that prevents the hypothesis of strong infiltration of surface waters, though locally this may occur (DANNEVILLE 1995).

**Table 6.** Mean variations in temperature and conductivity for the Ariège aquifer ground waters and 5 surface rivers from the same hydrological basin. Variations observed between October and April. Ariège aquifer: 1998-1999 and 1999-2000 data, surface rivers: 1992-1993 data (range between brackets).

	Temperature (°C)	Conductivity ( $\mu\text{S.cm}^{-1}$ )
Ariège	4.4 (9.4 – 13.8)	45.2 (171.8 – 217)
Volp	5.7 (9.2 - 14.9)	67 (316 – 383)
Lèze	5.2 (10.4 - 15.6)	125 (477 – 602)
Garonne	5.6 (9.9 - 15.5)	11 (261 – 272)
Hers	5.2 (10.8 - 16)	17 (425 – 442)
<b>Ariège aquifer</b>	<b>2.2 (12.5 – 14.7)</b>	<b>20 (581.2 – 601.6)</b>

These results are also strengthened by the high  $\alpha$ -probabilities from between-period ANOVA (p between 0.83 and 0.99), that encompass the 14 physico-chemical parameters monitored and thus demonstrate the constancy of global physico-chemical patterns at the scale of each station. Of course, these results have to be considered in their particular spatio-temporal context ; yet, they suggest a strong, overall environmental stability for all the stations.

## 2. Persistence of crustacean communities

All the sites were generally characterized by persistent communities, although this depended to a certain extent of what aspect of the community was considered.

Species total abundances had chaotic distribution between dates and wells and did not reveal any recognizable patterns whatever species were considered (cyclopoids, amphipods, isopods separately or

together). Peaks in number of individuals were punctually observed in some stations during the two-years survey, but they were not correlated with changes in measured physico-chemical parameters.

This situation is not rare in groundwater studies as great amount of biotic and abiotic factors are known to influence the invertebrates distributions in aquifers and result in a patchy distribution for the fauna (DANIELOPOL 1989, STRAYER 1994, PALMER et al. 1997, WARD et al. 1998, MAUCLAIRE 1999).

In contrast, investigating species diversity using classical descriptive approaches (e.g. taxonomic richness and Shannon-Weaver scores) revealed that assemblages had stable diversity patterns, with highly persistent species composition. On the whole, relative abundance patterns did not change across the periods: the majority of the stations had high Kendall's coefficients of concordance (W) for both cyclopoid and amphipod/isopod assemblages. For the stations having low ( $\leq 0.6$ ) W scores, a biased view of the overall faunal persistence occurred, as equal weight was given to both rare and common taxa in the computation of W: station 5, for instance, had one cyclopoid dominant species (*E. serrulatus*) with quasi-constant relative abundances between the four periods (91.1 to 99.3%) and several rare species collected in low abundance that induce Kendall's W of 0.5.

In the same way, stations 4 and 15 had one dominant species (*M. albidus* for station 4, *E. serrulatus* for station 15) with similar scores across all the periods except in October 1999, and very rare species that lower the W score.

Station 7, with the lowest Kendall's score (0.145) was characterized by the lowest number of collected invertebrates whatever the date (mean number of cyclopoid collected: 43 ; mean for all stations: 1900) and therefore variability has to be carefully considered.

Finally, a single station (station 10) was characterized by strong changes in assemblage composition, and this only occurred between the two first periods.

The same phenomenon occurred for macrocrustacean communities. In general, amphipods and isopods were collected in low abundance in the wells, and the lowest W scores were found in the sites with the lowest abundances (abundance  $\sim 10$ , cf. Table 7).

Thus, results from these stations (station 2, 7, 8, 9, 13 for amphipods/isopods assemblages and station 7 for cyclopoids) probably underestimate the overall faunal persistence and therefore have to be considered apart.

**Table 7.** Kendall's coefficient of concordance and number of specimens collected for the crustacean assemblages. n: mean number of individuals collected per survey in the 15 stations. In bold: lowest W scores and invertebrates abundances.

Station	Cyclopoid assemblages		Amphipod / isopod assemblages	
	Kendall's W	n	Kendall's W	n
1	0.868	1346	0.635	146
2	0.737	680	0	2
3	0.863	190	0.950	33
4	0.553	3494	0.929	280
5	0.518	954	0.503	68
6	0.689	818	0.890	30
7	<b>0.145</b>	<b>76</b>	<b>0.111</b>	<b>3</b>
8	0.635	12499	<b>0.457</b>	7
9	0.849	4670	<b>0.333</b>	<b>5</b>
10	0.532	1351	0.979	227
11	0.808	746	1	10
12	0.621	462	1	65
13	0.925	785	<b>0.351</b>	<b>11</b>
14	0.779	157	0.475	44
15	0.480	402	0.942	15

Assessing the community structure using an alternative approach (factorial analyses) also confirmed that stations had persistent faunal composition, at least for common taxa. For rare taxa, the presence / absence of a given species was in general consistent across the sampling periods (i.e. the same species were found again in the same stations), with variation in abundance ranks that may partly result from the low number of specimen collected, from one single individual to a few tens. This was of particular importance for amphipod and isopod taxa.

This persistence observed in our data may be only partly a consequence of the time scale of the study. Indeed, the taxa collected had shorter generation-time and species turn-over would have let time for changes in community composition to occur. Moreover, the sampling periods were very short in time and widely spaced out over the two-years period of the study. As a matter of fact, the faunal persistence in the Ariège aquifer is more likely to be the consequence of the habitat stability.

### 3. Comparisons from the literature.

Data from the literature (DEATH & WINTERBOURN 1994) report Kendall's W scores for benthic invertebrates from 0.38 to 0.55 in stable surface streams and from 0.46 to 0.66 in unstable ones ; correlation was significant between environmental stability and Kendall's coefficient.

Except for station 2, 7, 8, 9 and 13 for the reasons discussed above, our stations exhibited values from 0.48 to 0.93 for cyclopoid species, and from 0.48 to 1 for amphipods/isopods mostly with highly significant probabilities. Similar values were also found for interstitial harpacticoid communities in a French Pyrenean karstic system (21 taxa,  $W=0.89$ , ROUCH 1982) and for diverse interstitial crustacean assemblages in the hyporheic zone of two Pyrenean streams (Nert stream: 35 ostracod, isopod, amphipod, syncarid, cyclopoid and harpacticoid species,  $W=0.84$  (ROUCH 1995), Lachein stream: 7 cyclopoid taxa,  $W=0.83$  / 10 harpacticoid species,  $W=0.91$  (ROUCH 1988, ROUCH & LESCHER-MOUTOUÉ 1992)).

Our data are consistent with those from the literature that report higher W scores for ground waters than for surface waters. In agreement with the classical view emphasizing the predictability of underground systems in relation with their degree of isolation from surface influence, they suggest that an overall greater faunal persistence is more likely to be found in ground waters than in surface waters. This latter proposition in particular will require further testing and formal comparisons between surface and groundwater assemblages, and more rigorous methods will be needed in order to assess the overall stability of interstitial ecosystems.

### **3. Stability and dominance patterns**

Fairly uniform patterns were observed for both microcrustacean and macrocrustacean species. Assemblages were strongly dominated by one taxa, although more balanced assemblage may punctually occur. Moreover, the rare samples with relative abundance for the dominant species less than 0.75 coincided with stations where low invertebrate abundance was collected.

DEATH (1996) hypothesized that species distributions would be the reflect of competitive interactions and/or of abilities for species to withstand variable environmental conditions: “it therefore seems that if physical conditions at a site are such that any one species is greatly advantaged over others, either through competitive superiority or an ability to persist in the face of “harsh” physical conditions, a log series will result”. In this context, dominance patterns similar to those found over the area might result through two distinct processes.

On one hand, frequent and/or severe system perturbation would lead to non-balanced communities, such as frequently reported in cases of anthropic disturbances. Data from various, especially marine meio/macrobenthic communities, exhibited increases in dominance in disturbed sites, whereas non-disturbed sites presented more balanced assemblages (SHAW et al. 1983, LAMBSHEAD et al. 1983). The strong dominance patterns observed within the wells sampled could not be related to definite physico-

chemical perturbation in the aquifer ; in fact, groundwater quality did not seem to influence the distribution of interstitial crustaceans in this aquifer (see Chapter 2 of the present study). However, the parameters known to influence faunal distribution patterns are complex and many potential sources of disturbance may occur in aquifers. Massive irrigation associated to cereal production in particular might influence the crustacean distribution, as cyclopoid community differed between stations in relation with well utilisation (cf. Chapter 3). In the same way, high-discharge pumping experiments on a karstic system in the French Pyrénées led to long-term perturbation of hypogean harpacticoid communities, and one year later, species abundances and relative dominances had not recovered initial patterns (ROUCH et al. 1993). At the scale of the floodplain (regional scale), groundwater extraction may then be considered as a global disturbance for the interstitial crustacean communities and may be related to non-balanced dominance patterns in the sampled stations.

On the second hand, similar patterns of dominance may occur in cases where a strong environmental stability would be found. In such non-varying, highly-predictable systems, the predominance of an advantaged / more efficient species would result in communities characterized by low evenness in species abundance distribution through biotic interactions, e.g. competitive exclusion. This would be specially consistent with environmental data in the case of subterranean systems isolated from surface influence, as in this study. Unfortunately, field observations derived from abundance patterns and the lack of detailed information for hypogean species (species traits, biotic interactions) does not allow strong inferences for this type of ecological relationships

Yet, attempting to explain more precisely the processes and dynamics underlying these dominance patterns would be actually problematic in the context of our limited data set. As a matter of fact, investigations will have to be expanded prior to elucidate whether strong environmental stability or disturbance events may be responsible for the observed patterns of dominance in the Ariège aquifer.

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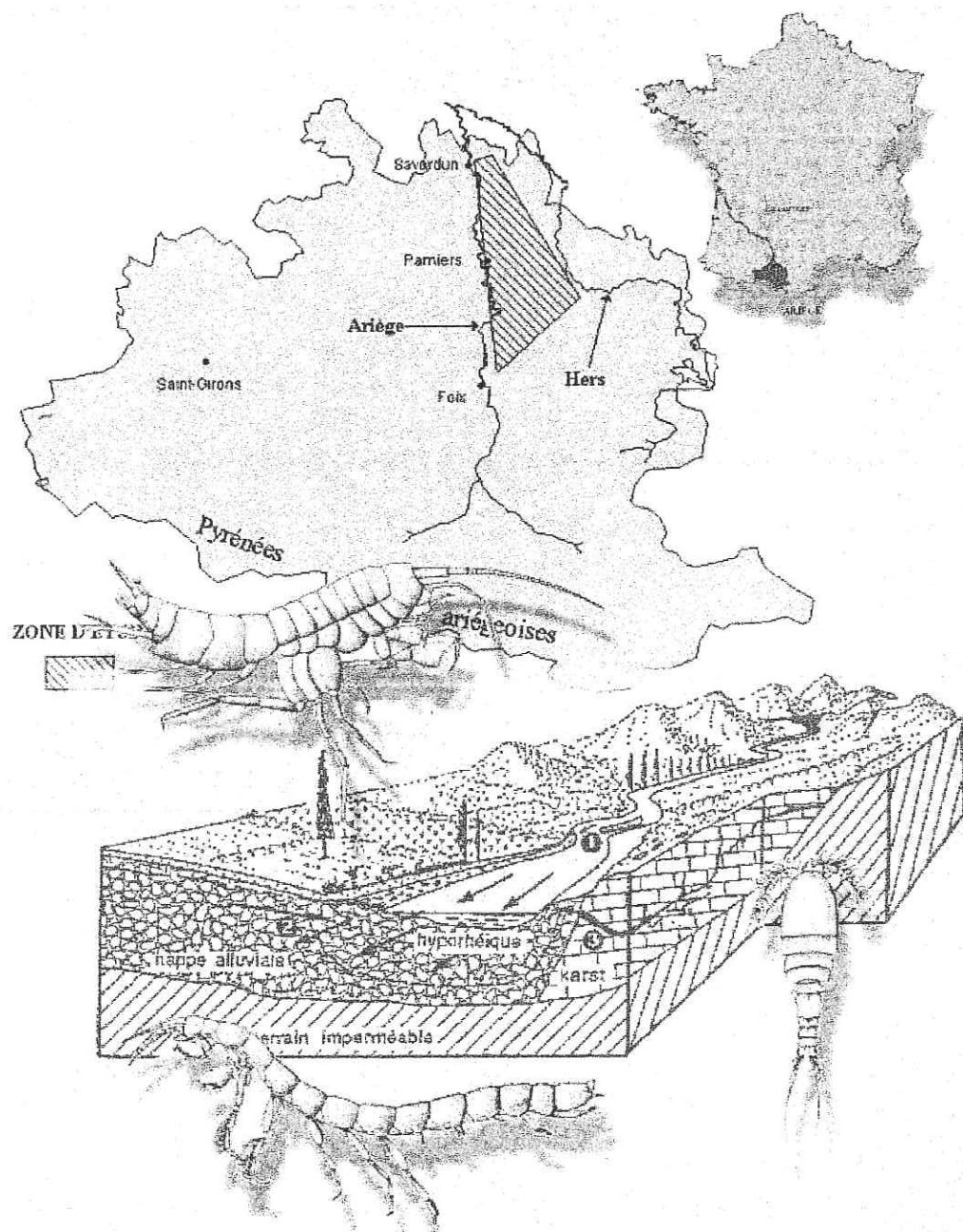
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## - CONCLUSION -





## RAPPEL, CONTEXTE ET OBJECTIFS.

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L'objectif principal de ce travail concernait l'étude de la dynamique de répartition et des structures de peuplement des communautés de crustacés souterrains à l'échelle d'un aquifère alluvial, en relation avec les paramètres du milieu. Interprétées dans un contexte d'évaluation de l'impact des perturbations anthropiques sur les peuplements interstitiels, ces recherches s'inscrivent dans un volet finalisé de l'écologie souterraine ; à ce titre, elles ont pour but de contribuer à la mise au point et au perfectionnement de méthodes d'évaluation biologique de la qualité des eaux souterraines.

Dans un contexte plus régional, ces recherches s'inscrivent également dans une approche d'évaluation de la biodiversité et d'étude des écosystèmes souterrains. L'acquisition de données faunistiques sur la nappe de l'Ariège, encore peu étudiée d'un point de vue biologique, constitue une contribution aux recherches en écologie souterraine entreprises en France depuis les débuts de la Biospéologie.

Les axes de recherche privilégiés au cours de cette étude ont été les suivants :

- **Etude de l'influence de la qualité de l'eau sur les structures des peuplements.**

En raison de la nature des contaminations observées dans la zone, l'aquifère de l'Ariège a été choisi afin de tester l'impact d'une pollution par les nitrates sur les communautés des crustacés interstitiels.

- **Etude de l'hétérogénéité spatiale des peuplements en relation avec les facteurs du milieu.**

L'existence de zones présentant des propriétés hydrogéologiques contrastées au sein du réservoir a permis d'étudier l'influence de la structure de l'aquifère et de son exploitation sur la répartition et les structures des assemblages interstitiels.

- **Etude de la distribution temporelle des peuplements en relation avec la variabilité du milieu.**

Caractérisé par des relations limitées avec les cours d'eau de surface, l'aquifère de l'Ariège a été choisi afin d'étudier l'hétérogénéité temporelle des assemblages dans un environnement à forte inertie et prédictibilité.

Les principaux résultats obtenus ont été présentés et discutés dans les 4 chapitres précédents ; ils sont résumés ici de manière synthétique.

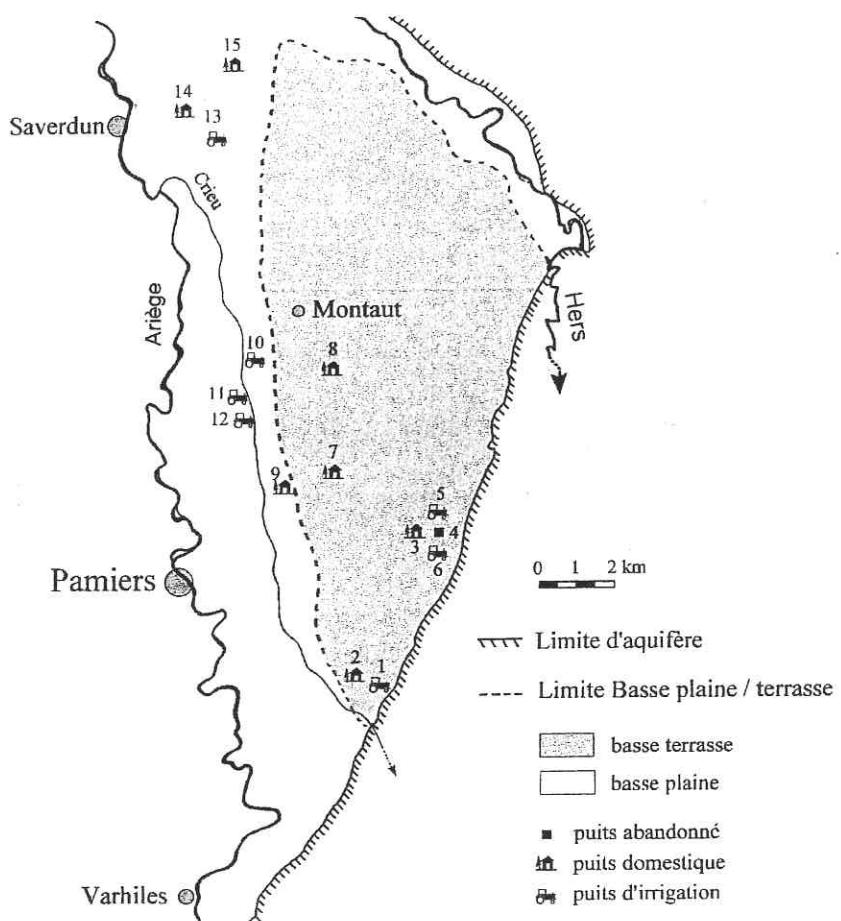
## A - STATIONS ECHANTILLONNEES ET PROTOCOLE.

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Les expérimentations ont été effectuées sur un ensemble de stations réparties sur une zone de 250 km<sup>2</sup> dans la plaine alluviale de l'Ariège (Pyrénées françaises). Les stations ont été retenues sur la base de leurs caractéristiques, de leur localisation et de leur utilisation variable à l'échelle de l'ensemble de l'aquifère : ouvrages de production d'eau potable, d'irrigation ou d'utilisation domestique. Deux ensembles de puits ont été échantillonnés au cours de cette étude :

- 9 captages d'eau servant à l'alimentation en eau potable de communes situées dans la basse plaine de l'Ariège, à l'occasion d'une campagne de prélèvements unique réalisée en avril 1998. En dépit de considérables efforts d'échantillonnage, les très faibles densités d'invertébrés collectées ont conduit à l'abandon de ces sites lors des campagnes ultérieures ; ces résultats, non présentés dans les chapitres précédents, sont exposés en annexe.
- 15 puits d'agriculteurs, répartis sur l'ensemble de la basse plaine et basse terrasse entre Varilhes et Saverdun, dont :
  - 7 puits utilisés pour l'irrigation des cultures (céréales, vergers), utilisés de façon intensive pendant les mois d'été de juillet à septembre en moyenne et répartis en majorité dans la plaine (stations 1, 5, 6, 10, 11, 12 et 13) ;
  - 7 puits de ferme, utilisés de façon modérée tout au long de l'année à des fins domestiques (consommation courante, arrosage des jardins potagers, bétail), essentiellement situés sur la terrasse (stations 2, 3, 7, 8, 9, 14 et 15) ;
  - 1 puits abandonné depuis environ 4 ans, situé en bordure de champ sur la terrasse et servant autrefois de puits d'irrigation (station 4).

Ces stations ont fait l'objet de 4 campagnes d'échantillonnage successives à un pas de 6 mois entre octobre 1998 et avril 2000, afin de couvrir deux années hydrologiques complètes (basse eaux / hautes eaux). Les prélèvements en vue des analyses physico-chimiques et faunistiques ont été effectués à l'aide de deux dispositifs complémentaires : une pompe mobile immergée au fond des puits et un filet phréatobiologique employé sur l'ensemble de la colonne d'eau (pour les détails, voir le chapitre 1).



**Figure 1** – Stations échantillonnées entre octobre 1998 et avril 2000 – contexte hydrogéologique (basse terrasse en grisé) et utilisation des puits.

**Tableau 1** – Récapitulatif des caractéristiques des 15 stations.

Station	Profondeur	Piézométrie	Localisation	Utilisation
1	9.40	3.02	terrasse	irrigation
2	8.70	6.09	terrasse	domestique
3	5.00	2.35	terrasse	domestique
4	9.60	1.9	terrasse	abandonné
5	11.00	1.45	terrasse	irrigation
6	8.10	0.46	terrasse	irrigation
7	4.20	2.6	terrasse	domestique
8	8.30	3.17	terrasse	domestique
9	9.20	6.15	plaine	domestique
10	9.30	5.7	plaine	irrigation
11	9.00	5.76	plaine	irrigation
12	9.90	7.83	plaine	irrigation
13	9.80	5.1	plaine	irrigation
14	5.40	3.95	plaine	domestique
15	8.20	6.3	plaine	domestique

## B - BILAN DES RESULTATS.

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### 1. Paramètres environnementaux de l'aquifère.

#### ▪ Hétérogénéité spatiale et temporelle

Dans la zone étudiée, la variabilité observée pour l'ensemble des variables physico-chimiques correspond essentiellement à des variations inter-sites de la qualité des eaux traduisant une hétérogénéité spatiale marquée. Les principaux paramètres étudiés ont été récapitulés dans le tableau 2.

**Tableau 2** – Variables environnementales pour les stations échantillonnées. Caractéristiques (moyennes, écarts-types, valeurs extrêmes) et tests de Kruskall-Wallis (ANOVA 1) en fonction des dates et des stations. Probabilités : \* < 0.05, \*\* < 0.005, \*\*\* < 0.0005.

Variables environnementales	Moyenne	Ecart-type	Minimum	Maximum	Périodes	Stations
Température (°C)	13.6	1.4	11.4	17.4	39.31***	1.98
Potentiel redox (mV)	393.7	57.5	263	587	25.37***	8
Conductivité ( $\mu\text{S.cm}^{-1}$ )	591.1	121.2	309	884	0.17	54.98***
Oxygène dissous	6.3	1.9	2.3	9.1	3.93	45.93***
pH	7	0.4	6	7.9	9.15*	38.86***
Dureté totale (°f)	25.6	7.6	10.9	39.1	1.14	52.88***
Alcalinité (°f)	15.5	7.4	3	28	0.34	56.17***
Calcium ( $\text{mg.l}^{-1}$ )	87.4	31.3	33.2	132.2	0.33	55.9***
Magnésium ( $\text{mg.l}^{-1}$ )	8.8	7.2	3.4	37.6	1.52	56.17***
Chlorures ( $\text{mg.l}^{-1}$ )	28.5	9.9	13.2	53.5	1.64	52.29***
Sulfates ( $\text{mg.l}^{-1}$ )	23.1	8.1	12.5	51.9	3.27	41.47***
Nitrates ( $\text{mg.l}^{-1}$ )	73.9	30.1	19.6	165.7	0.91	49.79***
Ammonium ( $\mu\text{g.l}^{-1}$ )	33.5	51.8	1	380	36.56***	5.63
Phosphates ( $\mu\text{g.l}^{-1}$ )	69	125.4	16	908	10.54*	37.43**

Des variations significatives de la qualité des eaux sont ainsi observées en fonction des puits échantillonnés pour toutes les variables à l'exception de la température, du potentiel redox et de l'ammonium (ANOVA 1, cf. tableau 2).

A cette variabilité associée à une forte structuration spatiale correspond une variabilité temporelle limitée : dans l'ensemble, les variations observées ne sont pas significatives entre les périodes d'échantillonnage, malgré les différentes saisons hydrologiques couvertes (ANOVA 1, cf. tableau 2).

Une évolution saisonnière est remarquée pour la température, le potentiel redox et le pH des eaux de l'aquifère ; l'ammonium et les phosphates présentent des variations traduisant une contamination ponctuelle (du puits 8 notamment) survenue au cours de la période d'étude.

En dépit de l'échelle limitée de cette étude, les résultats suggèrent une constance globale remarquable des paramètres environnementaux au sein de cet aquifère. Ce caractère inertiel, typique des systèmes souterrains, est une conséquence de l'isolement relatif du réservoir par rapport aux cours d'eau superficiels ; déjà déduite de l'observation des niveaux piézométriques, cette considération est valable à l'échelle de l'aquifère mais n'exclut cependant pas l'existence de variations locales dans l'intensité des échanges surface / aquifère (Chapitre 4).

#### ■ Qualité de l'eau et facteurs du milieu.

Les eaux du réservoir souterrain se caractérisent par un pH neutre, une oxygénation relativement importante associée à une minéralisation globale et à des teneurs en calcium et magnésium situées dans les limites habituellement rencontrées pour ce type d'aquifère. Des valeurs anormalement élevées en nitrates, ammonium, phosphates, chlorures et sulfates témoignent cependant d'un ensemble de phénomènes de contamination affectant l'ensemble de réservoir souterrain (chapitre 2).

De fait, les teneurs observées sur l'ensemble des stations correspondent à des eaux de qualité « médiocre, due aux activités humaines » selon les grilles de qualité des eaux souterraines établies par GULLY & RAMON (1980) et GUILLEMIN & ROUX (1992). L'analyse des variables physico-chimiques selon une approche statistique multifactorielle fait clairement apparaître deux ensembles de processus de minéralisation distincts en fonction de leur origine (naturelle ou anthropique) ; dans la zone étudiée, les chlorures, le magnésium et les sulfates apparaissent ainsi fortement corrélés avec les nitrates et constituent de ce fait des indices d'une contamination de l'aquifère d'origine majoritairement agricole.

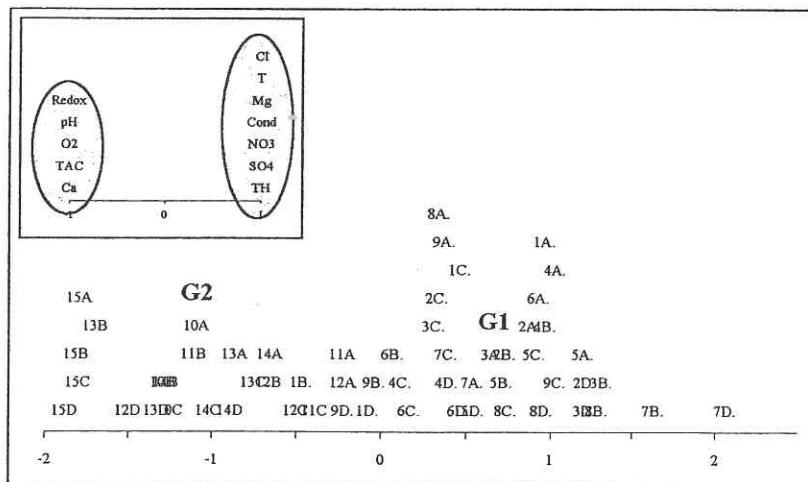
L'étude détaillée du stade et des causes des phénomènes de pollution observés dépassent largement le contexte de ce travail ; néanmoins, quelques remarques peuvent être faites dans le cadre des données recueillies.

L'approche multifactorielle employée afin de tester l'influence de facteurs du milieu sur les caractéristiques physico-chimiques des stations débouche sur une discrimination significative des stations en fonction de leur contexte hydrogéologique et de leur régime d'exploitation (Tableau 3).

**Tableau 3 – Analyses factorielles discriminantes basée sur les variables physico-chimiques des stations échantillonées. Critères de discrimination testés, corrélations, W, probabilités associées.**

	Utilisation des puits	Contexte hydrogéologique
Nombre de groupes	n=3	n=2
Echantillons bien classés	79.3 %	96.6 %
Corrélation :		
Axe F1	0.59	0.73
Axe F2	0.34	-
W (coefficient de Wilks) :		
Axe F1	64.6	65.07
Axe F2	20.5	-
Probabilité p associée :		
Axe F1	< 0.001	< 0.001
Axe F2	< 0.05	-

- **La localisation des puits** constitue un facteur permettant de distinguer deux groupes de puits (pouvoir discriminant 0.73,  $p<0.001$ , cf. tableau 3) : les puits de la basse terrasse, associés majoritairement aux variables de pollution nitrates, magnésium, chlorures et sulfates, et ceux de la basse plaine (variables associées : potentiel redox, pH, oxygène dissous, alcalinité et calcium, figure 3).

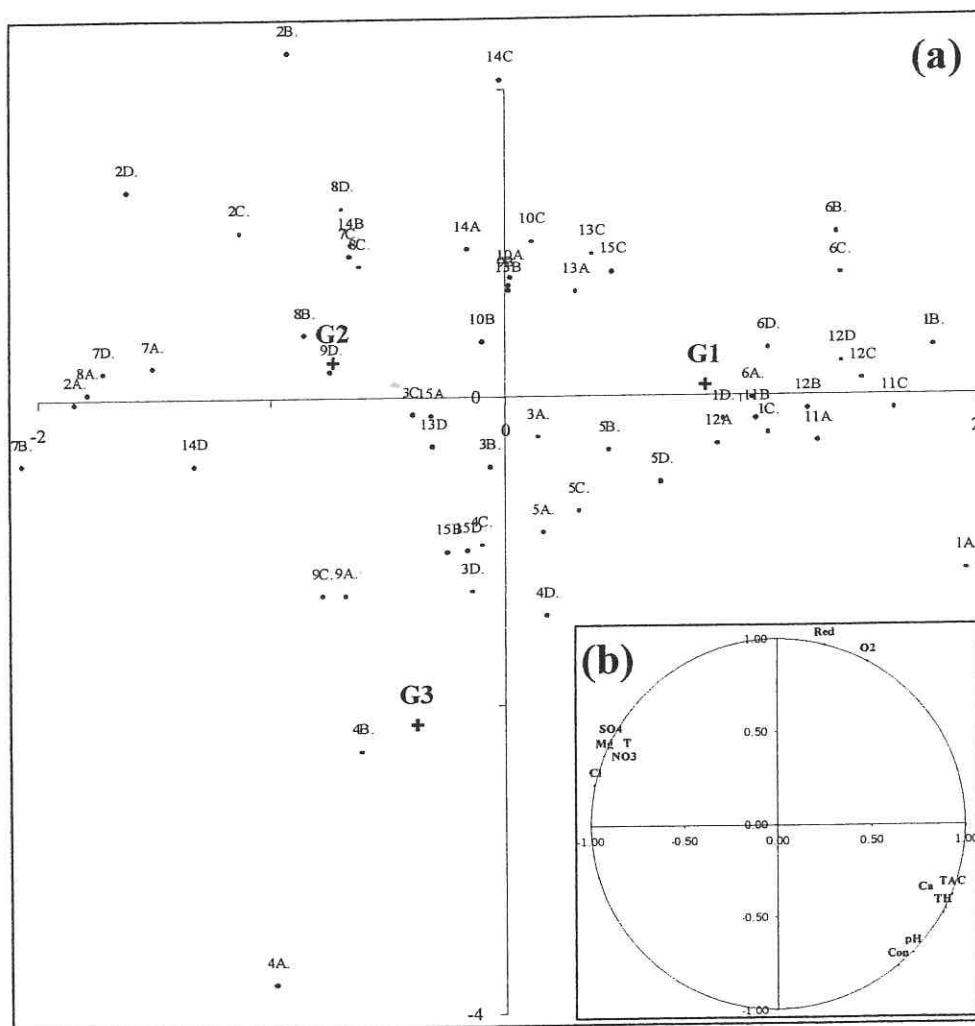


**Figure 3 – AFD des stations en fonction des variables physico-chimiques. Critère discriminant : localisation des puits. G1 : basse terrasse, G2 : basse plaine.**

- **La discrimination des stations selon leur utilisation** confirme cette tendance, dans la mesure où les transmissivités des alluvions conditionnent fréquemment l'usage des puits. Les puits d'irrigation apparaissent surtout associés aux variables de minéralisation naturelle, ceux à usage domestique aux variables de pollution (pouvoir discriminant sur F1/F2 : 0.59 / 0.34,  $p< 0.001 / <0.05$ , cf. figures 4a, 4b). Les stations les plus polluées correspondent ainsi à des puits situés dans

l'enceinte même des exploitations (cours de fermes) plutôt qu'à ceux situés plus à l'écart dans les champs.

A l'échelle de l'aquifère, l'hétérogénéité spatiale des paramètres physico-chimiques peut donc en partie être expliquée par le mode d'exploitation des ouvrages de captage échantillonnés et par leur contexte hydrogéologique ; néanmoins, une interprétation plus détaillée reste problématique en raison du nombre restreint de points d'échantillonnage et surtout du fait de la complexité des processus impliqués : d'après SEGUNIN (1980), « si les sols et le sous-sol (par ses propriétés conductrices variables) sont intervenus au tout début de la pollution en opérant une délimitation relativement stricte des zones polluées, cela n'est plus discernable actuellement ; de nombreuses années de pratiques agricoles néfastes et la circulation de l'eau dans le sous-sol ont fait peu à peu disparaître les traces de ces interventions en homogénéisant les teneurs ».



**Figure 4 – AFD des stations en fonction des variables physico-chimiques.**  
Critère discriminant : utilisation des puits. G1: puits d'irrigation, G2 : puits domestiques, G3 : puits abandonné.  
(a) : ordination des variables. (b) : ordination des stations.

## 2. Paramètres faunistiques.

### ■ Caractères généraux des assemblages.

Les communautés interstitielles de l'aquifère de l'Ariège sont largement dominées numériquement et spécifiquement par les microcrustacés (Copépodes cyclopides), formant des assemblages diversifiés (2-8 taxa par station) et collectés en abondance fortement variables selon les stations et les périodes (de 10 à plus de 32 000 individus).

Les macrocrustacés sont représentés par 11 espèces (0-6 par station), avec une dominance marquée des amphipodes niphargides (5 espèces dont une nouvelle en cours de description). La biodiversité, observée à l'échelle des stations (micro- / méso-échelle) ou de l'aquifère (macro-échelle) apparaît intermédiaire selon les données de la littérature (Chapitre 4).

Caractérisés par des stratégies écologiques reflétant des états adaptatifs différents vis-à-vis des biotopes souterrains, les trois types d'organismes habituellement rencontrés dans ce type de milieu sont présents dans l'aquifère de l'Ariège (stygoxènes, stygophiles, stygobies) ; cependant, la proportion relative des formes souterraines varie selon les groupes considérés.

Parmi les cyclopides, les espèces strictement inféodées aux eaux souterraines (stygobies) apparaissent spécifiquement minoritaires ; de même, leur abondance absolue est en général plus faible que celle des espèces épigées (à l'exception du groupe d'espèces *Diacyclops* spp.).

Chez les macrocrustacés au contraire, les espèces rencontrées sont majoritairement stygobies ; seule une espèce (*Proasellus meridianus*) peut être considérée comme non strictement inféodée au milieu souterrain.

### ■ Influence de la qualité de l'eau.

Comme cela avait été observé pour les paramètres physico-chimiques, les paramètres biologiques utilisés comme descripteurs des assemblages interstitiels (richesse taxonomique, indice de diversité de Shannon-Wiever, abondance absolue) présentent des différences significatives en fonction des stations (ANOVA 1, tableau 4).

Une hétérogénéité spatiale marquée, correspondant à des structures de communauté différentes, est ainsi trouvée quel que soit le groupe considéré (microcrustacés, macrocrustacés).

**Tableau 4** – Variables faunistiques pour les stations échantillonnées. Caractéristiques (moyennes, écarts-types, valeurs extrêmes) et tests de Kruskall-Wallis (ANOVA 1) en fonction des dates et des stations.  
Probabilités : \* < 0.05, \*\* < 0.01, \*\*\* < 0.001.

Variables faunistiques		Moyenne	Ecart-type	Minimum	Maximum	Périodes	Stations
<b>Microcrustacés</b>							
Richesse spécifique totale	3.54	1.34	2	8	3.68	25.48*	
stygobies	0.93	0.61	0	2	7.86*	20.71	
Indice de Shannon	0.83	0.58	0.06	2.03	3.34	30.32**	
Abondance absolue	1928	4906	10	32571	17.87***	28.69*	
<b>Macrocrustacés</b>							
Richesse spécifique totale	2.17	1.30	0	6.00	5.17	29.68**	
Indice de Shannon	0.68	0.61	0	2.11	4.68	29.43**	
Abondance absolue	64	96	0	424	0.96	48.22***	

Dans l'ensemble, il n'est pas observé de corrélations significatives entre la qualité de l'eau et les variables faunistiques. Avec des teneurs élevées dans toutes les stations, l'oxygène dissous, habituellement considéré comme un paramètre clé dans la répartition de la faune, ne constitue pas ici un facteur limitant, du moins à l'échelle globale de l'aquifère.

La contamination du réservoir par les nitrates et les ions associés n'affecte pas non plus les assemblages interstitiels (micro- ou macrocrustacés), malgré une gamme de variation relativement large pour les nitrates ; aucune sensibilité différentielle n'apparaît en fonction des espèces ou des types écologiques étudiés (organismes épigés ou hypogés) qui, dans le cadre de ces données, ne peuvent prétendre constituer des marqueurs biologiques de la qualité des eaux aux différents niveaux d'intégration étudiés (espèces, communautés).

Le recours aux abondances relatives des espèces comme descripteurs des structures d'assemblages apparaît néanmoins plus prometteur que l'utilisation de simples indices de diversité taxonomique ou d'abondance absolue, dans la mesure où ils permettent d'expliquer les structures faunistiques obtenues par d'autres paramètres environnementaux de l'aquifère (perméabilité des sédiments, régimes de pompage).

A l'échelle de l'aquifère, la qualité de l'eau ne constitue pas un paramètre majeur de structuration des peuplements interstitiels, du moins au stade actuel ; des effets sur la faune ne sont cependant pas à exclure dans l'hypothèse probable d'une dégradation de qualité de l'aquifère se poursuivant dans l'avenir. Il semble cependant difficile d'anticiper, dans le cadre de ces données, les effets d'une augmentation des taux de nitrate sur la composition et les structures des peuplements. De fait, les

structures de dominance apparaissent semblables entre les différents sites, quel que soit le niveau de contamination observé. Afin de préciser les réponses des communautés interstitielles à ce type de contamination, deux approches complémentaires sont notamment envisageables. Une première possibilité consiste à étudier la structures d'assemblages similaires (micro- et macrocrustacés) rencontrés au sein d'autres aquifères présentant des types de contamination analogues, à des niveaux contrastés. Les teneurs en nitrates de la zone étudiée se situant dans une gamme de 20-160 mg.l<sup>-1</sup>, de telles comparaisons pourraient alors être envisagées pour des aquifères s'écartant notablement de ces niveaux.

La seconde consiste en un suivi à plus long terme des peuplements de l'aquifère de l'Ariège, en relation avec le niveau de contamination du réservoir. Cette seconde possibilité présente l'avantage de permettre des comparaisons plus pertinentes en limitant au maximum les sources de variabilité ; dans une optique d'évaluation de la qualité biologique de l'aquifère, il s'agit en outre d'une approche plus à même de détecter les niveaux de sensibilité des organismes et d'éventuelles altérations des paramètres des communautés (changements de la diversité taxonomique ou fonctionnelle, des structures de dominance). Dans une telle optique, une fréquence d'échantillonnage bi-annuelle comme celle adoptée au cours de cette étude semble représenter un compromis tout à fait satisfaisant, en permettant de s'affranchir des variations environnementales à court terme tout en privilégiant une large répartition spatiale donc une meilleure représentativité des points de prélèvements.

#### ▪ **Structure des assemblages et paramètres du milieu**

Ces distributions faunistiques apparaissent fortement liées au contexte hydrogéologique des stations et, dans une moindre mesure, au régime d'exploitation des puits échantillonnes :

- Pour les microcrustacés comme pour les macrocrustacés, la distribution spatiale des structures de communautés se calque sur les divisions hydrogéologiques de l'aquifère, séparant les assemblages de la basse plaine de ceux de la basse terrasse. Cette structuration est cependant plus marquée chez les amphipodes et les isopodes que chez les cyclopides, sans doute en raison des gammes de taille respectives de ces organismes.
- Pour les cyclopides, les assemblages sont également influencés par le mode d'exploitation des stations : fréquence et intensité d'utilisation des puits modifient sensiblement la composition des communautés phréatiques ; à l'exception de *Speocyclops* sp., toutes les espèces stygobies sont en particulier majoritairement associées aux puits domestiques.

Ces constatations, si elles ne sont guère surprenantes, ont des implications concrètes sur les échantillonnages réalisés en milieu phréatique, dans la mesure où les résultats obtenus peuvent dépendre en grande partie des particularités des ouvrages considérés. Ainsi, lors de la campagne d'avril 1998, les puits échantillonnés (puits de forts diamètres, sollicités en permanence à des régimes soutenus) pourtant situés dans les mêmes zones n'ont permis de récolter qu'une faune pauvre et peu abondante (cf. annexe 1). L'importance des différents niveaux d'observation des phénomènes étudiés est ainsi soulignée, dans la mesure où les résultats obtenus au cours de cette étude ne sont représentatifs que d'une échelle spatio-temporelle donnée : les données disponibles à l'échelle des aquifères étant à l'heure actuelle en nombre limité, cette étude s'est volontairement attachée à décrire les structures de peuplements des crustacés interstitiels à l'échelle de l'ensemble de l'aquifère, avec un pas d'échantillonnage de 6 mois. Si la variabilité spatiale des peuplements est expliquée de façon satisfaisante à l'échelle de la plaine alluviale par les paramètres hydrogéologiques et hydrodynamiques de l'aquifère, certaines stations présentent cependant des compositions faunistiques atypiques (notamment station 5 pour les amphipodes et isopodes). L'étude complémentaire détaillée des paramètres de chaque station (granulométrie, nature des sédiments, écoulements de surface, contexte agricole, régime précis et volumes extraits des captages en activité) pourrait permettre d'obtenir une meilleure compréhension des facteurs structurant les peuplements à l'échelle locale (décimétrique).

Outre le régime d'utilisation, la taille des ouvrages constitue également un facteur à prendre en considération : dans les puits renfermant un volume important, la sphère d'aspiration des pompes utilisées se révèle incapable de déloger les organismes au-delà d'un certain rayon d'action. De fait, l'échantillonnage porte alors essentiellement sur la communauté phréatique, qui peut être représentative de conditions locales propres à la station, plutôt que sur les zones voisines de l'aquifère. L'échantillonnage en milieu interstitiel constitue toujours une difficulté majeure et une des pierres d'achoppement de l'écologie souterraine ; le recours à des prélèvements, suffisamment espacés dans le temps pour ne pas perturber les assemblages et effectués sur des piézomètres de petit diamètre semble représenter à l'heure actuelle un des meilleurs compromis disponibles.

Un tel phénomène « local » n'apparaît cependant pas dans cette étude volontairement réalisée à plus vaste échelle, dans la mesure où les structures de diversité et d'abondances observées localement au niveau des stations résultent de processus hydrogéologiques et hydrodynamiques clairement identifiés à l'échelle du réservoir. Les communautés de crustacés interstitiels peuvent ainsi être considérées comme de véritables indicateurs naturels des aquifères alluviaux, même si en l'occurrence seuls certains facteurs (transmissivité des alluvions, régime d'exploitation du réservoir, et non qualité physico-chimique de l'eau) interviennent ici sur les peuplements étudiés. Ces observations doivent également être replacées dans un contexte plus vaste, ce travail ne prétendant pas à une analyse exhaustive de l'ensemble des groupes taxonomiques et des paramètres du milieu. De fait, l'activité du

compartiment microbiologique, dont l'importance est aujourd'hui reconnue dans la structuration des communautés interstitielles, pourrait être explorée au niveau des stations en relation avec les paramètres faunistiques des assemblages d'invertébrés.

■ **Persistance des communautés et stabilité du milieu.**

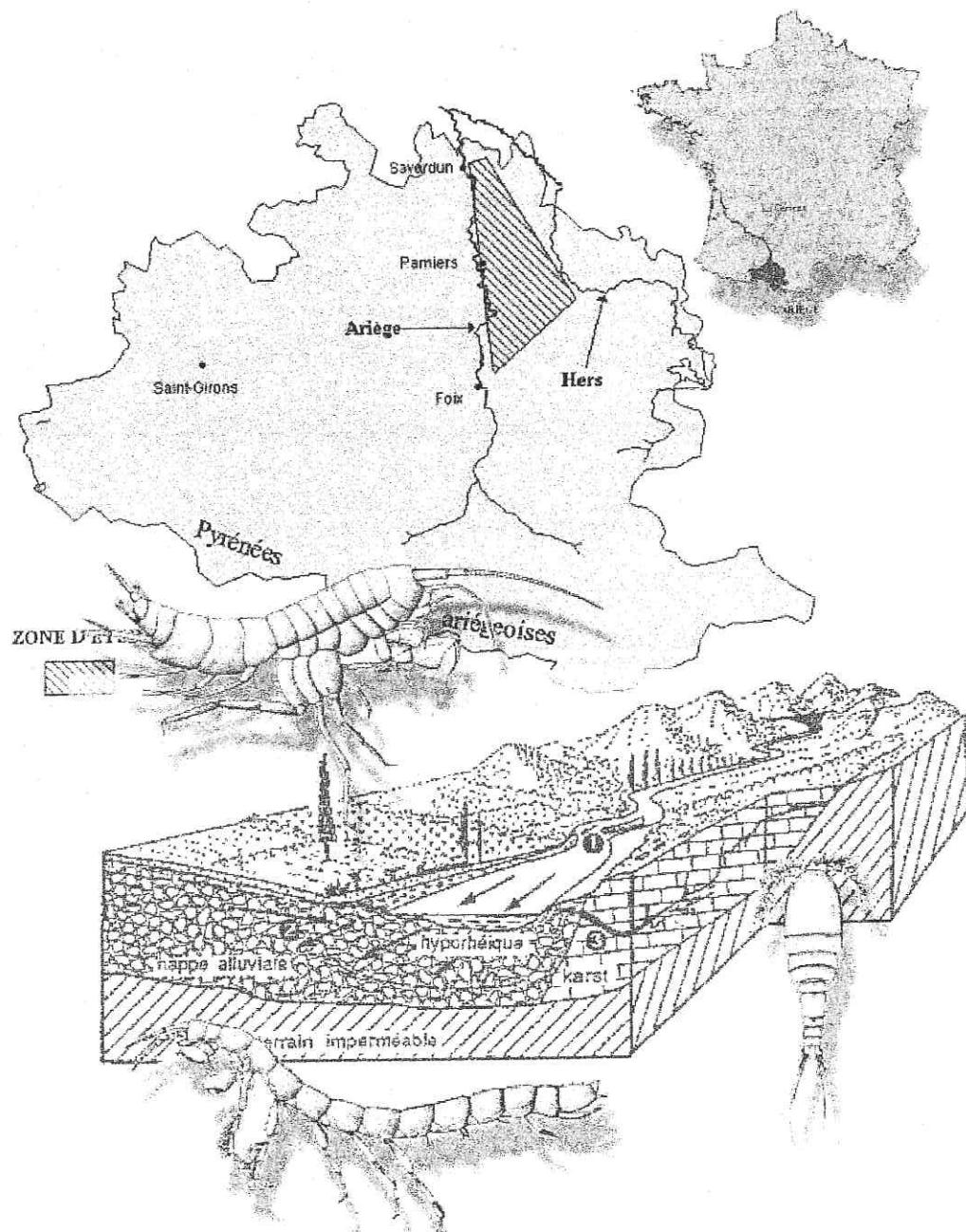
Dans l'ensemble, les paramètres faunistiques observés ne présentent pas de variations significatives entre les périodes d'échantillonnage (ANOVA 1, tableau 4), à l'exception des abondances absolues de cyclopides sujettes à des variations apparemment aléatoires. La structure et la composition des communautés de crustacés interstitiels apparaissent globalement stables à l'échelle temporelle considérée, c'est-à-dire sur une période de 18 mois.

En dépit de différences pouvant s'expliquer par les contraintes d'échantillonnage et de la variabilité intrinsèque aux processus vivants, une remarquable constance des assemblages s'observe tant pour les micro- que pour les macrocrustacés. Cette prévisibilité s'avère décrite de façon plus pertinente par les structures d'abondance relatives des espèces plutôt que par de simples descripteurs de diversité. Au sein des assemblages, les distributions de dominance sont en outre très généralement caractérisées par la présence d'une espèce largement dominante et de plusieurs espèces rares, sans que cette caractéristique puisse être expliquée de façon satisfaisante dans le cadre des données recueillies : ce type de structure fortement déséquilibrée au profit d'une, voire de deux espèces est analogues à celui observé pour certaines communautés benthiques marines en milieu perturbé. Néanmoins, il peut aussi être observé dans le cas de milieux prévisibles caractérisés par une stabilité environnementale marquée.

Ces caractéristiques faunistiques, valables aussi bien pour les assemblages de micro- et de macrocrustacés, semblent essentiellement être une conséquence directe de la stabilité physique marquée du milieu ; différents degrés de persistance pourraient alors être retrouvés selon les aquifères, en fonction du degré d'isolement par rapport à la surface. Une telle hypothèse, non vérifiable dans le cadre de cette étude portant sur un aquifère unique, ne constitue bien évidemment qu'un jalon préliminaire dans une perspective plus globale d'évaluation de la stabilité des écosystèmes souterrains. Le développement d'un indice « global » intégrant différents paramètres caractéristiques de la stabilité des systèmes serait susceptible de favoriser le développement d'études comparatives entre divers écosystèmes interstitiels, de façon analogue aux indices de stabilité (ex. « Pfankfuch index ») définis pour les cours d'eau de surface.

« Groundwater ecology is a young, synthetic science that merits much greater research investment » (STANFORD & GIBERT 1994). Ce travail n'a pas d'autre ambition que d'apporter une contribution, aussi modeste soit-elle, à ce vaste édifice.

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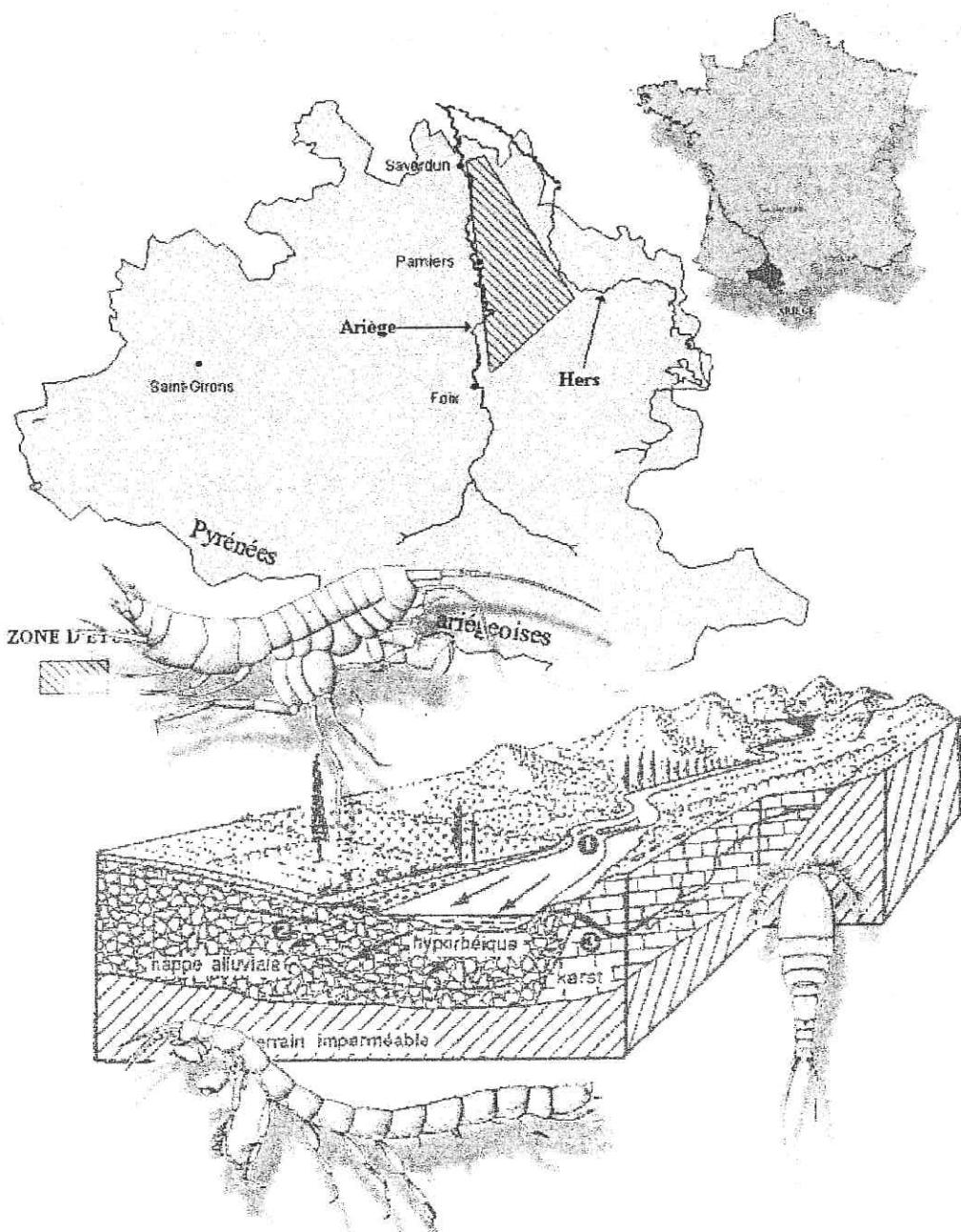
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## - ANNEXES -





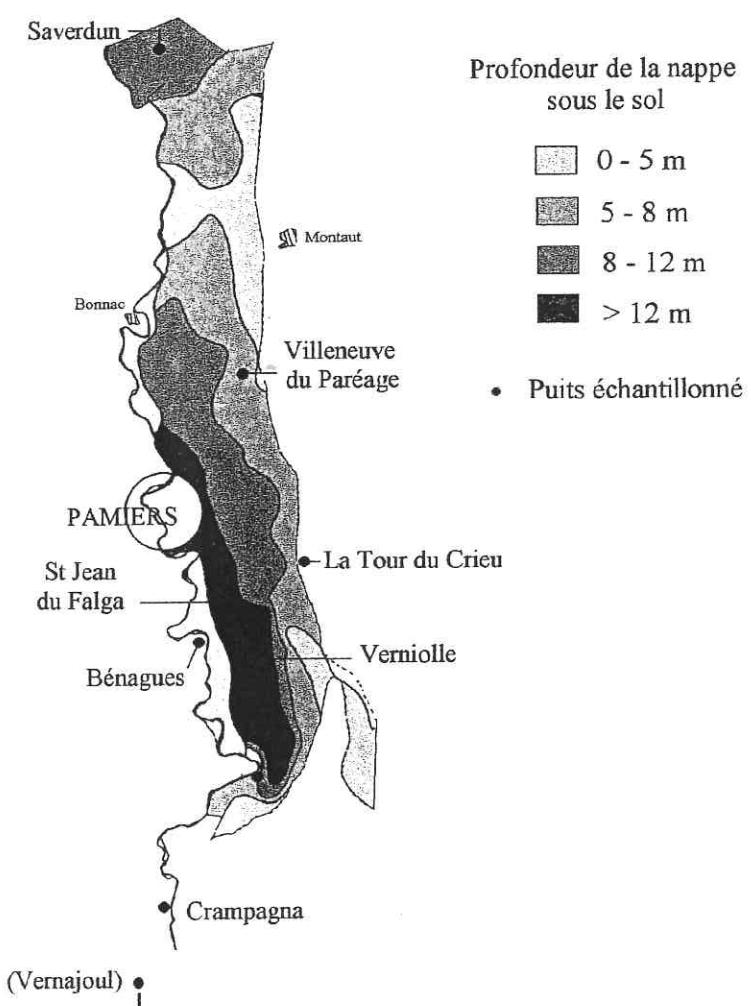
## ANNEXE 1 : Campagne d'Avril 1998.

Extrait des données de la première campagne d'échantillonnage réalisée en Avril 1998 sur 9 ouvrages de production d'eau potable localisés sur la basse plaine de l'Ariège. Les échantillons ont été prélevés au moyen d'une pompe immergée type FLYGT descendue au fond des puits.

Volume filtré : 10 m<sup>3</sup> par prélèvement.

Echantillonnage de la faune, prélèvements et analyse des paramètres physico-chimiques : protocole identique à celui des campagnes d'Octobre 1998 à Avril 2000 (pour les détails, se référer aux chapitres précédents).

- Localisation des 9 captages échantillonnés en Avril 1998 (puits AEP).



*Annexe 1 - Campagne d'Avril 1998.*

**Tableau 1.** Abondance des cyclopides collectés dans les puits AEP en Avril 1998.

	Graeteriella unisetigera	Diacyclops clandestinus	Diacyclops bisetosus	Eucyclops serrulatus	Tropocyclops prasinus	Paracyclops fimbriatus
BENAGUES	-	2	-	-	-	1
CRAMPAGNA	-	3	-	-	-	-
VARILHES	-	34	-	-	-	-
ST JEAN DU FALGA	-	-	-	-	-	-
VERNAJOUL	-	6	2	29	9	1
VERNIOILLE	-	17	-	-	-	1
VILLENEUVE DU P.	-	15	-	-	-	-
LA TOUR DU CRIEU	1	53	-	-	4	10
SAVERDUN	-	-	-	-	-	-

**Tableau 2.** Variables environnementales mesurées pour les 9 captages AEP en Avril 1998.

	Benagues	Crampa- gna	Varilhes	St Jean du Falga	Vernajoul	Verniolle	Vill. du Paréage	Tour du Crieu	Saverdun
Température (°C)	8,30	8,70	13,20	13,70	12,00	12,60	13,10	12,40	14,30
Oxygène (mg/l)	5,30	6,20	6,10	8,40	7,70	8,30	6,80	7,60	5,00
Conductivité (µS.cm <sup>-1</sup> )	131,00	210,00	502,00	590,00	571,00	625,00	487,00	462,00	642,00
pH	7,42	7,24	7,10	7,09	7,20	6,89	6,73	7,11	6,82
Potentiel redox (mV)	169	212	178	139	235	250	251	300	137
Dureté totale (°F)	6,42	13,64	25,10	28,30	30,50	29,70	21,54	17,12	30,62
Alcalinité (°F)	5,76	11,06	21,90	22,82	28,40	21,40	15,58	12,64	22,16
NH <sub>4</sub> (mg/l)	0,016	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01
PO <sub>4</sub> (mg/l)	< 0,15	< 0,15	< 0,15	< 0,15	< 0,15	0,15	< 0,15	0,37	< 0,15
DBO <sub>5</sub> (mg O <sub>2</sub> /l)	< 1	1,3	1	< 1	1	< 1	1,2	< 1	< 1
Ca <sup>2+</sup> (mg/l)	22,30	49,21	93,32	104,97	92,95	112,46	80,65	51,44	113,75
Mg <sup>2+</sup> (mg/l)	2,05	3,25	4,30	5,00	17,65	3,85	3,35	10,35	5,30
Cl <sup>-</sup> (mg/l)	1,30	2,85	6,40	11,85	4,05	21,10	15,95	12,40	18,90
SO <sub>4</sub> <sup>2-</sup> (mg/l)	8,10	20,65	18,90	19,85	10,15	16,30	21,25	47,55	22,30
NO <sub>3</sub> <sup>-</sup> (mg/l)	0,60	4,40	12,00	41,55	6,15	58,60	47,20	39,20	57,55

**ANNEXE 2 : Captages des campagnes Oct. 1998 – Avr. 2000**

Tableau récapitulatif des stations échantillonnées entre Octobre 1998 et Avril 2000.

n°	Lieu-dit	Coord. X (Lambert zone III)	Coord. Y	Propriétaire
1	Bordeneuve	545.506	3088.29	Mr DELPONTE Verniolle - 09 120 VARILHES
2	Bagatelle	544.674	3088.38	Mr SOULA Verniolle – 09 120 VARILHES
3	Baulussio	546.64	3092.07	Mr METGE Didier Le Carlaret – 09 100 PAMIERS
4	Baulussio	547.017	3092.3	Mr METGE Léon Le Carlaret – 09 100 PAMIERS
5	La Bouscarre	547.046	3092.56	Mr CRETE Alain Le Carlaret – 09 100 PAMIERS
6	Bramal	547.293	3091.6	Mr CRÉTÉ Alain Le Carlaret – 09 100 PAMIERS
7	Ferries d'en Haut	544.747	3094.03	Mr ROUCH Ferries-d'En-Haut - 09 100 PAMIERS
8	Péricou	544.391	3097.2	Mr EYCHENNE Péricou – 09 700 MONTAUT
9	Ferries de Bas	543.217	3093.78	Mr MONBLANC Jean-Claude Ferries-de-Bas – 09 100 PAMIERS
10	Les Espezats	542.346	3097.5	Mr METGE Les Espezats – 09 100 VILLENEUVE DU PAREAGE Mr CARDENTI
11	Lambrine	542.187	3095.9	Mr CARDENTI La Cabane – 09 100 VILLENEUVE DU PAREAGE
12	La Cabane	541.796	3096.42	Mr CARDENTI La Cabane – 09 100 VILLENEUVE DU PAREAGE
13	Caoucou	541.04	3104.48	Mr LEPAGNEY 21 Grand-rue – 09 700 SAVERDUN
14	Le Roussel	541.711	3106.65	Mr MASSE Péloque – 09 700 SAVERDUN
15	Péloque	540.664	3105.38	Mr MASSE Péloque – 09 700 SAVERDUN



## **ABSTRACT**

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The main topic of this work was to study the dynamics and structure of interstitial crustacean communities at the scale of a whole aquifer. Several faunistic (abundance, taxonomic and functional diversity) and environmental parameters (groundwater physicochemical variables, extraction regimes) were investigated simultaneously in a semi-confined, agriculturally-impacted alluvial aquifer located in the French Pyrénées (the Ariège aquifer). Sampling was carried out in fifteen wells scattered over the floodplain during four main field surveys occurring with a 6 months frequency. At the scale of the aquifer, a marked spatial heterogeneity was found for community parameters that could not be related to the spatio-temporal distributions of groundwater physico-chemical variables. The results suggested that environmental parameters could not be considered as major structuring factors for the interstitial communities in this aquifer, whatever variables (natural or anthropic, in particular nitrates) were considered. Variations in community structure were mainly related to the hydrological context of the stations and to the groundwater extraction regimes. Despite the intrinsic heterogeneity of interstitial aquifers resulting in local variability, the interstitial crustaceans thus constitute natural indicators of aquifer structure and utilization. A marked persistence of diversity/dominance patterns was also observed in relation with a strong, overall temporal environmental stability in this aquifer.

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**TITRE :** Les Crustacés souterrains, indicateurs naturels de l'aquifère alluvial de l'Ariège (France, Pyrénées).

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1816 CNRS / UPS / INP.

## **RÉSUMÉ**

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L'objectif principal de ce travail concerne l'étude de la dynamique et des structures de peuplement des crustacés souterrains à l'échelle de l'ensemble d'un aquifère.

Différents paramètres faunistiques (abondance, diversité taxonomique et fonctionnelle, dominance) et environnementaux (paramètres physico-chimiques, structure/exploitation du réservoir souterrain) ont été étudiés au sein d'un aquifère alluvial semi-confiné soumis à une pression anthropique marquée (pratiques agricoles intensives). Quatre campagnes principales de prélèvements ont été réalisées à 6 mois d'intervalle sur un ensemble de 15 ouvrages de captages situés dans la plaine de la basse Ariège (France, Pyrénées). L'analyse des paramètres écologiques des communautés fait apparaître une structuration spatiale marquée des assemblages à l'échelle de l'aquifère. Cette hétérogénéité n'est pas expliquée par les distributions spatiales ou temporelles des paramètres physico-chimique des eaux souterraines, quelles que soient les variables considérées (minéralisation naturelle ou anthropique). Dans l'ensemble, les variables environnementales, en particulier les nitrates dont les teneurs élevées constituent un marqueur de contamination des eaux souterraines, ne constituent pas un facteur de structuration majeur des communautés interstitielles.

Les résultats obtenus témoignent de variations significatives des structures de peuplements en fonction du contexte hydrogéologique des stations et, dans une moindre mesure, du régime d'exploitation des captages. Malgré l'existence d'une variabilité locale liée à l'hétérogénéité intrinsèque du milieu, les crustacés souterrains constituent ainsi des indicateurs naturels de la structure et de l'utilisation du réservoir. Les données mettent également en évidence une nette constance des structures de diversité/dominance des peuplements, associée à une forte persistance temporelle des paramètres environnementaux de l'aquifère.

## **MOTS-CLÉS**

Eaux souterraines, nappe alluviale, crustacés, structure des communautés.

**DISCIPLINE :** Hydrobiologie

## **LABORATOIRE**

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