Recovery of the macroinvertebrate community below a wastewater treatment plant input in a Mediterranean stream

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Abstract

We sampled chlorophyll *a*, benthic organic matter, and benthic macroinvertebrates in June 2001 in La Tordera stream (Catalonia, NE Spain), receiving a wastewater treatment plant (WWTP) input. Samples were collected in six equidistant transects in three reaches located upstream (UP), few m below (DW1), and 500 m below the WWTP input (DW2). Our first objective was to assess the effects of the point source on the structure and functional organization of the benthic macroinvertebrate community. Our second objective was to determine if the self-purifying capacity of the stream implied differences between the communities of the DW1 and the DW2 reaches. The WWTP input highly increased discharge, nutrient concentrations, and conductivity and decreased dissolved oxygen. At the DW1 and the DW2 reaches, taxa richness, EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera), and Shannon diversity decreased and gatherer relative density increased relative to the UP reach. At the UP reach, CPOM and FPOM standing crops were similar, whereas at the DW1 and the DW2 reaches CPOM was two times higher than FPOM. Detailed analysis showed that major changes in the benthic community occurred abruptly between 80 and 90 m downstream of the point source (middle of the DW1 reach). At this location, chlorophyll *a* concentration, density of macroinvertebrates, taxa richness, and scraper relative density increased, whereas gatherer relative percentage decreased. The macroinvertebrate community at the DW2 reach was comparable to that at the second middle of the DW1 reach (DW1B). The macroinvertebrate community at the DW1B and the DW2 reaches were quite similar to that at the UP reach, indicating that the recovery capacity of the stream from nutrient enrichment was high.

Introduction

Domestic sewage effluents represent one of the most common causes of degradation of water quality in stream ecosystems (Paul & Meyer, 2001). The effects of point sources attain special relevance in Mediterranean ecosystems where water is scarce. The actual Framework Directive of the European Community highlighted the need of considering biologic quality to provide information for the efficient and effective design of future monitoring programs (Council of the European Communities, 2000).

Karr & Dudley (1981) emphasized the need of considering biotic integrity in assessments of aquatic ecosystems, including taxa composition, diversity, and functional organization of living organisms. Using their approach will provide a broader understanding of the processes going on in altered streams. However, it is not clear what patterns should be expected below wastewater treatment plant (WWTP) effluents. Previous studies reported changes in taxa composition that implied a decrease in taxa richness and an increase in dominance because sensitive taxa were eliminated and resistant taxa were enhanced

(e.g., Hynes, 1978; Lenat & Crawford, 1994). Several studies found a decrease in total density of macroinvertebrates with increasing nutrient concentrations (Garie & McIntosh, 1986; Prenda & Gallardo-Mayenco, 1996), while others reported no changes (Jones & Clark, 1987; Roy et al., 2003) or even an increase (Hynes, 1978; Miltner & Rankin, 1998). Kerans & Karr (1994) hypothesized that human impact will also affect the relative percentage of functional feeding groups. They predicted that the percentage of shredders, scrapers, and predators would be diminished while the percentage of gatherers and filterers increased. Few studies considered functional organization in their assessments and their results are not consistent (Delong & Brunsen, 1998; Shieh et al., 1999a). Even less is known about the effects of point sources on standing crops of benthic organic matter (BOM) although it is a source of food and influences structure and function of streams (Hawkins & Sedell, 1981).

We examined the response of the macroinvertebrate community to a WWTP input in a Mediterranean stream in June 2001. We performed the study in summer, when the dilution capacity of Mediterranean streams is lowest and discharge of point sources has higher adverse effects (Gasith & Resh, 1999). We sampled benthic macroinvertebrates, periphyton chlorophyll *a*, and BOM at one reach located upstream of the WWTP input and two reaches located 60 and 500 m downstream. Our first objective was to assess the effects of the point source on the structure and functional organization of the benthic macroinvertebrate community. Our second objective was to determine if the self-purifying capacity of the stream implied differences between the community located few meters below the WWTP input and the community located 500 m downstream.

Materials and methods

Study site

The study was conducted in La Tordera stream in Catalonia (NE Spain; Fig. 1). At the sampling site (41° 41′ N, 2° 27′ E, 200 m a.s.l.), La Tordera is a 3rd-order stream draining a catchment of 80 km^2 dominated by a sclerophyllous forest of several species of *Quercus*. Small patches of irrigated crops are present in the lower part of the catchment, surrounding the urban area. The geology of the catchment is mainly siliceous and dominated by slates and fillites. The climate is typically humid Mediterranean, with mean air temperatures from 6 °C in January to 23 °C in August, and mean annual precipitation of 1071 mm, mostly occurring in spring and fall. Stream discharge is highly variable within a hydrologic year and among
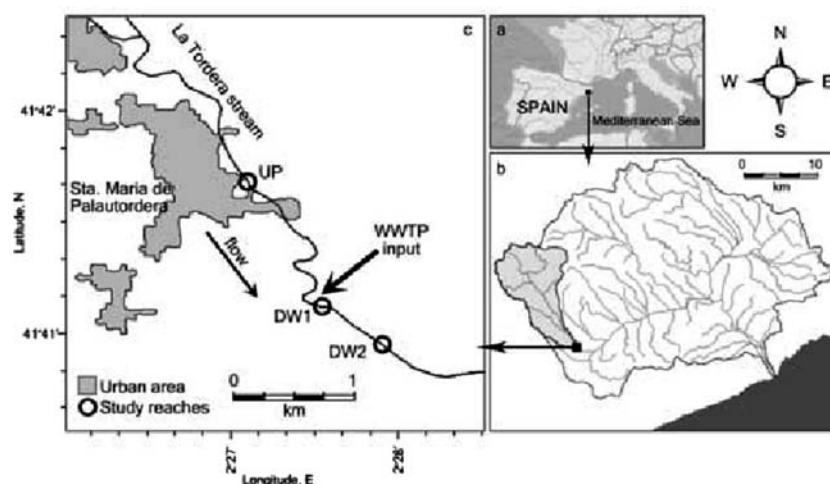

Figure 1. (a) Location of La Tordera stream in Catalonia, NE Spain, (b) La Tordera catchment and the subcatchment affecting the sampling site, highlighted in grey, and (c) location of the study reaches (UP, DW1 and DW2), in relation to the waste water treatment plant (WWTP) input.

years. Droughts are common from July to September in some sections of the stream and peak flows usually occur during spring and fall.

The upstream reach (UP) was located 1 km upstream of the WWTP. The second reach (DW1), started 60 m downstream of the WWTP input. The third reach (DW2), was located 500 m downstream of the WWTP input. The UP and the DW2 reaches were 100 m long, whereas the DW1 reach was 50 m long to avoid potential gradients. All study sites were run-riffle reaches with a slope around 1% and heterogeneous substrate dominated by cobbles, pebbles, and boulders. Mean air temperature in June 2001 was 21.6 °C and ranged from 10.1 to 35.7 °C. The accumulated rainfall in June 2001 was 20 mm and was preceded by 3 years of low precipitation. Any tributary joints the stream between the DW1 and the DW2 reaches and there are not diffuse sources under dry conditions (Merseburger et al., in press). We divided the DW1 reach into two subreaches (DW1A and DW1B) according to further analyses of macroinvertebrates, and periphyton chlorophyll *a*.

Physical and chemical parameters

We measured water velocity using a Neurtek Instruments® Miniair 2 flow meter, depth and width in six equidistant transects in the UP and the DW2 reaches. Discharge was calculated according to the velocity–area method described in (Gordon et al., 1992). We collected three water samples in six equidistant transects in the UP and DW2 reaches (18 samples per reach). Samples were filtered *in situ* through preashed Whatman® GF/F glass fiber filters and stored on ice. Ammonium (NH_4^+–N) concentration was analyzed on a Bran-Luebbe® Technicon Autoanalyzer II. Nitrate (NO_3^-–N) and soluble reactive phosphorus (SRP) concentrations were analyzed on a Bran-Luebbe® TRAACS 2000 Autoanalyzer, in the case of nitrate using the cadmium–copper reduction method, and in the case of SRP, using the molybdenum blue colorimetric method. Dissolved organic carbon (DOC) concentration was analyzed using a high-temperature catalytic oxidation (Shimadzu® TOC 5000 analyzer). Conductivity, dissolved oxygen (DO), pH, and water temperature were measured at hourly intervals during daytime and at 3-h intervals during nighttime over a 24-h period using a WTW® LF 340, WTW® Oxi 340-A, and a WTW® pH 340-A.

Periphyton chlorophyll a, primary producers, and benthic organic matter

We collected three samples of periphyton in six equidistant transects in each reach (18 samples per reach) from a known area of substrate. Samples were frozen for later analysis of chlorophyll *a* content in the laboratory. We determined chlorophyll *a* concentrations by spectrophotometry following extraction in 90% acetone according to Steinman & Lamberti (1996).

Filamentous algae, mosses, and vascular plants were taken from the samples by handpicking after invertebrate removal. Remaining BOM was separated into fine particulate organic matter (FPOM; 250 μm–1 mm) and coarse particulate organic matter (CPOM; > 1 mm) by sieving. Filamentous algae, mosses, vascular plants, FPOM, and CPOM, were dried at 60 °C until constant weight, weighed, ashed at 450 °C for 4.5 h and reweighed to obtain ash-free dry mass (AFDM).

Benthic macroinvertebrates

We collected two modified Surber samples (0.0625 m², 250 μm mesh size) in six equidistant transects in each reach (12 samples per reach). Samples were preserved in the field with 4% formaldehyde solution. Heavier inorganic substrates were removed by elutriation. All individuals bigger than 5 mm were removed by handpicking from the samples. Subsampling were done on an area basis if the remaining number of individuals was higher than 200 (Moulton II et al., 2000 ; Doisy & Rabeni, 2001). Small invertebrates were removed by handpicking with the aid of a dissecting microscope at 15× magnification. All invertebrates were identified up to the lowest practical taxonomic level. Relative contributions of taxa to each functional feeding group were assigned according to Tachet et al. (2000). Where taxa felt into two or more functional feeding groups, the abundance was proportionally apportioned to each group.

Data analysis

Total macroinvertebrate density, chlorophyll *a*, and standing crop of BOM and vascular plants were compared among reaches using one-way analysis of variance (ANOVA) procedures. Pairwise comparisons among group means were made using Tukey's studentized range test (HSD). Analysis of variance was done by using the statistical package SPSS (for Windows, version 11.0.1, SPSS Inc., Chicago, Illinois). The macroinvertebrate community parameters included density (individuals/m^2), taxa richness, EPT number (Lenat, 1983; Barbour et al., 1999), and Shannon diversity. Initial analysis using detrended correspondence analysis (DCA) showed that the macroinvertebrate data set had a gradient length shorter than two standard deviation units. Hence, we used linear models for further analysis as recommended by ter Braak & Šmilauer (1998). We carried out principal components analysis (PCA) on benthic macroinvertebrate data in order to ascertain the relative similarity among the three reaches. Ordination analyses (DCA and PCA) were carried out by using the CANOCO (for Windows, version 4.5, Centre for Biometry Wageningen, Wageningen, The Netherlands). Taxa present in only one sample were excluded from the analyses and macroinvertebrate densities were $\log_{10} (x + 1)$ transformed to stabilize variances and normalize the data sets.

Results

Physical and chemical parameters

Discharge and water velocity at the UP reach were much lower than at the DW2 reach (Table 1). Conductivity was three times higher at the DW2 reach than at the UP reach. At the DW2 reach, mean DO concentration was two times lower than at the UP reach and attained night values of 3 mg/l. The pH values at the reaches UP and DW2 were close to 7 and did not show a high daily range. The WWTP input largely increased concentrations of all nutrients. Nitrate and DOC concentrations increased 5 times below the WWTP input, while ammonium and SRP concentrations increased 50 and 60 times respectively.

Table 1. Mean values ± SE of discharge, water velocity, water temperature and chemical parameters at the UP and DW2 reaches

	UP	DW2
Q (l/s)	1.9 ± 0.4	54.4 ± 3.6
v (m/s)	0.01 ± 0.00	0.10 ± 0.01
T (°C)	19.8 ± 0.7	20.0 ± 0.4
	(13.5–26.6)	(16.1–23.3)
cond. (μS/cm)	175 ± 7	579 ± 5
DO (mg/l)	8.70 ± 0.23	4.43 ± 0.15
	(6.01–10.63)	(3.17–6.12)
pH	7.13 ± 0.06	7.06 ± 0.01
SRP (mg P/l)	0.04 ± 0.00	2.55 ± 0.05
NO_3^-–N (mg N/l)	0.54 ± 0.01	2.47 ± 0.05
NH_4^+–N (mg N/l)	0.06 ± 0.01	3.11 ± 0.04
DOC (mg/l)	1.73 ± 0.11	9.38 ± 0.15

Q = discharge, v = water velocity, T = water temperature, cond. = conductivity, DO = dissolved oxygen. Values in parenthesis indicate the daily range of temperature and dissolved oxygen.

Periphyton chlorophyll a, primary producers, and benthic organic matter

Mean chlorophyll *a* concentration was not significantly different among the three reaches in La Tordera stream in June 2001 (ANOVA, $p = 0.929$; Table 2). Standing crop of filamentous algae was greater than that of moss and vascular plants at the UP reach. Standing crops of filamentous algae, mainly *Cladophora glomerata*, were significantly different among reaches (ANOVA, $p = 0.048$) and, at 90% of confidence, were higher at DW2 reach than at the DW1 reach (HSD, $p = 0.051$). Biomass of the moss *Amblystegium riparium* was significantly lower at the UP reach than at the DW1 reach (HSD, $p = 0.035$). The DW2 reach had higher biomass of vascular plants (mainly *Apium nodiflorum* and *Callitriche* sp.) than the UP reach (HSD, $p = 0.015$).

Mean standing crop of CPOM was significantly higher at the DW1 reach than at the UP reach (HSD, $p = 0.017$; Table 2). In contrast, mean standing crop of FPOM did not differ significantly among reaches (ANOVA, $p = 0.109$). Standing crop of FPOM was similar to that of CPOM at the UP reach, but was only half at the DW1 and DW2 reaches.

Table 2. Mean values $\pm$ SE of chlorophyll *a* (Chl *a*; $n = 18$), filamentous algae, moss, vascular plants, CPOM, FPOM, and total macroinvertebrate density ($n = 12$) at the UP, DW1 and DW2 reaches

	UP	DW1	DW2
Chl *a* ($\mu g/cm^2$)	8.6 $\pm$ 2.07 (a)	13.59 $\pm$ 4.64 (a)	13.91 $\pm$ 4.99 (a)
Filamentous (g AFDM/m^2)	2.34 $\pm$ 1.3 (a)	1.89 $\pm$ 1.21 (a)	4.26 $\pm$ 1.04 (b[1])
Moss (g AFDM/m^2)	0.01 $\pm$ 0.01 (a)	1.77 $\pm$ 0.85 (b)	1.03 $\pm$ 0.35 (ab)
Vascular plants (g AFDM/m^2)	0.22 $\pm$ 0.18 (a)	5.76 $\pm$ 3.08 (ab)	17.99 $\pm$ 5.68 (b)
CPOM (g AFDM/m^2)	4.87 $\pm$ 1.18 (a)	13.61 $\pm$ 2.69 (b)	6.09 $\pm$ 1.69 (ab)
FPOM (g AFDM/m^2)	4.14 $\pm$ 1.12 (a)	6.10 $\pm$ 1.20 (a)	3.16 $\pm$ 0.61 (a)
Macroinvertebrate density (ind/m^2)	61641 $\pm$ 9405 (a)	167555 $\pm$ 36795 (b)	199835 $\pm$ 46688 (b)

Reaches sharing the same letter are not significantly different ($p > 0.05$) according to Tukey's multiple comparisons. Data were log ($x + 1$) transformed prior to analysis.[1]Mean biomass of filamentous algae was significantly different among the three reaches at $p = 0.05$ and significantly higher at the DW2 reach at $p < 0.10$.

Benthic macroinvertebrates

Sixty taxa were identified from the three sampled reaches. Thirteen macroinvertebrate taxa were recorded only at the UP reach (Appendix 1). Total macroinvertebrate density was significantly lower at the UP reach than at the reaches below the WWTP input (HSD, $p < 0.021$; Table 2). All samples downstream of the point source were dominated by the chironomid subfamilies Chironominae and Orthocladiinae, and the Oligochaete family Naididae. Tubificids were abundant at the DW1 reach as well. Most samples of the UP reach were dominated by cladocerans, Orthocladiinae, and Chironominae. Total number of taxa was higher at the UP reach mainly because of a higher number of EPT taxa (Table 3). The 2 reaches below the WWTP input had similar taxa richness and EPT richness. Shannon diversity was much higher at the UP reach than at the downstream reaches (Table 3).

The contributions of macroinvertebrate functional feeding groups to overall community differed among the three reaches (Fig. 2). Shredders (*Mystacides azurea*, *Elmis* sp.) were scarce at the UP and the DW2 reaches (0.09 and 0.005%, respectively) and absent at the DW1 reach. Scraper relative abundance was quite similar among the reaches and was dominated by Orthocladiinae and *Ancylus fluviatilis*. At the reaches below the WWTP input, macroinvertebrate functional group relative abundance was comprised primarily of gatherers (Chironomidae, Oligochaeta). Relative abundance of filterers

Table 3. Taxa richness, EPT richness and Shannon diversity at the UP, DW1 and DW2 reaches

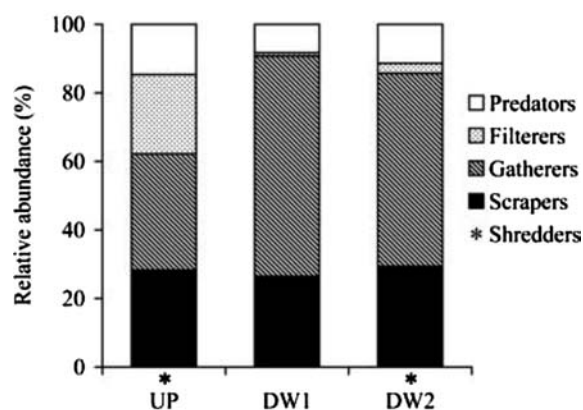|	UP	DW1	DW2
Taxa richness	52	41	39
EPT richness	16	8	8
Shannon diversity	3.64	2.97	2.67

Figure 2. Relative abundance of functional feeding groups for the study reaches. The presence of shredders is highlighted with an asterisk because of very low abundances. The DW1 reach was lacking in shredders.

(Cladocera) was low at the reaches below the WWTP input but contributed 23% of the functional group abundance at the UP reach. Abundance of predators was dominated by parasitic digeneans at the UP and DW2 reaches, whereas the reach DW1 was dominated by Tanypodinae and nematodes.

Ordination analyses

The first three axes of the PCA performed on macroinvertebrate densities for 48 taxa and 36 samples explained 54% of the variance (axis 1 = 31%; axis 2 = 12%; axis 3 = 11%). Axis 1 was positively associated with the snails *Lymnaea* sp. and *Radix* sp., the stonefly *Leuctra geniculata*, the mayfly *Caenis luctuosa*, the caddisflies *Mystacides azurea* and *Lepidostoma hirtum*, and Cladocera (Fig. 3a). Axis 1 was also negatively associated with the leeches *Erpobdella* sp. and *Helobdella stagnalis*, the oligochaetes Tubificidae and Naididae, the mayflies *Baetis rhodani* and *B. fuscatus*, Nematoda and the chironomid subfamilies Orthocladiinae and Chironominae. Axis 2 was best
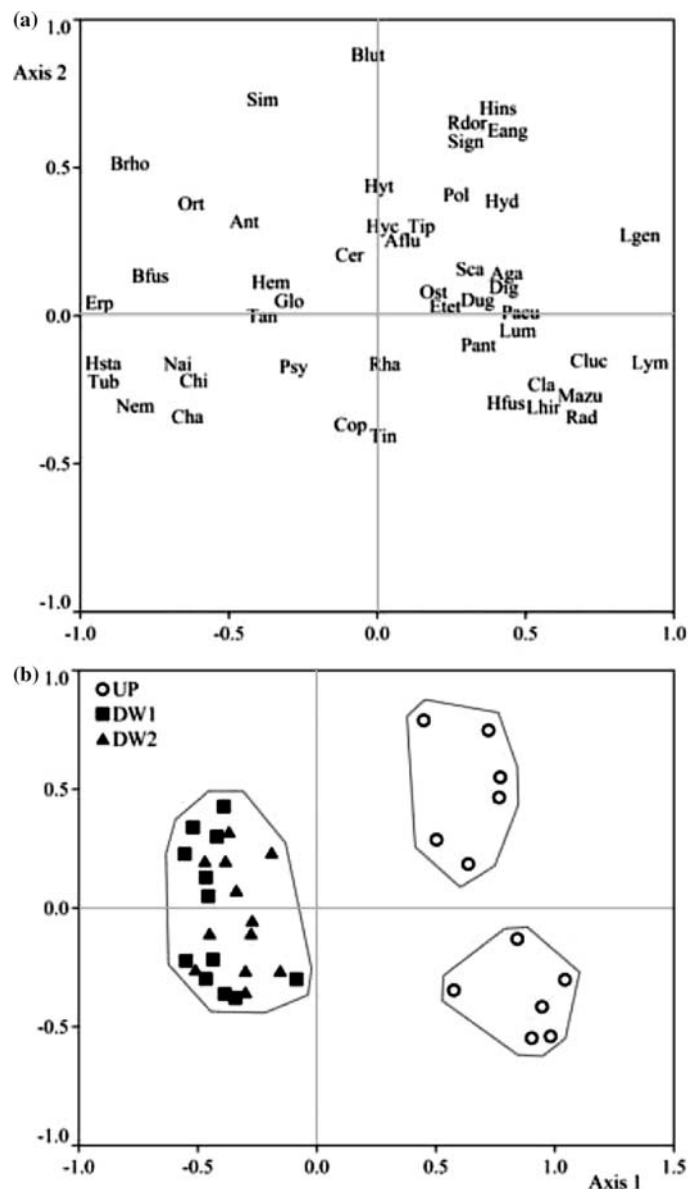

Figure 3. Axis 1 and 2 of the principal components analysis (PCA) performed for 48 taxa collected in the 3 reaches (UP, DW1 and DW2) in June 2001. (a) Ordination plot for taxa relationships based on $\log_{10} (x + 1)$ transformed densities (ind/m^2). (b) Ordination plot for samples of the three reaches. See Appendix 1 for taxon codes.

explained by a positive relationship with *B. lutheri*, Simuliidae, *Hydropsyche instabilis*, *Rhyacophyla dorsalis*, *Ecdyonurus angelieri*, and *Serratella ignita*. Axis 3 was best explained by a negative relationship with microcrustaceans (Ostracoda, Copepoda, Cladocera) and Digenea (Fig. 4a). In the site plot, axis 1 separated the samples according

to their location with respect to the WWTP input (Fig. 3b). Axis 2 scattered the samples based on microhabitat characteristics. At the UP reach, samples were separated into 2 clusters according to their taxa composition. At the top were located macroinvertebrates that prefer large substrata sizes and moderate water velocities (*Hydropsyche*
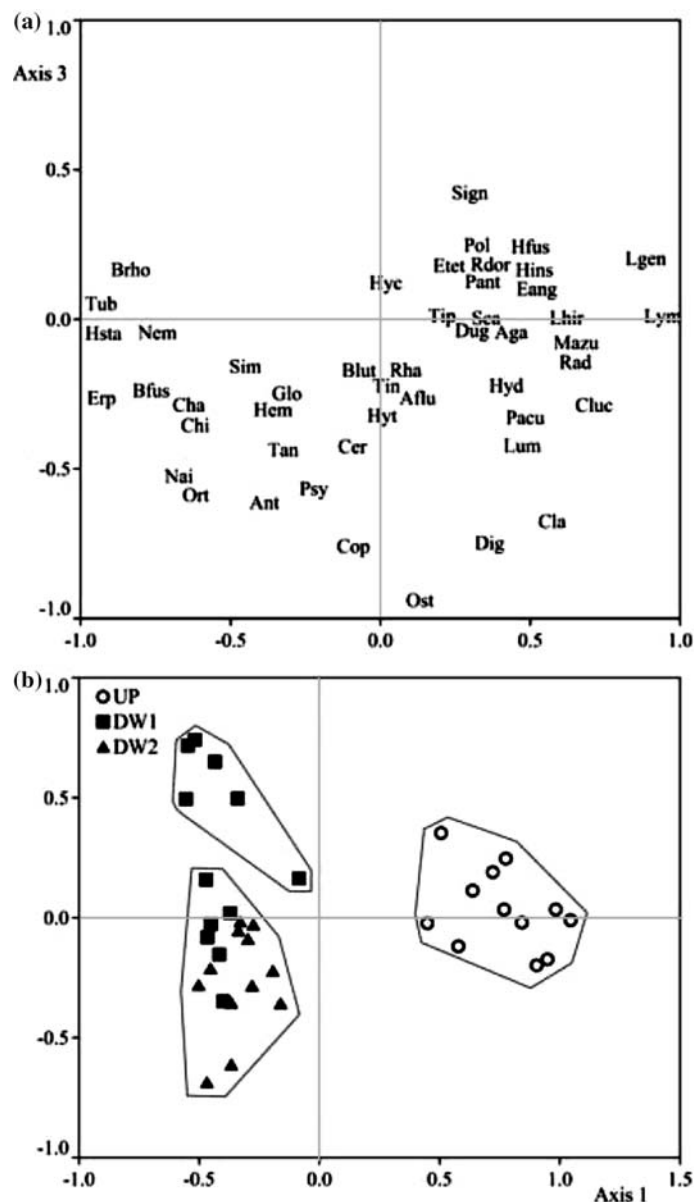

Figure 4. Axis 1 and 3 of the principal components analysis (PCA) performed for 48 taxa collected in the 3 reaches (UP, DW1 and DW2) in June 2001. (a) Ordination plot for taxa relationships based on $\log_{10} (x + 1)$ transformed densities (ind/m^2). (b) Ordination plot for samples of the three reaches. See Appendix 1 for taxon codes.

instabilis, Rhyacophila dorsalis, Ecdyonurus angelieri). At the bottom were located taxa with preference to low water velocities, vascular plants and BOM (*Radix* sp., *Lepidostoma hirtum, Mystacides azurea, Cladocera*). Axis 3 split the samples of the DW1A subreach from the samples of the DW1B and DW2 reaches (Fig. 4b).

Gradient below the WWTP input

Detailed analysis of the DW1 reach revealed a strong gradient occurring within this reach. We divided this reach into two reaches according to chlorophyll *a* concentrations and macroinvertebrate structure and functional organization of each transect of the reach. We named DW1A subreach the first three transects of the DW1 reach

(from 60 to 80 m below the WWTP input) and DW1B subreach the last three transects (from 90 to 110 m below the WWTP input).

Chlorophyll *a* was 20 times higher at the DW1B subreach that at the DW1A subreach (HSD, $p = 0.003$; Fig. 5a). The DW1B subreach had significantly higher standing crops of CPOM and FPOM than the other three reaches (HSD, $p < 0.008$ and $p < 0.010$, respectively; Table 4). The DW1A subreach showed low biomass of filamentous algae, moss, and vascular plants.

Total macroinvertebrate density was similar between the DW1A and the UP reaches (HSD, $p = 0.957$) and between the DW1B and the DW2 reaches (HSD, $p = 0.416$; Fig. 5b). In contrast, total macroinvertebrate density in the DW1B and the DW2 reaches was significantly higher to that in

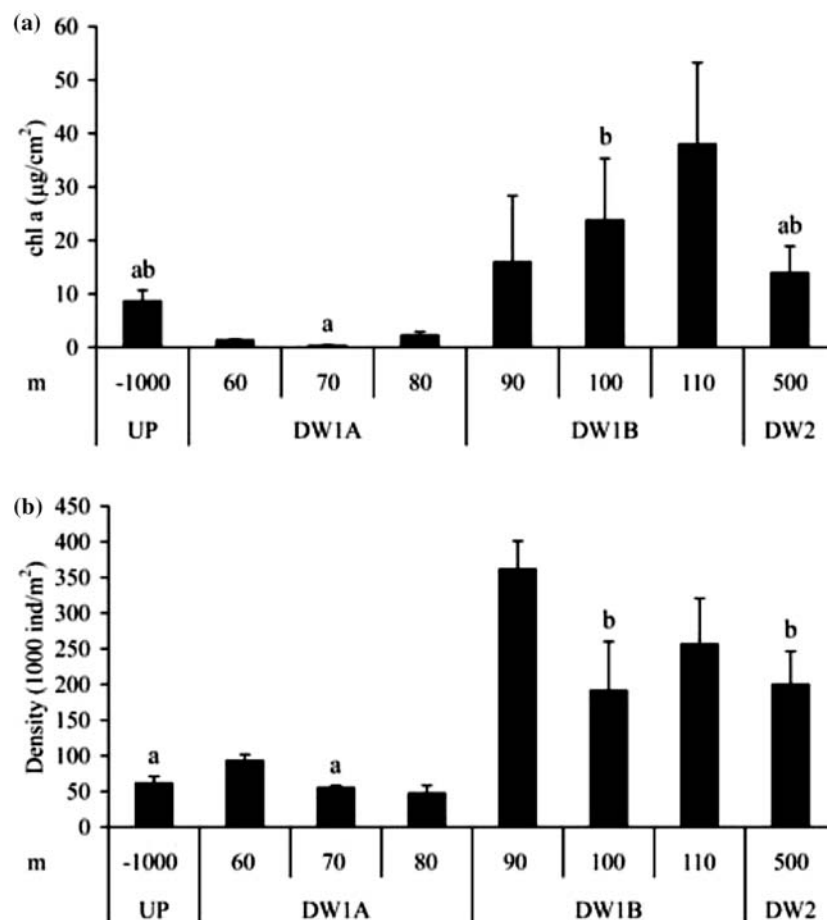*Figure 5.* Mean values ($\pm$SE) of (a) periphyton chlorophyll *a* (μg/cm^2) and (b) total density of macroinvertebrates (ind/m^2) for the three reaches. Values are given per each transect of the DW1 reach. Distance from the WWTP input are indicated in m.

Table 4. Mean values $\pm$ SE of filamentous algae, moss, vascular plants, CPOM and FPOM (g AFDM/m^2) at the UP, DW1 and DW2 reaches

	UP ($n = 12$)	DW1A ($n = 6$)	DW1B ($n = 6$)	DW2 ($n = 12$)
Filamentous (g AFDM/m^2)	2.34 $\pm$ 1.3 (ab)	0.05 $\pm$ 0.05 (a)	3.72 $\pm$ 2.25 (ab)	4.26 $\pm$ 1.04 (b)
Moss (g AFDM/m^2)	0.01 $\pm$ 0.01 (a)	0.05 $\pm$ 0.03 (ab)	3.49 $\pm$ 1.42 (c)	1.03 $\pm$ 0.35 (b)
Vascular plants (g AFDM/m^2)	0.22 $\pm$ 0.18 (a)	0.14 $\pm$ 0.14 (ab)	11.38 $\pm$ 5.39 (ab)	17.99 $\pm$ 5.68 (b)
CPOM (g AFDM/m^2)	4.87 $\pm$ 1.18 (a)	5.81 $\pm$ 1.47 (a)	21.42 $\pm$ 2.3 (b)	6.09 $\pm$ 1.69 (a)
FPOM (g AFDM/m^2)	4.14 $\pm$ 1.12 (a)	2.85 $\pm$ 0.35 (a)	9.35 $\pm$ 1.4 (b)	3.16 $\pm$ 0.61 (a)

Reaches sharing the same letter are not significantly different ($p > 0.05$) according to Tukey's multiple comparisons. Data were log ($x + 1$) transformed prior to analysis.

the DW1A and the UP reaches (HSD, $p < 0.041$). Taxa richness was lower at the DW1A (31 taxa) than at the DW1B (37 taxa). At the DW1A subreach, Shannon diversity was comparable to that at the UP reach (3.27 bits) while at the DW1B subreach was similar to that at the DW2 reach (2.79 bits). At the DW1A, relative percentage of gatherers was higher than at the DW1B (75 and 62%, respectively) while relative percentage of scrapers was lower (13 and 30%, respectively). Relative percentage of predators and filterers was similar between the DW1A and the DW1B subreaches. However, the dominant predator group at the DW1A was Nematoda while at the DW1B was Tanypodinae.

Discussion

In June 2001, the WWTP input significantly increased nutrient concentrations, organic matter, and discharge of La Tordera stream. The summer period in Mediterranean streams affected by WWTP effluents lead to the worst conditions for biota because stream dilution is lower (House & Denison, 1997; Gasith & Resh, 1999). At La Tordera stream, degradation of water quality was moderate compared to studies performed in nearby streams (Martí et al., 2004). The increase of nutrients and organic matter enhance respiration (Steinman & Lamberti, 1996; Miltner & Rankin, 1998) and lead to low DO concentrations, especially at night (Mulholland et al., 2001). Macroinvertebrates are not directly affected by nutrient enrichment but by the induced changes in DO concentrations (McCormick et al., 2004). The outlet of the WWTP effluent into the stream not only increased nutrient concentrations but also water availability. The resulting increase of discharge may convert a temporary stream into a permanent one (Gasith & Resh, 1999). Previous studies established that temporary streams hold higher taxa richness because of temporal heterogeneity (Dieterich & Anderson, 2000).

Our results support previous findings that continuous source of nutrients and organic matter supplied by WWTPs usually enhances algal biomass (Paul & Meyer, 2001). However, at the DW1A subreach, algal biomass was significantly lower than at the DW1B subreach although shading and nutrient concentrations were quite similar. Previous research found that WWTP inputs enhance heterotrophic microorganisms (Masseret et al., 1998; Paul & Meyer, 2001), and that the derived low DO concentrations can reduce autotrophy (McCormick & Laing, 2003). Surprisingly, major changes in periphyton chlorophyll *a* occurred within the 10 m that separated the two halves of the DW1 reach (A and B). Such abrupt recovery did not match with previous studies, which reported recovery gradients several km long (Hynes, 1978; Prenda & Gallardo-Mayenco, 1996). In this sense, high water temperature may play an important role as it accelerates metabolic processes, and consequently, self-purification.

Point sources can dramatically increase dissolved and particulate organic carbon concentrations (Paul & Meyer, 2001), but little is known about the effects on BOM. Our results show a higher accumulation of both CPOM and FPOM at the DW1B subreach than in the rest of the reaches. The WWTP input may increase the

standing crop of FPOM because it supplies DOC and nutrients that may enhance primary producers and consumers. The fact that although DOC and nutrient concentrations were higher at all downstream reaches but FPOM was only increased at the DW1B subreach, suggest that DOC did not represent a significant source of FPOM. At the DW1B and DW2 reaches, vascular plants and moss may represent an important source of CPOM during die-back (Allan, 1995), but this does not explain why CPOM is higher only at the DW1B subreach since canopy cover was similar in the two reaches. The lack of primary producers at the DW1A subreach translated in little retention of the particulate organic matter (POM) entering the subreach, which was transported downstream. At the DW1B subreach, vascular plants and mosses act as matrix that retains POM (Stream Bryophyte Group, 1999; Koetsier & McArthur, 2000) entering this reach. At the DW2 reach, vascular plants supply a retention capacity similar to that of the DW1B subreach, but standing crop of BOM was lower because was also retained throughout the length between the DW1B and the DW2 reaches. The ratio CPOM to FPOM was much higher at all the downstream reaches that at the upstream reach indicating that leaf litter processing rates were influenced by the point source (Braioni et al., 1997). Previous studies reported that shredders significantly increase the conversion of CPOM to FPOM (Wallace & Webster, 1996).

Therefore, decline of shredders at the downstream reaches may be in charge of higher CPOM/FPOM ratios.

The WWTP input caused important changes in the composition of the benthic community. Sensitive taxa, such as EPT taxa, decreased or disappeared while tolerant taxa, mainly chironomids and oligochaetes, increased in abundance. Both, elimination of taxa and increase of dominance, led to lower diversity values at all the downstream reaches. These results agree with those of previous studies (e.g., Garie & McIntosh, 1986; Prenda & Gallardo-Mayenco, 1996; Shieh et al., 1999b) which found that taxa richness and diversity decreases with increasing human influence. The DW1B and DW2 reaches had the greatest total macroinvertebrate densities. Meanwhile, total macroinvertebrate density at the DW1A subreach was similar to that at the UP reach. Several studies maintained that human impact decreases total density of macroinvertebrates (Garie & McIntosh, 1986; Prenda & Gallardo-Mayenco, 1996). In contrast, other studies found that total density of macroinvertebrates is not affected by urbanization (Jones & Clark, 1987; Roy et al., 2003), or even increase under certain nutrient enrichment (Hynes, 1978; Miltner & Rankin, 1998). The subsidy-stress hypothesis (Odum et al., 1979) proposed that certain community parameters, such as density, are enhanced at low levels of usable inputs but degrade at higher levels
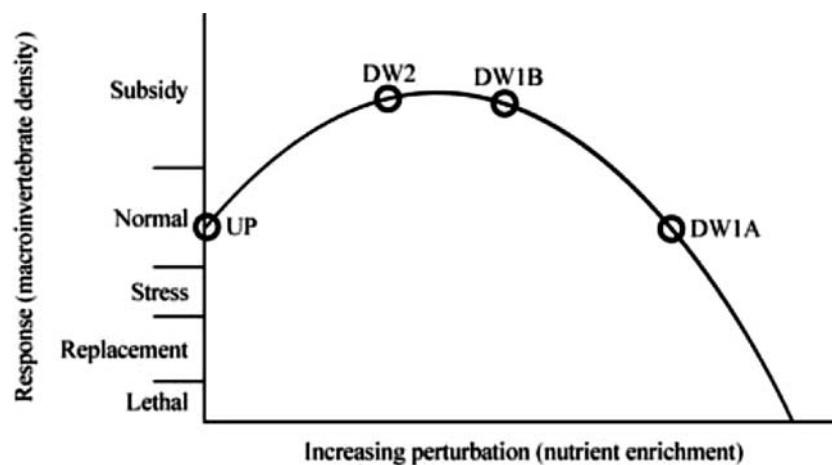

Figure 6. Potential location of macroinvertebrate density at the UP, DW1A, DW1B, and DW2 reaches along the subsidy-stress curve. Modified from (Odum et al., 1979).

(Fig. 6). Linear relationships between nutrient concentrations and macroinvertebrate density might be explained by too narrow nutrient concentration ranges. According to the subsidy-stress hypothesis, macroinvertebrate density at the DW1A subreach was similar to that at the UP reach but was located in the downward region of the subsidy-stress curve (Fig. 6). Meanwhile, the DW1B and the DW2 reaches were located close to the peak of the subsidy-stress curve, with higher macroinvertebrate densities but closer to the UP reach. Ordination analysis supported this assumption, as the DW1B and DW2 reaches were more comparable to the UP reach than the DW1A subreach. Ordination analysis separated the samples of the UP reach from all those taken downstream of the point source in the first axis. Such disjointing indicates that the WWTP input clearly affected taxa composition and densities of benthic macroinvertebrates. The lower spreading of the downstream samples along axis 2, which was related to microhabitat variables, suggests an increase of generalist taxa. As the point source act as a major factor on determining community characteristics, other environmental conditions have less weight in the distribution of macroinvertebrates.

As predicted by Kerans & Karr (1994), human impact decreased the relative percentage of shredders and predators, while increased the relative percentage of gatherers. The decrease in relative percentage of scrapers was observed only at the DW1A subreach, and relative abundance of filterers was higher at the UP reach. At the DW1B and DW2 reaches, scrapers were more abundant than at the UP reach, in apparent response to a higher periphyton biomass (Wallace & Webster, 1996). A higher abundance of algae allowed scrapers have relative abundance similar to the UP reach. At the DW1A subreach, chlorophyll *a* appeared to limit density of scrapers as seen in previous studies (Hart & Robinson, 1990). Surprisingly, relative percentage of filterers was much lower at the reaches below the WWTP input than at the UP reach. The fact that this result is in contradiction with the findings of previous research (Roy et al., 2003) may be explained by constraints of the functional feeding grouping. In streams with stable substrate and enough current velocity,

organic seston enhance filter-feeding invertebrates (Wallace & Merritt, 1980). At the study reaches, the bed substrate was dominated by cobbles, pebbles, and gravel, providing sufficient stable substrate to let filter feeders attach. Certainly, WWTP effluents increase quantity and quality of transported organic matter (Paul & Meyer, 2001). On the other hand, water velocity was probably too low to guarantee a rate of suspended organic matter to enhance attached filterers so much. In contrast, at the UP reach water velocity was even lower than at all the downstream reaches and greatly favored filtering microcrustaceans (Cladocera).

In conclusion, the obtained results show that, under summer conditions, the studied stream can present a high but limited self-purification capacity. Nutrient concentrations derived from the WWTP input clearly affected periphyton, BOM, and macroinvertebrates. The macroinvertebrate community showed an unexpectedly significant recovery between 80 and 90 m below the WWTP input. However, 500 m downstream the macroinvertebrate community did not changed at all indicating that self-purification was overwhelmed. Future research considering diel and temporal variability within the first hundred of meters below WWTP inputs will certainly increase our knowledge about the mechanisms involved in stream self-purification. The obtained information will improve the development of future management plans in stream ecosystems.

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Appendix

Appendix 1. Codes, mean densities in ind./m^2 $\pm$ SE of macroinvertebrate taxa recorded at the UP, DW1A, DW1B, and DW2 reaches

Taxa	Code	UP ($n = 12$)	DW1A ($n = 6$)	DW1B ($n = 6$)	DW2 ($n = 12$)
Ecdyonurus angelieri	Eang	202 ± 95	0 ± 0	0 ± 0	0 ± 0
Epeorus torrentinum	Etor	1 ± 1	0 ± 0	0 ± 0	0 ± 0
Baetis fuscatus	Bfus	11 ± 8	338 ± 141	418 ± 175	370 ± 59
Baetis lutheri	Blut	2116 ± 1334	1063 ± 672	1878 ± 885	1169 ± 538
Baetis rhodani	Brho	1783 ± 700	6768 ± 845	8815 ± 1267	4896 ± 634
Serratella ignita	Sign	328 ± 105	69 ± 34	119 ± 48	0 ± 0
Caenis luctuosa	Cluc	2397 ± 469	149 ± 50	167 ± 64	449 ± 125
Habrophlebia fusca	Hfus	61 ± 28	22 ± 16	3 ± 3	7 ± 3
Leuctra geniculata	Lgen	51 ± 18	0 ± 0	0 ± 0	0 ± 0
Calopteryx virgo	Cvir	0 ± 0	3 ± 3	0 ± 0	0 ± 0
Haliplus sp.	Hal	1 ± 1	0 ± 0	0 ± 0	0 ± 0
Scarodites sp.	Sca	13 ± 9	0 ± 0	0 ± 0	0 ± 0
Agabus sp.	Aga	27 ± 14	0 ± 0	3 ± 3	0 ± 0
Elmis sp.	Elm	9 ± 9	0 ± 0	0 ± 0	0 ± 0
Oulimnius sp.	Oul	9 ± 9	0 ± 0	0 ± 0	0 ± 0
Hydropsyche instabilis	Hins	36 ± 20	0 ± 0	0 ± 0	0 ± 0
Polycentropus sp.	Pol	3 ± 2	0 ± 0	0 ± 0	0 ± 0
Tinodes sp.	Tin	51 ± 25	33 ± 33	121 ± 31	27 ± 14
Rhyacophila dorsalis	Rdor	8 ± 5	0 ± 0	0 ± 0	0 ± 0
Hydroptila sp.	Hyt	24 ± 11	0 ± 0	50 ± 34	43 ± 15
Lepidostoma hirtum	Lhir	14 ± 8	0 ± 0	0 ± 0	0 ± 0
Mystacides azurea	Mazu	38 ± 19	0 ± 0	0 ± 0	9 ± 9
Psychodidae	Psy	9 ± 9	0 ± 0	17 ± 17	52 ± 16
Dixa sp.	Dix	0 ± 0	0 ± 0	21 ± 21	0 ± 0
Simuliidae	Sim	174 ± 95	92 ± 33	699 ± 333	338 ± 140
Ceratopogoninae	Cer	35 ± 20	0 ± 0	133 ± 49	28 ± 15
Forcipomyinae	For	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Tanypodinae	Tan	1592 ± 304	1694 ± 241	11157 ± 2236	3326 ± 1227
Chironominae	Chi	6320 ± 2471	12826 ± 2925	62859 ± 9472	14214 ± 4167

Appendix 1. Continued

Taxa	Code	UP ($n = 12$)	DW1A ($n = 6$)	DW1B ($n = 6$)	DW2 ($n = 12$)
Orthocladiinae	Ort	9827 ± 5885	5816 ± 2184	75497 ± 21918	54365 ± 15143
Tipulidae	Tip	3 ± 2	0 ± 0	0 ± 0	9 ± 9
Limonidae	Lim	1 ± 1	0 ± 0	0 ± 0	0 ± 0
Hemerodromiinae	Hem	0 ± 0	0 ± 0	176 ± 82	11 ± 9
Clinocerinae	Cli	9 ± 9	0 ± 0	0 ± 0	0 ± 0
Rhagionidae	Rha	1 ± 1	0 ± 0	3 ± 3	3 ± 2
Tabanidae	Tab	0 ± 0	0 ± 0	17 ± 17	0 ± 0
Anthomyidae	Ant	30 ± 20	17 ± 17	138 ± 28	143 ± 41
Cladocera	Cla	13448 ± 4313	201 ± 119	1477 ± 302	1396 ± 344
Copepoda	Cop	1142 ± 431	313 ± 133	2268 ± 381	1553 ± 254
Ostracoda	Ost	598 ± 92	85 ± 85	607 ± 188	4045 ± 807
Gammaridae	Gam	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Hydracarina	Hyc	203 ± 51	170 ± 22	205 ± 43	152 ± 51
Potamopyrgus antipodarum	Pant	43 ± 20	55 ± 49	0 ± 0	12 ± 8
Bythiospeum sp.	Byt	0 ± 0	0 ± 0	67 ± 67	0 ± 0
Ancylus fluviatilis	Aflu	5064 ± 1449	2411 ± 631	4047 ± 961	3915 ± 677
Lymnaea sp.	Lym	942 ± 307	36 ± 36	50 ± 34	0 ± 0
Radix sp.	Rad	32 ± 15	0 ± 0	0 ± 0	1 ± 1
Physella acuta	Pacu	1171 ± 342	181 ± 74	315 ± 143	353 ± 144
Naididae	Nai	6108 ± 866	14237 ± 4179	62836 ± 26823	81668 ± 26343
Chaetogaster spp.	Cha	1211 ± 454	2860 ± 1095	24287 ± 11987	3052 ± 759
Tubificidae	Tub	46 ± 32	10626 ± 3359	4699 ± 1570	6411 ± 1491
Lumbriculidae	Lum	427 ± 138	73 ± 48	280 ± 188	314 ± 97
Eiseniella tetraedra	Etet	38 ± 21	5 ± 3	30 ± 22	1 ± 1
Glossiphonia sp.	Glo	1 ± 1	16 ± 13	0 ± 0	52 ± 27
Helobdella stagnalis	Hsta	0 ± 0	1307 ± 563	601 ± 314	788 ± 204
Erpobdella sp.	Erp	8 ± 8	282 ± 89	327 ± 57	1048 ± 218
Nematoda	Nem	270 ± 104	3094 ± 895	4877 ± 889	1317 ± 181
Hydra sp.	Hyd	270 ± 85	61 ± 52	238 ± 137	105 ± 58
Dugesia sp.	Dug	17 ± 12	0 ± 0	0 ± 0	9 ± 9
Digenea	Dig	5419 ± 1040	340 ± 194	367 ± 165	14181 ± 3016