ECOSYSTEMS

Transplanting macrophytes as a rehabilitation technique for lowland streams and their influence on macroinvertebrate assemblages

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Abstract: Lowland streams are usually affected by river engineering works that produce the loss of habitat heterogeneity. Our aim was to assess the transplantation of macrophytes with different complexity into a lowland stream which was dredged and widened. *Stuckenia pectinata* and *Hydrocleys nymphoides* were collected at an extraction site and installed at a transplant site. The growth and coverage of macrophytes beds were quantified. Taxonomic richness, Shannon-Wiener diversity, abundance, composition and proportion of functional feeding groups of the macroinvertebrate assemblage presented in macrophyte beds were assessed between sites and species. The growth of both macrophytes did not differ significantly between sites and the coverage of transplanted beds increased, therefore they established at the transplant site within a short period. Regarding to macroinvertebrate assemblage, only the functional feeding groups did not show differences between sites. Moreover, the proportion of predators presented differences between macrophytes at the same site, with *H. nymphoides* having a higher proportion. Our study showed that this technique is suitable for reintroducing these species and is applicable in rehabilitation projects that promote the restoration of habitat heterogeneity deteriorated by river engineering works. Also, we highlight the importance of incorporate macroinvertebrate functional traits to assess the ecological status after rehabilitation.

Key words: restoration, aquatic plants, macroinvertebrates, S*tuckenia pectinata*, *Hydrocleys nymphoides*.

INTRODUCTION

Although streams and wetlands provide essential biological and economic services (Millennium Ecosystem Assessment Board 2005), over the last century they have been seriously threatened by human activity, such as water quality degradation and flow regulation (Allan 2004, Elosegi et al. 2010). The implementation of river engineering projects produce significant changes in flow velocity and erosion of bed and bank material (Elosegi & Sabater 2013). As a consequence, streams have lost their habitat heterogeneity and have become deeper and wider (Brooker 1985, Elosegi et al. 2010). Moreover, these practices have had negative effects on birds, fish, phytoplankton, and periphytic and epipelic algae assemblages and have caused reductions in the density of invertebrates and the loss of aquatic plants (Lewis et al. 2001, Licursi & Gómez 2009, Cabrita 2014, Grygoruk et al. 2015, Kjelland et al. 2015).

Currently, lowland streams are usually highly modified and managed predominantly for their drainage roles (Licursi & Gómez 2009, Suren 2009). These streams are characterised by

the development of dense and rich macrophyte assemblages (Giorgi et al. 2005, Rodrigues Capítulo et al. 2010). However, because of river engineering works, they have lost these assemblages and the channel simplification has reduced the probability of their recolonization by fragments of plants or seeds (propagules, Riis 2008).

Macrophytes have been described as biological engineers (Sand-Jensen 1997). They affect the sediment and nutrient dynamics, and are highly efficient at removing a variety of contaminants from the water (Guittonny-Philippe et al. 2015, Bonanno et al. 2017). Furthermore, macrophytes increase the physical complexity of the streams and provide habitat for macroinvertebrates (Cortelezzi et al. 2013). The effect of aquatic macrophytes on their associated macroinvertebrate assemblage depends on their structural complexity, also known as architecture (Lillie & Budd 1992). Complex macrophytes can increase the resources for macroinvertebrates, like microhabitats (Mcnett & Rypstra 2000), protection against predators (Warfe & Bermuta 2004) and food (Phiri et al. 2012). Therefore, complex macrophytes may support higher abundance and richness of macroinvertebrate than simple macrophytes (Taniguchi et al. 2003, Warfe et al. 2008, Cremona et al. 2008).

Despite the numerous benefits that aquatic plants provide to lowland streams and their potential for rehabilitation, only a few studies have assessed the feasibility of their reintroduction by transplantation in these water courses (Larned et al. 2006, Riis et al. 2009, Suren 2009, Paz et al. 2018). These studies have reported contradictory results, depending on the macrophytes species and stream conditions. Therefore, it is necessary to improve this technique for future restoration projects. Moreover, it is important to evaluate the ecological status of watercourses after rehabilitation; for this

purpose, macroinvertebrates are considered good indicators of changes in the environment (Bonada et al. 2006, dos Reis Oliveira et al. 2019). This assemblage is an essential component of the aquatic ecosystem because contribute to the processing of particulate organic matter, regulating primary production and providing food for fishes (Reece & Richardson 2000, Spänhoff & Arle 2007).

We analyzed the growth and the changes in macroinvertebrate assemblage of two transplanted macrophytes with different structural complexity in a stream modified by river engineering works. We used the richness, diversity, abundance, and proportion of functional feeding groups (FFGs) to evaluate the changes in macroinvertebrate assemblage after the transplant. Our hypotheses were: 1-both macrophytes grow after the transplant, 2- the macroinvertebrate assemblage descriptors do not differ between transplanted and extraction (control) sections and 3- more complex macrophytes present high richness, diversity, and proportion of FFGs of macroinvertebrates.

Our results will be useful not only to improve the transplantation technique for future management and rehabilitation of lowland streams but also contribute to understand how macroinvertebrate assemblages change depending on macrophyte complexity.

MATERIALS AND METHODS

Study area

The experiment was conducted in 2015, in a lowland stream of the Pampean ecoregion, which is located in central eastern Argentina. The Martín stream, a second-order watercourse (Figure 1), that has been affected by river engineering works. As a result, this stream has a highly simplified and uniform channel. This stream mainly crosses a suburban area and the
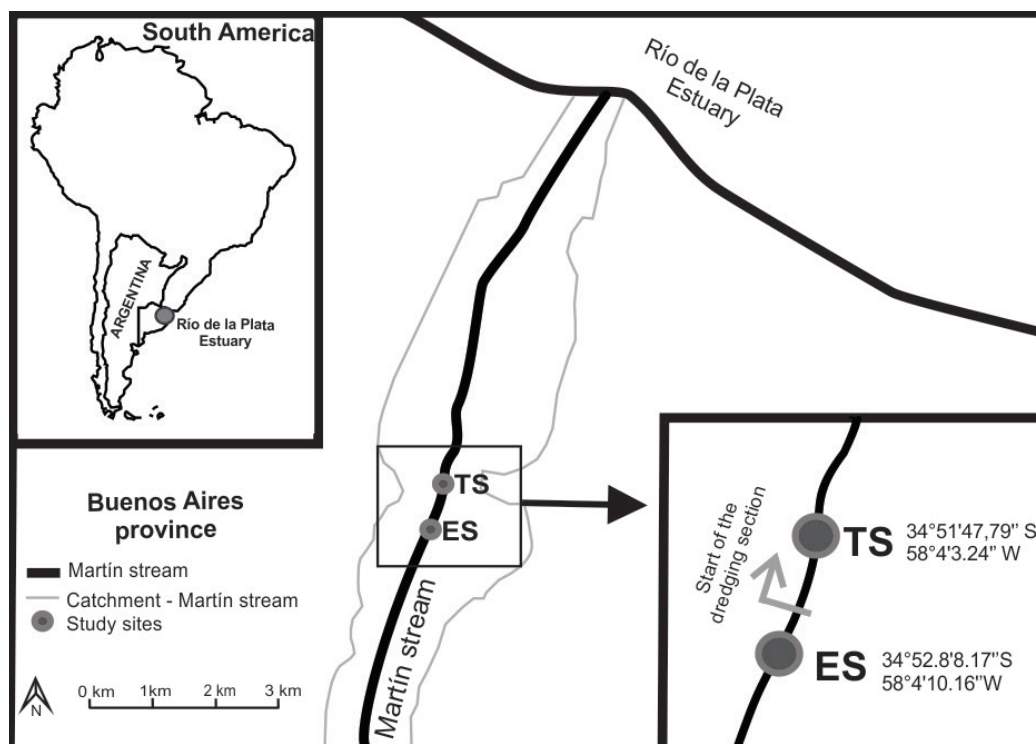
Figure 1. Map of the study area showing the location of the transplant site (TS) and the extraction site (ES), modified from Hurtado et al. 2006.

land use in the catchment is mainly peri-urban and agricultural (Cochero et al. 2015).

Two sites were established in a section of the Martín stream that cross the Municipal Ecological Park in La Plata. One was located upstream where macrophytes were collected (extraction site, ES); this site was characterised by a high coverage of aquatic plants, a pronounced sinuosity and availability of source populations of colonists. The other site (transplant site, TS), which was downstream, had recently been deepened, widened and straightened. Additionally, the aquatic plants had been removed from the stream bed and as a result, the heterogeneity was decreased. The transplant site was characterised as shallower (depth = 0.28 ± 0.07 m) and narrower (width = 14.97 ± 0.47 m) than the ES (depth = 0.06 ± 0.02 m and width = 11.2 ± 5.49 m).

In each sampling site, macrophytes and macroinvertebrates were sampled four times (every 20 days) from October to November (spring). The first sampling occasion occurred immediately after transplant (day 0).

Selected macrophytes

We selected two abundant species at ES, *Stuckenia pectinata* (L.) Börner [= *Potamogeton pectinatus* (L.)] (fennel pondweed, Potamogetonaceae) and *Hydrocleys nymphoides* (Hump. and Bonpl. ex Willd.) Buchenau (waterpoppy, Alismataceae). However, before starting the experiment, we calculated their fractal dimension (D) using ImageJ software (Rasband 1997–2008, Ferreiro et al. 2011) to verify that they had different complexities. We found that *S. pectinata* is more complex than *H. nymphoides* (D = 1.63 and D = 1.53, respectively).

Transplantation of aquatic plants

We collected specimens of the two species from the ES with a garden spade, taking care not to damage the roots and shoots, and placed them in trays (45 x 35 x 10 cm; Riis et al. 2009, Paz et

al. 2018, Figure 2a). The total area transplanted was of 0.05 m² in the case of *S. pectinata*, and 0.26 m² in the case of *H. nymphoides.* In order to exclude the effect of the substrate, sediment from the same stream was used to line the trays (Riis et al. 2009, Choudhury et al. 2015). The trays were immediately installed at the TS, forming three beds of approximately 135 x 105 cm by species (two trays conformed one bed); they were planted in the stream at depths of 0.20 to 0.40 m, depending on the species. Mean water velocity was between 0.04 and 0.09 m s^{-1} to ensure favorable light exposure and conditions (Lauridsen et al. 2003).

The transplantation was carried out following the recommendations of Riis et al. (2009): a) choosing a stream with shallow water (<1 m) and unshaded conditions for sustainable macrophytes growth, b) selecting macrophytes naturally present in the region and in upstream reaches, c) using trays of at least 20 x 30 x 5 cm, and d) transplanting during the growth season. The distribution pattern of the trays was selected following the recommendations of Bal et al. (2011) (Figure 2b).

Macrophytes monitoring and analysis

To evaluate macrophytes growth, 12 specimens of each species (at both sites) were marked. The shoot length of each specimen (SL) was measured throughout the experiment and growth was calculated as increase in length (LI) (LI [cm] = final SL – initial SL) (Choudhury et al. 2015).

Coverage of each transplanted bed was calculated following the methodology proposed by Pan et al. (2007). A series of images captured by a Nikon 3100 camera on each sampling date were taken, including cover at the TS immediately after transplantation. These images were processed using ImageJ version 1.51 R. to quantify the coverage of each bed. The increase in coverage (m²) was calculated based on the difference between the final and initial cover of each bed and for both species (ΔCoverage [m^2] = final coverage – initial coverage).
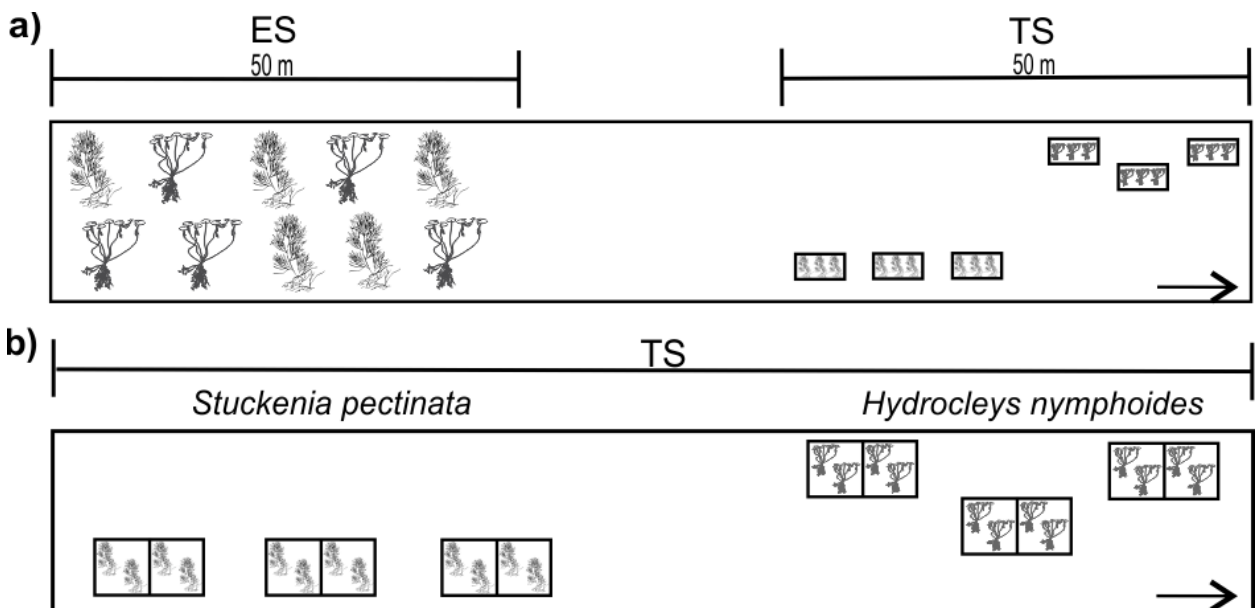

Figure 2. Tray distribution in the stream: a) plan view of tray distribution in the section of Martín Stream showing the extraction site (ES) and transplant site (TS); b) section of the TS magnified with details of the trays' disposition. The arrows indicate the direction of flow.

Macroinvertebrate assemblages

We collected one replicate from each macrophyte bed (three beds per species) using a hand net (0.018 m^2; 500 μm pore size) at both sites and on each sampling occasion. The samples were fixed *in situ* with 5% formaldehyde and the organisms sorted. In the laboratory, the specimens were counted and identified under a stereomicroscope to the lowest possible taxonomic level using taxonomic keys (Barbour et al. 1999).

We calculated the taxonomic richness and Shannon-Wiener diversity index and we assigned each taxa to a functional feeding group (FFG) using available references (Cummins et al. 2005, Allan & Castillo 2007, Merritt et al. 2008). The relative frequency values of each FFG at each site and on each sampling occasion were calculated using macroinvertebrate densities (ind m^{-2}).

Data analysis

We performed a Student's *t*-test (α = 0.05) to compare macrophyte growth (LI) between sites. The comparisons between species was not possible due to both macrophytes have different life form. However, we only analyzed the values up to day 40 because all the marks on the specimens were lost. Additionally, two of the three transplanted beds of S. *pectinata* were damaged by acts of vandalism, which caused the loss of one of the two trays of each bed. The damage prevented calculation of the coverage of these beds, but sampling was performed in the remaining tray of each bed.

We analyzed Shannon-Wiener diversity index at both sites and macrophytes with a linear model and a Gaussian error distribution (link: identity). Models for Taxonomic richness and each FFG were first fitted with a Gaussian error distribution (link: identity), since model residuals were not normally distributed (Shapiro test: p < 0.01), they were refitted using alternative distributions more suited to the response data. Specifically, we used generalized models with the Poisson error distribution for Taxonomic richness (link: log) and Binomial error distribution (link = logit) for FFG proportions.

The predictor variables were the macrophyte species (*S. pectinanta* and *H. nymphoides*), time (days 0, 20, 40, and 60), sites (ES and TS) and its interactions. All models were fitted with a random effect "patches" (intercept) because of the lack of independence of the data. We considered models with all possible combinations of predictor variables and evaluated the models using Akaike's Information Criterion corrected for small sample size (AICc) (Burnham & Anderson 2002). This resulted in 10 candidate models for each response variable corresponding to all possible combinations including one general model with all predictors (global model) and a base model without predictors (null model). A null model was useful for assessing the relative explanatory power of models containing predictors of interest. Model comparisons were made with ΔAICc, which is the difference between the lowest AICc value (i.e., best of suitable models) and AICc from all other models. Models with ΔAICc ≤ 2 have substantial support from the data (Burnham & Anderson 2002). The AICc weight of each model (wi) was also calculated to evaluate support for estimates of predictor variables, parameters with good support have high wi values (near to 1). Once we selected the suitable model, we calculated 95% confidence intervals of parameter estimates and performed a Tukey test to determine differences between levels of the fixed variable

To analyze the composition of macroinvertebrate assemblage for each sampling day, site and macrophyte we performed a multidimensional scaling (MDS), in this case we performed a Principal Coordinate Analysis (PCoA).

Since our data set was zero inflated because of a large number of real zeros and species with high densities was necessary to carried out a standardization process. We consider that the most appropriate was the standardization by ranges (0-1). This standardization leads the data to a range between 0 and 1 allowing comparison without masking the existing differences. Onces the data was standardized used ranges, Euclidean distance could be used for the analysis, this distance meets all the mathematical properties which is more in order to our objectives. Additionally, we tested significative differences among all samples from sites and macrophytes using a "Permutational Multivariate Analysis of Variance" (PERMANOVA, 999 random permutation) with Bonferroni correction.

We performed all the analyses using 'R' version 3.5.2 (R Core Team 2018) with MASS (Venables & Ripley 2002), MuMIn (Bartón 2013), lme4 (Bates & Maechler 2010), Multicomp (Hothorn et al. 2013), vegan (Oksanen et al. 2013) and ape (Paradis & Schliep 2019) packages.

RESULTS

Macrophyte monitoring and analysis

The macrophyte growth at both sites were similar, LI of the two species at the two sites did not show significant differences (p = 0.295, gl = 9 for *S. pectinata* and p = 0.458, gl = 7 for *H. nymphoides;* Table I); nevertheless, this value was higher at the ES.

The increase in the coverage of *S. pectinata* at the TS at the end of the experiment was 0.37 m^2, while the increase in coverage of *H. nymphoides* was 0.34 ± 0.10 m^2 (Figure 3).

Macroinvertebrate assemblages

A total of 29 invertebrate taxa were collected from Martín Stream. The most abundant taxa found on *S. pectinata* during the experiment were Ostracoda (29%, of total density), *Heleobia* spp. (21% of total density), and *Hyalella curvispina* (17% of total density). In contrast, the most abundance taxa on *H. nymphoides* were *Hyalella curvispina* (15% of total density), Oligochaeta (13% of total density), Ostracoda (13% of total density), and *Heleobia* spp. (12% of total density). Twelve taxa were gathering-collectors, eight predators, five scrapers, three filtering-collectors. Free-living aquatic nematodes were not included in the FFG analysis due to the controversies in the FFG classification (Moens et al. 2006) and the scarce number of specimens found.

The results from the linear models indicated that the most important predictor for taxonomic richness and Shannon-Wiener diversity was the site, with a 47% and a 46% of the variation respectively (wi = 0.74 and wi = 0.80, Table II).

Table I. Mean ± Standard deviation of shoot lengths (SL, cm) and length increase (LI = final SL– initial SL, cm) at the extraction (ES) and transplant (TS) sites during the experiment.

	SL						LI	
	DAY 0		DAY 20		DAY 40			
	ES	TS	ES	TS	ES	TS	ES	TS
S. pectinata	6.33 ± 1.51	5.80 ± 2.77	9.00± 4.43	9.8 ± 3.35	11.00 ± 4.82	7.80 ± 1.48	4.67±4,63	2.00±2.91
H. nymphoides	29.14 ± 4.37	23.75 ± 8.30	37.5 ± 16.10	27.67 ± 9.61	35.57 ± 15.39	23.00 ± 5.35	4.83±8.13	1.00±1.00

In both cases, ES presented significantly higher values for both macrophytes (z = -4.76; p < 0.001; N = 48; z= -3.65; p < 0.001 ; N=48). The FFG proportions found are presented in Figure 4. The suitable model for predators indicated that the macrophytes species was the only important predictor, with a 48% of the variation (wi = 0.63; Table II). *Hydrocleys nymphoides* presented the highest proportion of predators in both sites (z = 2.68; p = 0.02; gl = N = 48). The variation of the collectors-filterers were explained by the predictor site and the macrophyte (wi = 0.35; Table II). However, there were no significant differences between levels. The best model for the proportion of collector-gatherers and scrapers was the null model (Table II). Therefore, the explanatory variables used in the analyzes were poor descriptors of the proportion of these groups.

The macroinvertebrate assemblage composition of each macrophytes across sites during the experiment are shown in Figure 5 (PCoA). On day 0, the assemblage of *S. pectinata* at TS was different from the others macroinvertebrate assemblages sampled (Figure 5a). However, the PERMANOVA analysis did not show significant differences between macrophytes and sites on the transplant day (day 0). On day 20, only the assemblage of *H. nymphoides* was different between sites (Figure 5b) which was supported by the PERMANOVA results (F = 4.69, p = 0.024). On day 40 and 60 both macrophytes presented different assemblage between sites (Figure 5c and 5d respectively). However, only the assemblage of *H. nymphoides* on day 40 was significantly different between sites, while the assemblage of *S. pectinata* was significantly different for day 60 (F = 6.62, p = 0.030).

a)

b)
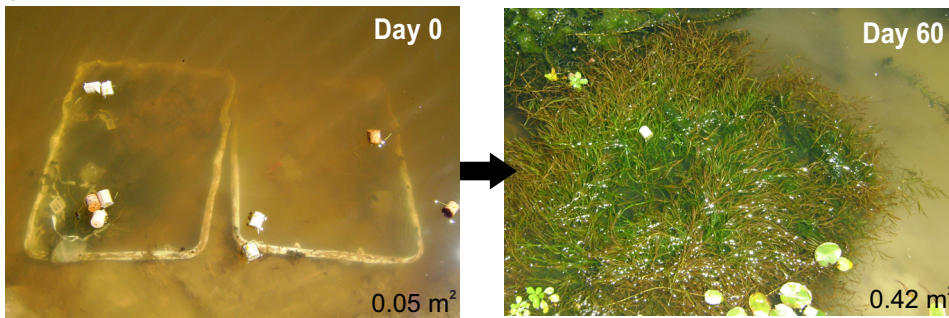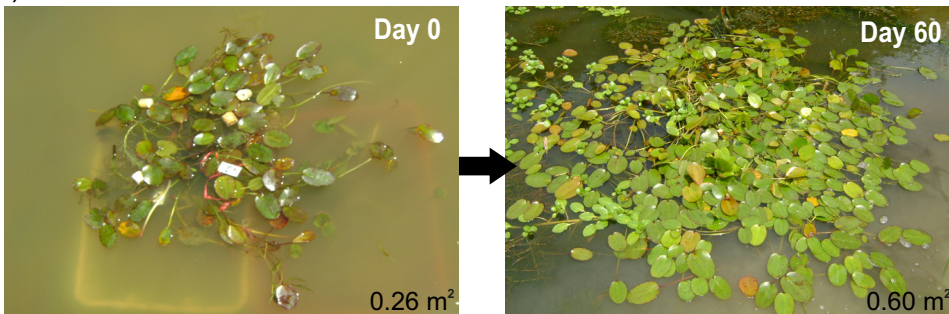
Figure 3. Coverage of the transplanted beds at the transplant site on days 0 and 60: a) *S. pectinata;* b) *H. nymphoides.* Increase in coverage (ΔC) was calculated as follows: final coverage – initial coverage.

Table II. The linear models explaining variation in: A- taxonomic richness (GLM), B- Shannon-Wiener diversity (LM), C- predators (GLM), D-.gathering-collectors (GLM), E- Scrapers (GLM), F = filtering-collectors (GLM). The null model, the global model, and models with strong support (ΔAICc ≤2) are provided. Models are listed in decreasing order of importance. S = Site; M= macrophytes; T = time.

| Response variable	model	Explanatory variables[a]	ΔAICc	wi	R2
A= taxonomic richness	**1**	**S**	**0.00**	**0.74**	**0.47**
	2	M+S	2.27	0.24	0.46
	null	-	20.96	0.00	-
	global	all variables	23.65	0.00	0.65
B= Shannon-Wiener	**1**	**S**	**0.00**	**0.80**	**0.46**
	null	-	3.68	0.13	**-**
	global	all variables	74.07	0.00	0.64
C = Predator	**1**	**M**	**0**	**0.63**	**0.48**
	2	M+S	2.29	0.19	0.48
	null	-	4.49	0.06	-
	global	all variables	208.65	0.00	0.70
D = Collector-gatherers	**null**	**-**	**0.00**	**0.41**	**-**
	1	S	1.07	0.24	0.05
	global	all variables	23.34	0.19	0.66
E = Scrapers	**null**	**-**	**0.00**	**0.45**	**-**
	1	S	1.66	0.19	0.01
	global	all variables	26.32	0.00	0.56
F = Collectors-filteres	**1**	**M+S**	**0**	**0.35**	**0.28**
	2	S	0.57	0.26	0.14
	3	M+S+T	1.89	0.14	0.52
	null	-	3.08	0.07	-
	global	all variables	23.01	0.00	0.58
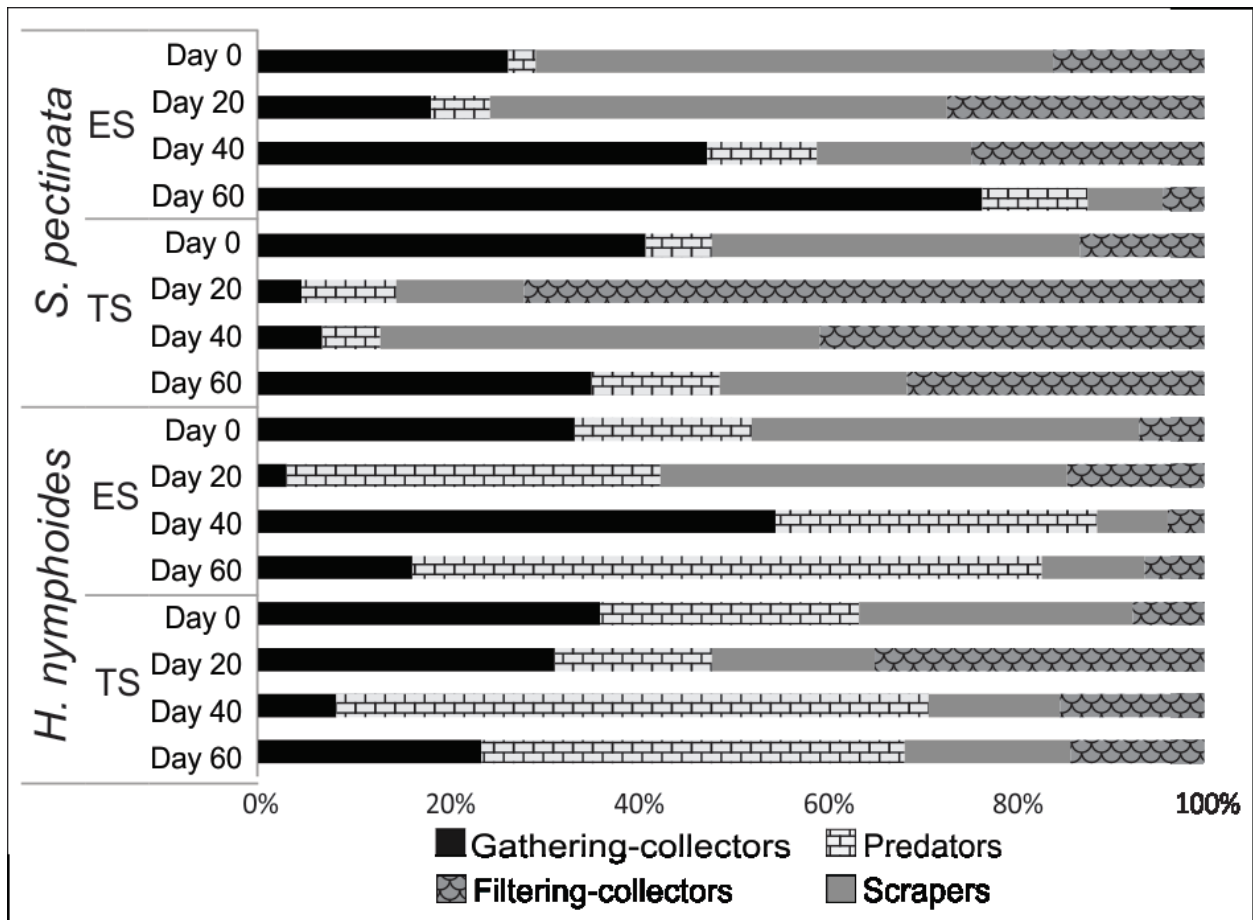
Figure 4. Proportion of functional feeding groups (FFGs) on *S. pectinata* and *H. nymphoides* at extraction (ES) and transplant (TS) site for each day.

DISCUSSION

The relatively quick growth of *S. pectinata* and H. nymphoides at the transplant site, which was deepened, widened, straightened and their aquatic plants removed, demonstrated that the reintroduction of these macrophytes is possible after a short period of time. This conclusion is based on the similar values of shoot length increase found at both sites and the increase in coverage of transplanted beds during the experiment.

The results found for *S. pectinata*, were in accordance with Larned et al. (2006) that reported similar values for increase in the patch area after 90 days to those we found

after 60 days. Moreover, Lauridsen et al. (2003) considered that *S. pectinata* established itself quickly after transplantation because it anchored easily to the sediment. No studies have used *H. nymphoides* in transplants, which indicates that this experiment is the first approximation of the utilization of this species in a transplant experiment. Evaluating the growth of different macrophytes species after a transplant experiment in lowland streams is essential to improve this technique for future management and rehabilitation projects.

The response and recolonisation of macroinvertebrates assemblage in a short time depends on the availability of source populations of colonists upstream of the
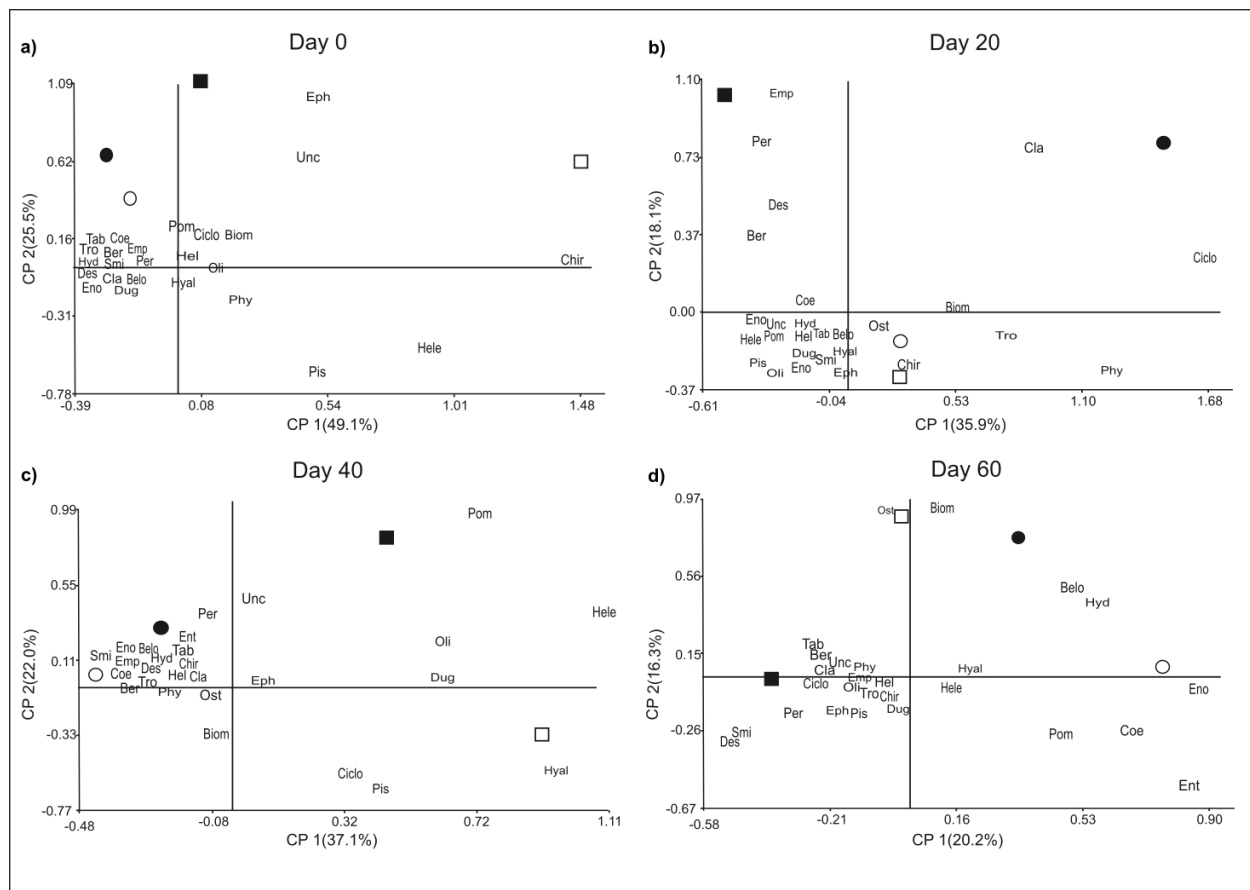
Figure 5. Principal Coordinate Analysis (PCoA) for each day showing the spatial ordination of the two sites (ES: circle and TS: square) and both macrophytes (*H. nymphoides*: black, *S. pectinata:* white) in relation to their macroinvertebrate abundance. a) Day 0; b) Day 20; c) Day 40; d) Day 60. Oli: Oligochaeta; Ent: Entomobryidae; Smi: Sminthuridae; Eno: *Enochrus* sp.; Hyd: *Hydrophilus* sp*.; Ber: Berosus* sp.; Tro: *Tropisternus* sp.; Chir: Chironomidae; Tab: Tabanidae; Eph: Ephydridae; Emp: Empididae; Hyal: *Hyalella curvispina;* Dug: Dugesiidae; Hel: *Helobdella*spp*.; Des: Desmopachria* sp*.;* Bel: *Belostoma* sp.; Per: *Perithemis* sp*., Coe:* Coenagrionidae; Ciclo: Cyclopoidea; Hele: *Heleobia spp.;* Phy*: Physa* sp.; Pom: *Pomacea canaliculata;* Bio*: Biomphalaria* sp.; Unc: *Uncancylus* sp.; Pis: *Pisidium* sp*.;* Ost: Ostracoda; Cla: Cladocera; Nem: Nematoda.

rehabilitated site (Al-Zankana et al. 2019). Nevertheless, our results showed differences in the structural metrics of the assemblages between sites in a short time despite the presence of a nearly source of specimens. These differences could indicated that the characteristics of the site produced by the river engineering works could be a determining factor in macroinvertebrate assemblage composition. Other authors transplanted macrophytes, and transplanted sections presented low values of diversity and the invertebrate colonization was

limited by site features (Larned et al. 2006). Additionally, most studies that improve habitat complexity did not find higher values of richness and diversity of macroinvertebrates (Palmer et al. 2010). Conversely, Miller et al. (2010) and Verdonschot et al. (2016) found significantly higher values of richness and diversity in restored sections of streams but with a longer follow-up period. Therefore, macroinvertebrate structural metrics did not always responds to the increase in habitat heterogeneity at the site, while other aquatic biota may respond. Paz et al.

(2018) transplanted macrophytes and assessed the change of epiphytic biofilm in a section of the stream affected by river engineering works. These authors found that the biofilm developed in transplanted macrophytes had similar features to those from a non-dredging site after three months. On the other hand, Kail et al. (2015) and Al-Zankana et al. (2019) highlight that the use of functional metrics of macroinvertebrate assemblage would be more appropriate to analyze the effects of restoration measures than structural metrics. In our study, we did not find differences in the proportion of FFGs between extraction and transplanted site, indicating assemblages with similarities in functional composition after three months.

Structural complex macrophytes are often related to higher richness and diversity of macroinvertebrates (Warfe et al. 2008, Bell et al. 2013), because they affect the food supply by trapping detritus, alter epiphyte availability (Phiri et al. 2012), and offer greater protection against possible predators (Dionne & Folt 1991, Cheruvelil et al. 2002). However we found that richness and diversity did not show differences between macrophytes with different structure complexity, in agreement with the results of McAbendroth et al. (2005) and Ferreiro et al. (2011). On the other hand, we found a higher proportion of predators on the most simple species study (*H. nymphoides*). However, few studies of the abundance of this group in macrophytes with different complexity have been addressed (Bell et al. 2013, Paice et al. 2015). Both authors found that structurally more complex macrophytes supported a higher density of predators. Nevertheless, many studies have assessed the predation rate, explaining that predation is less frequent in complex macrophytes than in simpler ones, due to the protection against predators (Dionne & Folt 1991, Cheruvelil et al. 2002, Warfe & Barmuta 2004).

The structural metrics used in this study were not appropriate for identifying similarities between sites after three months. However, functional metrics could be more suitable to assess the macroinvertebrate assemblage after a rehabilitation project in a short term. Similarly, these metrics could be useful to evaluate differences between macrophytes with different structure complexity. Nevertheless, further research are necessary to assess how functional features respond after restoration measures.

In conclusion, the transplant technique of *S. pectinata* and *H. nymphoides* is feasible and promotes their reintroduction in lowland streams. Transplantation of these macrophytes could be useful as a tool in management and rehabilitation projects to restore the habitat of lowland streams deteriorated by river engineering works. Although, we highlight the importance of incorporating functional traits to assess the ecological status of watercourses after rehabilitation.

Acknowledgments

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