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Designing a standardised sampling method for invertebrate monitoring: a pilot experiment in a motorway retention pond

Olivier Scher[1,*], Kate E. McNutt[2] and Alain Thiéry[3]

[1] Fédération des Parcs naturels régionaux de France, 9 rue Christiani, 75018 Paris, France.
[2] Botanical, Environmental & Conservation Consultants, 27 Upper Fitzwilliam street, Dublin 2, Ireland. katemcnutt97@gmail.com
[3] UMR CNRS 6116-IMEP, 3 Place Victor Hugo, Case 37, 13331 Marseille cedex 03, France. alain.thiery@univ-provence.fr

[*] Corresponding author: olivier.scher@gmail.com

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ABSTRACT

Designing a standardised sampling method for invertebrate monitoring: a pilot experiment in a motorway retention pond

The implementation of the European Water Framework Directive revealed the necessity to develop new tools designed for freshwater ecosystems monitoring. As a new assessment approach employing invertebrate monitoring, three artificial substrates (two benthic and one pelagic) were tested for 7, 14, 21 and 35 days of exposure in a motorway retention pond located in Southern France. Two of these artificial substrates appeared to sample too narrow a range of taxa, which was confirmed by two-way ANOVA tests and diversity and evenness indices. Samples taken by the remaining artificial substrate, composed of six plastic plant stems fixed on a 15 × 15 cm square tile, were representative of the species assemblage found in the stormwater retention ponds. The use of an artificial substrate as a standardised method for long term invertebrate monitoring in ponds holds much potential.

Key words: Artificial substrate, aquatic invertebrates, highway, retention pond, diversity indices.

RESUMEN

Diseño de un método de muestreo estandardizado para el seguimiento de invertebrados: experimento piloto en una balsa de retención de autopista

La puesta en marcha de la Directiva Marco Europea del Agua requiere el desarrollo de nuevos protocolos de muestreo para el seguimiento de los ecosistemas acuáticos continentales. Como nuevas aproximaciones al seguimiento de invertebrados acuáticos se probaron tres sustratos artificiales (dos bénticos y uno pelágico) con un tiempo de exposición de 7, 14, 21 y 35 días en una balsa de retención en una autopista del Sur de Francia. Dos de estos sustratos capturaron selectivamente un reducido número de taxones específicos, lo que fue confirmado con un análisis ANOVA de dos factores y los índices de diversidad y equitabilidad. Las muestras obtenidas con el sustrato artificial compuesto por seis pies de plantas de plástico fijadas en una baldosa cuadrada de 15 × 15 si que resultaron representativas de la asociación de especies característica de las balsas de retención de aguas en las autopistas. El uso de sustratos artificiales como método estandardizado para el seguimiento a largo plazo de los invertebrados acuáticos en balsas y charcas es de un gran potencial.

Palabras clave: *Sustratos artificiales, invertebrados acuáticos, autopistas, balsas de retención, índices de diversidad.*

INTRODUCTION

During the last few decades many stormwater retention ponds have been dug alongside French motorways following the key aim of the European Water Framework Directive (WFD) to achieve a good ecological water status in Europe by 2015 (European Commission, 2000). These basins act as “road pollutant traps” by collecting surface runoff during rainstorm events (chronic pollution) or by confining accidental and localised pollution. They also play an important role in regulating water level during the drainage process. However, the ecological processes within them are poorly understood and few studies have been conducted upon them (Wren *et al.*, 1997; Bishop *et al.*, 2000a, b; Scher & Thiéry, 2005).

The need for practical long term monitoring tools in aquatic ecosystems responds to the objective of the WFD. This is particularly important for stakeholders (public or private) who have to continuously monitor pond ecosystems in order to identify potential disturbance linked to their use (retention, treatment, etc.). Unfortunately, such tools are as yet still rare and not adequately designed for non specialists (Oertli *et al.*, 2005). Moreover, a long term monitoring approach involves regular sampling in the one pond which could disturb the ecosystem when using net sampling, a commonly used method. All these arguments show that room exists to develop a sampling method that is (1) designed for non specialists, (2) useful in engineered or disturbed environments, (3) low impact on ecosystems and (4) standardised. Artificial substrates, already used in several aquatic ecosystems, could respond to these expectations.

Cairns (1982) defined an artificial substrate as “a device placed in an aquatic ecosystem to study colonization by indigenous organisms”. They have been intensively used to study colonization processes (estimation of richness, abundance of macroinvertebrates, etc.) especially in lotic systems (reviewed in Rosenberg & Resh, 1982; Benoit *et al.*, 1998) but only a few studies exist for shallow lentic habitats (Clarke *et al.*, 1997; Muzzafar & Colbo, 2002).

These devices offer real advantages when compared to net sampling since they are useful in habitats that are difficult to sample (such as those with dense macrophyte beds or concentrated filamenteous algae), they are low cost, low-impact (passive sampling), standardised and can be used for assessing species richness as well as changes in abundance (Buikema & Voshell, 2001). Nevertheless, they could demonstrate some disadvantages such as selectivity and potential loss of organisms during retrieval of substrates (Rosenberg & Resh, 1982).

Artificial substrates used in the study of lentic systems have encompassed a range of designs according to the objectives of the various studies, these include rock-bags (De Pauw *et al.*, 1997; Clarke *et al.*, 1997; Muzzafar & Colbo, 2002), leaf/wood litter bags (France, 1997; Pope *et al.*, 1999) wood snags (Thorp *et al.*, 1985), imitation pondweeds (Jeffries, 1993; Benoit *et al.*, 1998), multiplate samplers (Francis & Kane, 1995) and cement balls in wire baskets (Schmude *et al.*, 1998). In the present study we chose substrates made of imitation pondweeds (as used in lentic ecosystems by Jeffries (1993) and Benoit *et al.* (1998)), a scrubbing brush (never tested) and pan scourers (used in marine habitats to capture meiofauna by Smith & Rule (2002) and Attila *et al*. (2003)).

The aim of our study was to design a sampling method using three different artificial substrates (two benthic and one pelagic) placed in a motorway retention pond over a five week period. The advantage of designing a sampling method in a motorway pond is the homogeneous conditions offered by this ecosystem (Scher, 2005). We first compared the artificial substrates on the basis of their invertebrate assemblages in order to evaluate (1) the exposure period necessary to reach equilibrium communities and (2) to assess differences in richness and diversity. We then compared our results with previous sampling efforts conducted in the same pond the previous year in order to choose the artificial substrate most effective at sampling the invertebrate community of the studied retention pond.

This pilot experiment provides an initial insight into the efficiency of three artificial substrates in sampling aquatic invertebrates and their potentiality for long term monitoring studies.

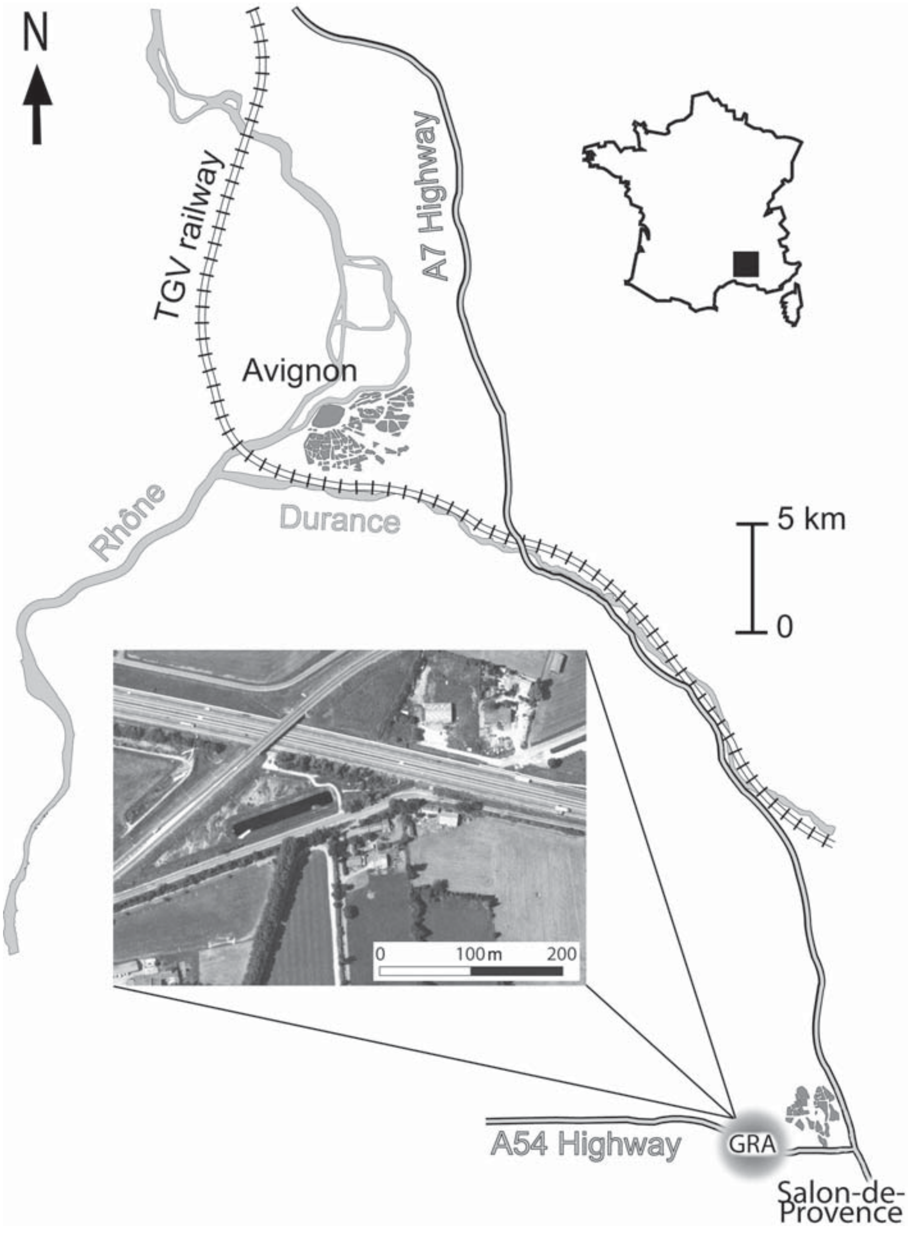

Figure 1. Map of study area showing the sampling site. *Mapa del área de estudio indicando el punto de muestreo.*

METHODOLOGY

Study site

The study was carried out on a stormwater retention pond, namely Grans (GR), located in South-Eastern France, near Salon-de-Provence (43°37′43″N / 05°03′36″E, 70 m a. s. l.). The pond is about 120 × 15 m with a total surface area of 1825 m^2 and an average depth of 60 cm (Fig. 1). It is filled by storm water and by surface runoff from the adjacent A54 motorway (ASF, Autoroutes du Sud de la France) during rainstorms. Violent storm events at the end of summertime and a drought period are the main characteristics of the study area, which is under a Mediterranean climate (Blondel & Aronson, 1999). The ground of the basin is composed of a PEHD (High Density Poly-Ethylene) membrane covered with a thin layer (range 0-5 cm) of sediment without pebbles or stones. Only hydrophytes (*Chara globularis* Thuille, *Chara vulgaris* L., *Potamogeton pectinatus* L.) and filamentous algae are present.

Materials

Three types of artificial substrates were tested: two benthic ones (the Plastic plant, PL, and the Scrubbing brush, BR) placed upon the ground and a single pelagic one (the Pan scourer, PE) maintained in the water column. The PL substrate (Fig. 2) was made up of a square tile (15 × 15 cm) to which four plastic stems (39 cm long) of the model "Indian Cabomba"®, as well as two 30 cm long stems and one 20 cm long stem of the model "Ambulia"® were affixed. These two models, showing the appearance of *Chara* species, are manufactured by Aqua-Nature®. When submerged, the stems always stand vertically in the water column. The square tile was used as a weight to keep the substrates at their chosen positions. As with the PL, the BR substrate (Fig. 2), made of a scrubbing brush (16 × 6 cm with a height of 3.5 cm), was fixed to a square tile. The final substrate, the PE (Fig. 2), was made of two pan scourers (15 × 9 × 0.7 cm) that were inter-locked in their middle to form a cross. It was maintained at a chosen depth (about 20 cm under the surface) by a string secured to a stone.

A total of twenty four samplers were placed in the pond on the 15th of April 2003. Each of their positions was randomly chosen to produce an unpredictable distribution. For each of the four tested exposure lengths (7, 14, 21 and 35 days), two substrates of the same type were removed in order to allow comparisons of intra-sampler variability. As such a total of six substrates were removed from the basin on each date, placed directly into a ziplock® bag and preserved in 7 % formaldehyde. Utmost care was taken to prevent the loss of invertebrates when retrieving sampling devices (Rosenberg & Resh, 1982).

A set of five samples collected every four weeks in the same retention pond during the year 2002 (from March 13 to June 12) was used as a control for sampling procedure. These samples

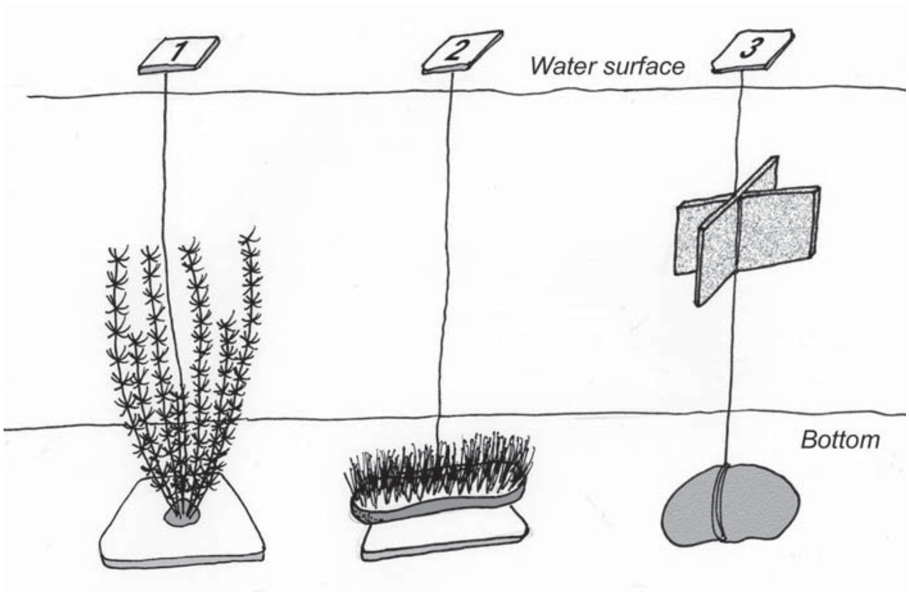

Figure 2. Schematic of the three artificial substrates described in this study with (1) Plastic plant, *PL*, (2) Scrubbing brush, *BR* and (3) Pan scourer, *PE. Esquema de los tres sustratos artificiales empleados en este estudio con (1) Plantas de plástico, PL, (2) Cepillo de barrer suelos,* BR *y (3) Estropajo de fibra limpia-cacerolas.*

included three replicates from each date and were collected using a net of a 125 μm mesh-size with an opening of 21 cm. Total distance swept by the net, from bottom to surface, was 4 m.

Invertebrate samples were then sorted in the laboratory and identified to the lowest taxonomic level according to Tachet *et al.* (2002). When the number of organisms in a sample was excessive (i.e., as often with Oligochaeta, Cladocera and Ostracoda), they were estimated using a Dollfus tank that provides a 10 % to 11 % count error for zooplankton (Pont, 1983).

Abiotic data (electrical conductivity measured in $\mu S \cdot cm^{-1}$ and temperature in °C) were measured *in situ* in a single point centred in the pond with a WTW® (LF_{91}) device. Depth variation (cm^{-1}) was measured with a graduated ruler placed at the deepest location in the water body. Water samples were preserved in a refrigerated box in order to carry them to the laboratory for analysis. Anions were analysed by chromatography on DIONEX DX120 and cations by atomic absorption on SPECTRA AA 640 VARIAN®. Water samples were analysed for herbicides (glyphosate and its by-products) by the Departmental Laboratory of Drôme (LDA 26) by liquid chromatography using fluorogenic labelling.

Analysis

Community structure on each substrate was analysed using several methods:

- Taxonomic diversity: total number of taxa collected on each substrate;
- Jaccard's coefficient (S_j): a measure of similarity in species composition between two communities (Lincoln *et al.* 1998);
- Shannon-Wiener index (H') and Evenness (E): these indices provide information on the structure and regularity of different samples;

Numeric data were then transformed into density per litre due to the impossibility of estimating the surface of the PL substrate. For the plastic plant substrate, each stem was considered as a cylinder and all stems were summed while the two other samplers were considered as cubic volumes. The total volume of each substrate was estimated as 1.583 l for PL, 0.336 l for BR and 0.189 l for PE. Density data were then log $(x+1)$ transformed to stabilise variances. Only the ten most abundant invertebrate taxa found in the substrate and net samples were used in the analyses. Two-way ANOVAs were used to test differences among the samples from the three artificial substrates. Substrate type and length of exposure were the two factors tested. The REGWQ post-hoc test was applied to rank treatment factors. Analyses were conducted using XLSTAT© for Windows© software (Addinsoft, 2003).

We finally assessed the colonization dynamics on artificial substrates by drawing accumulation curves and calculating the number both of taxa acquired and of taxa lost at each substrate between each sampling occasion (day 14, 21 and 35).

RESULTS

Chemical data

The principle results are presented in Table 1. During the five weeks of the experiment, temperature, measured in the morning (between 9

Table 1. Main physical and chemical characteristics of the studied pond during the experiment. Measurements were taken in a unique point centred in the pond. Annual mean refers to previous data published in Scher & Thiéry (2005). *Principales características físicas y químicas de la balsa durante el experimento, medidas en un punto central. Medias anuales extraídas de Scher & Thiéry (2005).*

	Conductivity	Temperature	Depth	HCO_3^-	Cl^-	NO_3^-	SO_4^{2+}	Ca^{2+}	Glyphosate	AMPA
	($\mu S \cdot cm^{-1}$)	(°C)	(cm)	($mg \cdot L^{-1}$)					($\mu g \cdot L^{-1}$)	
day 7	224	16.4	60	41.48	37	1	3.35	11.95	0.9	< 0.1
day 14	284	20.4	58	54.9	53	0	3.05	13.69	0.33	0.17
day 21	344	23	55	75.64	55	0	3.25	21.11	0.28	< 0.1
day 35	321	23	60	67.1	50	0.9	4.6	19.43	0.25	< 0.1
Annual mean	*303*	*17.6*	*59*	*53.66*	*48.98*	*1.16*	*4.48*	*20.14*	*0.21*	*0.17*

and 10 a.m.), increased continuously from 16 °C to 23 °C. Conductivity followed depth variation with an increase in the concentration of the major ions (i.e. HCO_3^-, Cl^- and Ca^{2+}) following drops in water level and their subsequent dilution during the final two weeks when the basin was filled by rain water and runoff (day 21 to 35). Nutrients such as nitrogen, phosphorus and iron were barely detected in the stormwater retention pond. Glyphosate molecules along with their by-product, the AminoMethylPhosphonic Acid (AMPA), were found in the water column at low concentration (0.17 to 0.9 $\mu g \cdot l^{-1}$) during the experiment period. Physical and chemical variations of water during the experiment did not differ from those recorded during the 2002 survey (Mann-Whitney U-test, $p > 0.05$). With regards to the hydrology of the studied basin, an outflow originating from the stormwater retention pond and draining into the surrounding landscape was occasionally observed during the previous survey. When this occurred, it was always resulting from a large rainstorm event. Despite the dry summer climate of the studied area, this pond has never dried up since it was dug in 1996 (ASF, unpublished data).

Biological data

Taxonomic diversity

The three artificial substrates were largely dominated by Oligochaetea, Cladocera and Chironomidae taxa. The total number of organisms on each substrate ranged from 351 to 1560 on the PL, 580 to 4058 on the BR and from 123 to 1176 on the pelagic. In the 2002 survey of Grans pond, about 80 % of the total organisms caught by the net sampling were cladoceran. Taxonomic richness ranged from 34 (BR), 35 (PL) and 40 (PE) taxa to 38 taxa in the previous Grans survey. When species composition among the different surveys was examined, several differences became apparent. *Lymnaea* was found solely during the 2002 GR survey and was never sampled by the artificial substrates. Coleoptera were often absent from all sampling methods, with the exception of the pelagic substrate after a colonization period of 21 days. Zygoptera were quick to colonize all artificial substrates and were found during the entirety of the experiment, while Anisoptera appeared only after 14 weeks of exposure on the BR and the PE. Chironomid taxa were apparently much synchronised, showing the same pattern of appearance/disappearance in all substrates. All substrates showed an increase in the number of taxa during the two first weeks, followed by stabilization or a decrease during the two final weeks (Tab. 2).

Table 2. Evolution of Jaccard's coefficient of similarity (S_j) of each substrate type (PL = plastic plant, BR = scrubbing brush, PE = pan scourers) between 7 days of exposure and all tested exposure lengths (14, 21 and 35 days). *Evolución del coeficiente de similitud de Jaccard* (S_j) *en cada tipo de sustrato (PL=plantas de plástico, BR=cepillo de barrer suelos, PE=estropajo de fibra limpia cacerolas) entre los 7 días y los diferentes periodos de exposición 14, 21 y 35 días).*

	S_j Coefficient		
	day 7 to 14	day 7 to 21	day 7 to 35
PL	0.75	0.64	0.59
BR	0.61	0.38	0.45
PE	0.63	0.58	0.56

Jaccard's coefficient of similarity

Jaccard's coefficient of similarity was used firstly within artificial substrates to assess community differences among replicates at each date. This coefficient, S_j, was always higher than 0.5, indicating a high similarity between each of the two compared samples (Mann-Whitney *U*-tests, $p > 0.05$ in all cases). These results confirmed that replicates of each substrate at each sampling date always showed similar species patterns. Then, by looking at these assemblages during the experiment period, we observed that, for each tested substrate, S_j decreased from day 7 to day 35 with a maximum decrease between day 14 and day 21 (Tab. 2) without reaching significance (Mann-Whitney U-test, $p > 0.05$). The taxa assemblages (coded 0 when a taxon was absent and 1 when present) of each substrate during all experiment period were compared to the taxa pool of this stormwater retention pond found during the previous survey (2002; $n = 15$; 5 dates × 3 replicates). Similarities among these communi-

Table 3. Evolution of Richness (*S*), Shannon's index (*H'*) and Evenness (*E*) of each substrate type (PL = plastic plant, BR = scrubbing brush, PE = pan scourers) in relation to exposure length. *Evolución de los índices: riqueza de especies (*S*), diversidad de Shannon (*H'*) y equitabilidad (*E*) para cada sustrato (PL, CR, PE como en la figura 2).*

Substrate	Day	Number of taxa (*S*)	Shannon's index (*H'*)	Evenness (*E*)
PL	7	24	2.612	0.57
	14	31	3.373	0.68
	21	27	2.801	0.59
	35	26	2.226	0.47
BR	7	22	1.163	0.26
	14	27	2.199	0.46
	21	17	0.557	0.14
	35	22	1.155	0.26
PE	7	20	1.608	0.37
	14	29	1.286	0.26
	21	29	1.441	0.30
	35	30	1.049	0.21

ties were found to be about 72 % between the net sampling and the pelagic substrate (PE) and greater than 81 % between the net sampling and each of the other two substrates (BR and PL).

Shannon diversity

The Shannon diversity index (*H'*) revealed great disparity between substrates (Tab. 3). During the colonization period *H'* varied from 2.23 to 3.37 on the PL substrate while it was lower for the other two substrates: 0.56 to 2.20 for the BR and 1.05 to 1.61 for the PE. As for the Shannon index, Evenness (*E*) was higher in the PL than in the latter two.

ANOVAs

The effects of substrate type and of colonization time on the abundance of the ten most abundant taxa were evaluated (Tab. 4). Substrate type had a significant effect on the abundance of seven of these taxa while length of exposure was only significant for Ostracoda ($p = 0.024$) and *Cloeon dipterum* ($p = 0.029$). The REGWQ post-hoc test ranked substrate types according to their principle effect. Oligochaetea ($p = 0.030$) and Cyclopoid ($p = 0.036$) appeared to be more abundant on the BR while Cladocera ($p = 0.016$), Ostracoda ($p = 0.003$), *Cloeon dipterum* ($p = 0.010$), Heteroptera ($p = 0.010$) and Hydracarina ($p = 0.010$) were more abundant on the pelagic substrate (PE). For all of these taxa, the PL substrate appeared to be the most indiscriminate (often ranked in last position in the REGWQ post-hoc test).

Five taxa (Oligochaetea, Cladocera, Heteroptera and Chironomidae) were examined more thoroughly at a lower taxonomic level with 2-way ANOVAs and REGWQ post-hoc tests (Tab. 5).

Table 4. Two-way ANOVAs (substrate type and exposure length) and REGWQ post-hoc test results for the densities of the most abundant taxa (PL = plastic plant, BR = scrubbing brush, PE = pan scourers). Statistical differences ($P < 0.05$) in the REGWQ test are ranked from highest to lowest (High-Low) density. *Resultados del ANOVA de dos factores (tipo de sustrato y tiempo de exposición) y RGWQ pruebas post-hoc para las densidades de los taxones más abundantes (PL, CR, PE como en la figura 2). Las diferencias estadísticas (*P *< 0.05) de las pruebas RGWQ están ordenadas de mayor a menor.*

	Substrate type (PL, BR, PE)		Exposure length (7, 14, 21, 35 days)	
main taxa	*F* value	REGWQ (High-Low)	*F* value	REGWQ (High-Low)
Oligochaetea	6.611*	BR – (PE, PL)	2.106	
Cladocera	8.944*	PE – (BR, PL)	2.001	
Ostracoda	18.940**	PE – (BR – PL)	6.708*	(14, 35) – (35, 21, 7)
Cyclopoidae	6.063*	BR – (PE – PL)	2.277	
Cloeon dipterum	10.906**	PE – (BR, PL)	6.137*	(35, 14) – (14, 21) – 7
Caenis	0.775		0.327	
Zygoptera	1.479		0.262	
Heteroptera	11.030**	PE – (PL, BR)	0.435	
Chironomidae	4.106		1.679	
Hydracarina	10.990**	PE – (PL, BR)	4.241	

* = $P < 0.05$; ** = $P < 0.01$

Table 5. Two-way ANOVAs (substrate type and exposure length) and REGWQ *post-hoc* test results for the densities of most significant taxa (PL = plastic plant, BR = scrubbing brush, PE = pan scourers). Statistical differences ($P < 0.05$) in the REGWQ *post-hoc* test are shown for the groups separated by hiphens and ranked from highest to lowest (High-Low) mean density. *Resultados del ANOVA de dos factores (tipo de sustrato y tiempo de exposición) y pruebas* post-hoc *RGWQ para las densidades de los taxones más significativos (PL, CR, PE como en la figura 2). Las diferencias estadísticas (*P *< 0.05) de las pruebas* post-hoc *RGWQ se muestran para los grupos separados por guiones y ordenados de mayor a menor densidad media.*

		Substrate type (PL, BR, PE)		Exposure length (7, 14, 21, 35 days)	
	taxa	*F* value	REGWQ (High-Low)	*F* value	REGWQ (High-Low)
Oligochaetae	*Chaetogaster*	11.436**	PE – (PL, BR)	7.057*	14 – (7, 35, 21)
	Lumbriculidae	5.227*	BR – (PL, PE)	1.076	
	Tubificidae	14.472**	BR – (PL, PE)	0.183	
Cladocera	*Chydorus sphaericus*	8.914*	PE – (BR, PL)	9.288*	(14, 7) – (35, 21)
	Pleuroxus aduncus	9.629*	PE – (PL, BR)	1.966	
Heteroptera	*Plea leachi*	6.897*	PE – (PL, BR)	0.886	
	Naucoris maculatus	26.180***	PE – (PL, BR)	9.189*	(21, 35) – (14, 7)
Chironomidae	Tanypodinae A	2.755		5.592*	(21, 35) – (14, 7)
	Tanypodinae B	0.884		13.474**	35 – (14, 21) – (21, 7)
	Orthocladiinae	9.836*	PE – (PL, BR)	4.018	
	Tanytarsini	2.681		7.167*	35 – (21, 14) – (14, 7)
	Chironomus	88.949***	BR – (PL, PE)	1.542	

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

The Oligochete *Chaetogaster* ($p = 0.009$) was highly selected by the pelagic substrate PE, having achieved a maximum abundance after 14 days of exposure ($p = 0.022$), while the Tubificidae ($p = 0.005$) and Lumbriculidae ($p = 0.048$) were preferentially found in the scrubbing brush, BR. Within the Cladocera, two genus, *Chydorus* ($p = 0.016$) and *Pleuroxus* ($p = 0.013$), were mostly found on the PE, particularly for *Chydorus* during the first 14 days of colonization ($p = 0.022$). Two true bug species were selected by the PE substrate, *Plea leachi* ($p = 0.028$) and *Naucoris maculatus* ($p = 0.001$) which were more abundant during the last two weeks of exposure ($p = 0.012$). Finally, the Chironomidae were also shown to sometimes be selected for, firstly *Chironomus* were found to be highly selected by the BR ($p < 0.001$) and also Orthocladiinae ($p = 0.013$) by the PE. Tanypodinae were separated into two types according to the aspect of their head capsule i.e., long (type A) or short (type B). Tanypodinae type B ($p = 0.004$), type A ($P = 0.036$) and Tanitarsini ($p = 0.021$) were mostly present on all substrates by the end of the experiment (day 35).

Colonization time

Only 14 days of exposure were required in order to account for 90 % of total taxa caught on the two benthic substrates, while 21 days were necessary for the pelagic substrate (Fig. 3). Moreover,

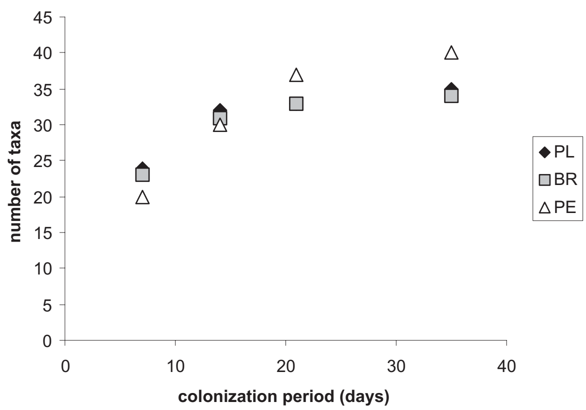

Figure 3. Cumulative curve of substrate taxa richness in relation to exposure length. Total number of unique taxa is shown. PL refers to plant substrate, BR to scrubbing brush and PE to pan scourer. *Curva acumulada de la riqueza de especies en cada sustrato en relación con el tiempo de exposición. PL, BR y PE como en la figura 2.*

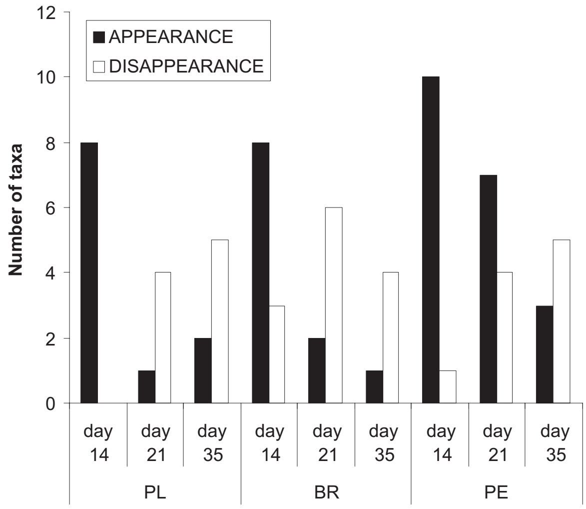

Figure 4. Histogram presenting taxa appearance and disappearance on each substrate in relation to exposure length. Appearance refers to each new taxa found after each exposure period: 7 to 14 days, 14 to 21 and 21 to 35, while disappearance refers to each lost taxa after each exposure period. PL refers to plant substrate, BR to scrubbing brush and PE to pan scourer. *Histogramas mostrando la aparición y desaparición de taxones en cada sustrato en relación con el tiempo de exposición. Aparición se refiere al número de taxones nuevos encontrados después de cada tiempo de exposición: desde 7 a 14 días , desde 14 a 21 y desde 21 a 35 y desaparición se refiere al número de taxones perdidos durante dichos periodos. PL, BR y PE como en la figura 2.*

many new species had appeared on the substrates after 14 days of exposure while many subsequently disappeared after 21 and 35 days (Fig. 4).

DISCUSSION

The three artificial substrates (two benthic and one pelagic) tested for five weeks in a motorway retention pond in southern France showed contrasting results in terms of invertebrate sampling efficiency and representativeness of the whole invertebrate community.

As reviewed by Rosenberg & Resh (1982), most substrates appear to be selective for different aquatic invertebrates. In the current experiment, organism selectivity was noticed in two of the three tested substrates. The PE was highly selective for *Chaetogaster* which is described as a swimmer and a predatory Oligochete (Tachet *et al.*, 2002). This substrate was also preferentially chosen by two Cladocerans, *Chydorus sphaericus* and *Pleuroxus aduncus*, this last species mainly found on the floating leaves of hydrophytes (Amoros, 1984). *Plea leachi* and *Naucoris maculatus*, two predators, were more commonly found on the PE, as was also the case for the Orthocladiinae subfamily mainly composed of microphyte grazers. The complex structure of the pan scourers to which were fastened numerous egg-clutches, probably laid by heteropterans, offers a large attachment surface to invertebrates and microphytes. The BR benthic substrate, made of a scrubbing brush, was principally selective for oligochetes such as Lumbriculidae and Tubificidae. This last taxa feed on bacteria which decompose organic matter (Tachet *et al.*, 2002). The BR also contained numerous individuals of *Chironomus*, a key genus in eutrophic or disturbed environments (Johnson *et al.*, 2001, Péry *et al.*, 2003). This substrate, located on the ground, was rapidly enveloped by organic matter and filamentous algae. Such material supplies a great source of food for scraper species and additionally creates abundant spaces in which organisms can hide. The last substrate, the PL, mock aquatic plants made from plastic, appeared to be very indiscriminate with no particular selectivity for any taxa making it potentially a good candidate for long term monitoring. This was also confirmed by diversity and evenness indices, always higher for the PL substrate compared to the two other ones. However, molluscs were absent from all artificial substrates despite occurring in net samples. Such a discrepancy could be explained by the time necessary for molluscs to colonize the substrates (particularly in the case of Bivalves such as *Sphaerium*) and by their possible loss during the retrieval of the samplers, due to their lack of an adaptation for "clinging", which has also been noticed for crustaceans (Rabeni & Gibbs, 1978).

The efficiency of artificial substrates to collect macroinvertebrates is also dependent on the length of the colonization period (Rosenberg & Resh, 1982). Indeed De Pauw *et al.* (1986) recommended a 3 week exposure period due to their observation that some species only colonized substrates after 2-3 weeks while others tended to disappear after 4 weeks which is consistent with our observations. The pelagic substrate, PE, had the biggest turnover in species composition. This may be explained by its suspension in the water column which favours its colonization by very mobile organisms such as Coleoptera and Heteroptera. Only active swimmers could easily reach such a pelagic device. These species also explain the difference in richness observed between this last substrate and the net sampling where three coleopterans species were lacking. In all substrates, even though the species composition tended to stabilise after 35 days of exposure, equilibrium did not appear to have been reached as is often observed in such experiments (De Pauw *et al.*, 1986; Peckarsky, 1986). Conversely, colonization was shown to be mainly under the influence of drift processes in lotic systems (Mackay, 1992; Boothroyd & Dickie, 1991; Mihaljevic *et al.*, 1998). This highlights the importance of the artificial substrate type on colonization in lentic systems.

This pilot study aimed at designing and evaluating an artificial substrates based methodology that could be useful in long term monitoring of pond ecosystems. The need for simple and practical monitoring methods has increased these last years (following the Water Framework Directive recommendations), creating room for investigation by scientists. Our previous results suggest that artificial substrates have real potential for pond invertebrate monitoring: they are (1) usable by non specialists, (2) are useful in engineered or disturbed environments, (3) have a low impact on ecosystem since only artificial substrates are collected and (4) are standardised.

However, even if the artificial plant substrate, PL, seems to fulfil monitoring expectations in our pilot experiment, it is now necessary to test this substrate (1) during different seasons in order to take into account species with different life cycles, (2) to design a multi-site protocol in order to evaluate its robustness in various ecosystems and (3) to compare it with hand-net sampling on the basis of time and effort required to assess the same invertebrate community.

Finally, such methods are a fruitful avenue of investigation in the future, particularly for environmental hazard assessment in aquatic habitats.

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REFERENCES

ADDINSOFT. 2003. XLSTAT© version 6.1.9. http://www.xlstat.com.

AMOROS, C. 1984. Crustacés cladocères. *Bull. Mens. Soc. Linn. Lyon*, 53: 1-63.

ATILLA, N., M. A.WETZEL & J. W. FLEEGER. 2003. Abundance and colonization potential of artificial hard substrate-associated meiofauna. *J. Exp. Mar. Biol. Ecol.*, 287: 273-287.

BENOÎT, H. P., J. R. POST, E. A. PARKINSON & N. T. JOHNSTON. 1998. Colonization by lentic macroinvertebrates: evaluating colonizaion processes using artificial substrates and appraising applicability to the technique. *Can. J. Fish. Aquat. Sci.*, 55: 2425-2435.

BISHOP, C. A., J. STRUGER, D. R. BARTON, L. J. SHIROSE, L. DUNN, A. L. LANG & D. SHEPERD. 2000a. Contamination and Wildlife communities in stormwater retention ponds in Guelph and the greater Toronto area, Ontario, 1997 and 1998. Part I-Wildlife communities. *Water Qual. Res. J. Can.*, 35: 399-435.

BISHOP, C. A., J. STRUGER, D. R. BARTON, L. J. SHIROSE, L. DUNN, A. L. LANG & D. SHEPERD. 2000b. Contamination and Wildlife communities in stormwater retention ponds in Guelph and the greater Toronto area, Ontario, 1997 and 1998. Part II-Contamination and biological effects of contamination. *Water Qual. Res. J. Can.*, 35: 437-474.

BLONDEL, J. & J. ARONSON. 1999. *Biology and Widlife of the Mediterranean Region*. Oxford: Oxford University Press. 328 pp.

BOOTHROYD, I. K. G. & B. N. DICKIE. 1991. Macroinvertebrate drift and community colonization on perpex artificial substrates in the Ohinemuri River, New-Zealand. *N. Z. J. Mar. Freshwat. Res.*, 25: 167-176.

BUIKEMA, A. L. & J. R. VOSHELL. 2001. Toxicity studies using freshwater benthic macroinvertebrates. In: *Freshwater biomonitoring and benthic macroinvertebrates*. Rosenberg, D.M. & V.H. Resh (eds): 344-398. Kluwer Academic Publishers, Norwell, USA.

CAIRNS, J. 1982. *Artificial substrates.* Ann Arbor: Science Publishers. 279 pp.

CLARKE, K. D., R. KNOECHEL & P. M. RYAN. 1997. Influence of trophic role and life-cycle duration on timing and magnitude of benthic macroinvert response to whole-lake enrichment. *Can. J. Fish. Aquat. Sci.*, 54: 89-95.

DE PAUW, N., D. ROELS & A. P. FONTOURA. 1986. Use of artificial substrates for standardized sampling of macroinvertebrates in the assessment of water-quality by the Belgian Biotic Index. *Hydrobiologia*, 133: 237-258.

FRANCE, R. L. 1997. Macroinvertebrate colonization of woody debris in canadian shield lakes following riparian clearcutting. *Conserv. Biol.*, 11: 513-521.

FRANCIS, D. R. & T. C. KANE. 1995. Effect of substrate on colonization of experimental ponds by Chironomidae (Diptera). *J. Freshwat. Ecol.*, 10: 57-63.

JEFFRIES, M. 1993. Invertebrate colonization of artificial pondweeds of differing fractal dimension. *Oikos*, 67: 142-148.

JOHNSON, R. K., T. WIEDERHOLM & D. M. ROSENBERG. 2001. Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. In: *Freshwater biomonitoring and benthic macroinvertebrates*. D. M. Rosenberg & V. H. Resh (eds.): 40-158. Kluwer Academic Publishers, Norwell, USA.

LINCOLN, R., G. BOXSHALL & P. CLARK. 1998. *A dictionary of Ecology, Evolution and Systematics.* Cambridge: Cambridge University Press. 361 pp.

MACKAY, R. J. 1992. Colonization by lotic macroinvertebrates – a review of processes and patterns. *Can. J. Fish. Aquat. Sci.*, 49: 617-628.

MIHALJEVIC, Z., M. KEROVEC, V. TAVCAR, & I. BUKVIC. 1998. Macroinvertebrate community on an artificial substrate in the Sava River: long-term changes in the community structure and water quality. *Biologia*, 53: 611-620.

MUZAFFAR, S. B. & M. H. COLBO. 2002. The effects of sampling technique on the ecological characterization of shallow, benthic macroinvertebrate communities in two Newfoundland ponds. *Hydrobiologia*, 477: 31-39.

OERTLI, B., AUDERSET JOYE, D., CASTELLA, E., JUGE, R., LEHMANN, A. & J.-B. LACHAVANNE. PLOCH : A standardized method for sampling and assessing the biodiversity in ponds. *Aquatic Conserv.: Mar. Freshw. Ecosyst.*, 15: 665-679.

PECKARSKY, B. L. 1986. Colonization of natural substrates by stream benthos. *Can. J. Fish. Aquat. Sci.*, 43: 700-709.

PÉRY, A. R. R., V. SULMON, R. MONS, P. FLAMMARION, L. LAGADIC & J. GARRIC. 2003. A model to understand the confounding effects of natural sediments in toxicity tests with *Chironomus riparius*. *Environ. Toxicol. Chem.*, 22: 2476-2481.

PONT, D. 1983. *Recherches quantitatives sur le peuplement de copépodes, cladocères et ostracodes des rizières de Camargue*. PhD Thesis. Université d'Aix-Marseille I. 161 pp.

POPE, R. J., A. M. GORDON & N. K. KAUSHIK. 1999. Leaf litter colonization by invertebrates in the littoral zone of a small oligotrophic lake. *Hydrobiologia*, 392: 99-112.

RABENI, C. F. & K. E. GIBBS. 1978. Comparison of two methods used by divers for sampling benthic invertebrates in deep rivers. *J. Fish. Res. Board Can.*, 35: 332-336.

ROSENBERG, D. M. & V. H. RESH. 1982. The use of artificial substrates in the study of freshwater benthic macroinvertebrates. In: *Artificial Substrates*. J. Cairns (eds.): 175-235. Ann Arbor Science Publishers, Ann Arbor, USA.

SCHER, O. 2005. *Les bassins d'eau pluviale autoroutiers en région Méditerranéenne: Fonctionnement et Biodiversité. Evaluation de l'impact de la pollution routière sur les communautés animales aquatiques*. PhD Thesis, Université d'Aix-Marseille 1, France. http://tel.archives-ouvertes.fr/tel-00109123/fr/

SCHER, O. & A. THIÉRY. 2005. Odonata, Amphibia and environmental characteristics in motorway stormwater retention (Southern France). *Hydrobiologia*, 551: 237-251.

SCHMUDE, K. L., M. J. JENNINGS, K. J. OTIS & R. R. PIETTE. 1998. Effects of habitat complexity on macroinvertebrate colonization of artificial substrates in north temperate lakes. *J. N. Am. Benthol. Soc.*, 17: 73-80.

SMITH, S. D. A. & M. J. RULE. 2002. Artificial substrata in a shallow sublittoral habitat: do they adequately represent natural habitats or the local species pool? *J. Exp. Mar. Biol. Ecol.*, 277: 25-41.

TACHET, H., P. RICHOUX, M. BOURNAUD & P. USSEGLIO-POLATERA. 2002. *Invertébrés d'eau douce*. Paris : CNRS Editions. 588 pp.

THORP, J. H., E. M. MCEWAN, M. F. FLYNN & F. R. HAUER. 1985. Invertebrate colonization of submerged wood in a cypress-tupulo swamp and blackwater stream. *Am. Midland Nat.*, 113: 56-68.

WREN, C. D., C. A. BISHOP, D. L. STEWART & G. C. BARRETT. 1997. *Wildlife and contaminants in constructed wetlands and stormwater ponds: current state of knowledge and protocols for monitoring levels and effects in wildlife*. Canadian Wildlife Service, Ontario region. 106 pp.