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# The Impact of Herbicides on Benthic Organisms in Flooded Rice Fields in Southern Brazil

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## 1. Introduction

Agricultural production is always directly associated with the use of agrochemicals to control the harmful organisms that attack the crops and reduce the harvest. In spite of their benefits, the use of agrochemicals usually causes great problems, considering that these often-toxic chemicals are used in large quantities over large areas, and generally persist in the environment for some time (Prime et al., 2005).

As a consequence of agrochemical application, water quality and aquatic biodiversity have been compromised due to the destructuring of the physical and chemical environment and alteration of the natural dynamics of the biological communities (Goulart & Callisto, 2003). According to Biggs et al. (2007), it is highly important to regulate the use of an agrochemical and its action against non-target organisms in the aquatic environment. However, specific data on the occurrence and population dynamics of aquatic organisms in agricultural areas are very limited.

Studies by Mesléard et al. (2005) indicate that the use of herbicides, insecticides, and fertilizers can modify the feeding pattern and alter the development of animal communities present in rice fields, especially the invertebrates. Because they are sedentary organisms and have relatively short life cycles (compared to fish), benthic macroinvertebrates are considered good indicators of water quality. Due to their short life cycles, they express more rapidly the changes in the environment through changes in structure of their populations and communities (Rosemberg & Resh, 1993). Because they also have great biological diversity, they tend to exhibit a greater variability of responses to different kinds of environmental impacts (Rosemberg & Resh, 1993). Another aspect refers to adaptive strategies to environmental instabilities of the environment, in general, resilience and persistence. A resilient biota can rapidly recolonize areas disturbed by flooding; and a persistent biota demonstrates a good capacity to resist disturbances (Winterbottom et al., 1997).

The use of herbicides can indirectly influence the zoobenthic community, since, as seen in experiments carried out by Moreby & Southway (1999), the use of selective herbicides against a species of weed is essential to conserve the invertebrates that feed on plants. The use of broad-spectrum herbicides risks negative effects on the food chain of these herbivores and thus causing an imbalance in the community.

The herbicide Quinclorac (3, 7 - dichloroquinoline -8-carboxylic acid) has a relatively high persistence in rice crops compared to other herbicides (Reimche et al., 2008). Marchezan et al. (2003) found Quinclorac in the water of rivers in central Rio Grande do Sul in considerable concentrations, sufficient to harm the local benthic community.

Other products, that are widely used in irrigated rice fields in southern Brazil are the herbicides Bispyribac-sodium {sodium 2,6-bis-[(4,6-dimethoxypyrimidin-2-yl)benzoate} and a formulated mixture of the herbicides Imazethapyr and Imazapic [(RS)-5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid and (RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methyl nicotinic acid]. However, there are no data available about their effect on the environment (MAPA, 2009).

Considering that irrigated rice fields are large shallow lakes during the growing season, and tend to shelter a considerable aquatic fauna, studies in these areas can contribute considerably to elucidate the real impact of the agrochemicals used in this crop. The aim of this study was to investigate the impact of the use of commercial formulations of the mixture of the herbicides Imazethapyr + Imazapic (ONLY®), Bispyribac-sodium (NOMINEE 400SC), and Quinclorac (FACET PM) on the density and abundance of different groups of benthic organisms present in irrigated areas. The influence of agrochemicals on water quality as indicated by selected physical and chemical parameters, and the period of action of these agrochemicals on the community were evaluated.

## 2. Material and methods

The study was carried out in an experimental marshy area belonging to the Plant Technology Laboratory of the Federal University of Santa Maria, Rio Grande do Sul, Brazil, during the 2007/08 irrigated-rice growing season. The block was constituted by four plots (treatments) and each experimental unit measured 8 x 6 m, including refuge (48 m<sup>2</sup>). In these areas the following herbicides were used (treatments): TO - Imazethapyr (75.ha<sup>-1</sup>) + Imazapic (25.ha<sup>-1</sup>) (ONLY®); TB - Bispyribac-sodium(50.ha<sup>-1</sup>) (NOMINEE 400 SC); TQ - Quinclorac (375.ha<sup>-1</sup>) (FACET PM); and TC - Control.

The experimental units were separated by embankments with irrigation and individual drainage. The rice seed IRGA 422 CL was sowed at a density of 130 kg per ha, in a direct system, using a sowing machine with rows separated by 0.17 m. The fertilizer was applied according to the soil analysis and recommendations (SOSBAI, 2005). The herbicides were applied on the dry soil, with rice in the three-to-four-leaf stage.

The area was irrigated one day after the application of the post-emergent herbicides, and the water level was maintained at 0.10 m during the entire experiment. To avoid loss of water due to lateral infiltration, a wide guard embankment was constructed, keeping a canal with water between the plots and around the experiment, with the same hydraulic load as the plot.

The benthic macroinvertebrates were collected with the help of a cylindrical PVC sampler, 0.10 m in diameter (area 0.01 m<sup>2</sup>) and 0.10 m long. The collections were made 28 and 84 days after the entry of water into the plots; these dates correspond to the growing period and the pre-harvest (drying the area to begin the harvest). For each treatment and on each day, 12 samples were collected.

The sampling points, in each treatment, were chosen in a universe of 42 points that corresponded to one sample per m<sup>2</sup> of the area cultivated.

After the collection, the material was suitably packed, labeled, and taken to the laboratory, where it was washed in 0.25 mm sieves and repacked in plastic bottles, adding Rose Bengal stain. After 20 minutes in the stain, the material was fixed with absolute ethyl alcohol.

After fixing, the animals were sorted and identified to the lowest taxonomic level possible, using specialized references (Fernandez & Dominguez, 2001; Costa et al. 2006). For each sample, the population density of each taxonomic group was recorded. All the material was stored in the collection of the Carcinology Laboratory of UFSM. The species were separated into four trophic guilds: predator, detritivore, filter-collector or catcher-collector, and herbivore scraper or perforator. This classification was based on Merritt & Cummins (1996), Callisto and Esteves (1998), Marinoni (2001), and Silva et al. (2009a).

The water was collected for analyses of the physical and chemical parameters on days 3, 7, 14, 21, 28, 42, 56, and 84 after the plots were flooded, comprising the entire period of cultivation. In each sampling period, the following analyses were done: pH, with a Hanna pH meter (HI8424); total hardness, according to APHA (1992); and temperature and dissolved oxygen (YSI oximeter model Y5512). The turbidity was measured with a PoliControl turbidimeter.

During the cultivation, samples of water were collected 1, 2, 3, 7, 14, 21, 28, 42, 56, 77, 84, and 90 days after the plots were flooded; day 90 was the day of harvest. After each collection, the labeled samples were taken for chemical analysis in the Group of Research Analyses of Residues and Pesticides laboratories of UFSM. The concentrations of herbicides were determined by means of High Performance Liquid Chromatography, with detection by the arrangement of diodes (HPLC - DAD), using methanol and water as the mobile phase and a C-18 column, according to the method described by Zanella et al. (2002).

The physical and chemical data were evaluated through a two-way ANOVA (time and treatment), and the means were compared by a *t* test ( $p < 0.05$ ). A PCA analysis was used to assess possible correlations between the physical and chemical parameters and the pesticides applied in the field.

The density data for the main groups were submitted to a Shapiro-Wilk analysis, and after satisfying the criteria and/or were transformed for data standardization, they were submitted to a one-way ANOVA, and the means were compared by a *t* test ( $p < 0.05$ ). An ANOSIM was applied to assess if there was similarity in the macroinvertebrate community composition among the treatments and the sampling days. This test was also used to assess if there was similarity in the fauna composition of the trophic guilds among the treatments and the sampling days. The Bray-Curtis index was used to construct the similarity matrix. For these analyses, the programs PAST 1.82b (Hammer et al., 2001) and BioEstat 5.0 (Ayres et al., 2007) were used. To assess the period when the herbicides might affect the benthic community, a PRC (Principal Response Curve) was constructed, using the statistical program R (vegan package).

### 3. Results

#### 3.1 Abiotic data

The abiotic data were measured on specific dates, which covered the entire period of rice culture. The statistical analyses showed no differences among treatments, but there were differences among the sampling days (Table 1). The PCA showed no correlation among the parameters analyzed. The highest dissolved oxygen content was recorded on day 42,

11.90mg.L<sup>-1</sup>, in the treatment with Quinclorac. The pH remained between 6 and 7, during almost the entire experiment; although the variability was low, the differences among sampling days were significant (Table 1).

The water temperature varied from 34.2°C on the first day of experiment to 17.7°C on the last one, in January and April, respectively. Hardness was highest on days 21 and 28, with

	O <sub>2</sub> D (mg.L <sup>-1</sup> )							
	3 c	7 a	14 c	21 b	28 a	42 d	56 cd	84 a
TO	6.90	3.50	5.43	4.50	4.10	11.40	7.40	3.90
TB	6.50	3.60	7.27	6.00	2.90	11.10	7.30	3.40
TQ	6.60	4.10	5.98	6.30	4.50	11.90	6.60	3.30
TC	7.50	4.40	6.86	4.20	5.40	11.70	9.10	2.50
	pH							
	3 cd	7 e	14 de	21 ab	28 c	42 bc	56 bc	84 a
TO	6.81	7.30	7.08	6.83	6.73	6.60	6.40	6.37
TB	6.74	6.90	7.17	6.81	6.62	6.52	6.74	6.15
TQ	6.73	7.19	7.03	6.22	6.60	6.25	6.47	6.21
TC	6.78	7.04	6.82	5.98	6.58	6.44	6.49	6.12
	Water Temperature in °C							
	3 f	7 e	14 c	21 b	28 c	42 d	56 d	84 a
TO	34.20	26.67	22.38	20.23	22.13	23.77	23.60	17.80
TB	33.53	26.57	22.42	20.23	22.10	23.57	23.33	17.80
TQ	33.67	26.63	22.56	20.70	22.27	23.67	23.50	17.75
TC	33.23	26.63	22.50	20.70	22.70	23.57	23.53	17.70
	Hardness (mg.L <sup>-1</sup> de CaCO <sub>3</sub> )							
	3 a	7 bc	14 ab	21 c	28 c	42 <sup>nb</sup>	56 a	84 a
TO	17.00	32.00	28.00	40.00	40.00	36.00	24.00	36.00
TB	22.00	36.00	20.00	40.00	40.00	32.00	28.00	32.00
TQ	26.00	32.00	24.00	40.00	36.00	32.00	20.00	24.00
TC	22.00	24.00	24.00	36.00	32.00	36.00	20.00	24.00
	Turbidity (NTU)							
	3 c	7 c	14 bc	21 ab	28 c	42 c	56 c	84 b
TO	22.73	14.00	14.80	6.60	23.90	14.60	17.10	15.90
TB	17.00	18.20	17.60	4.52	23.10	24.20	28.90	8.47
TQ	18.77	17.50	18.40	4.86	18.50	17.00	15.40	6.19
TC	22.40	15.20	12.80	3.92	11.10	9.45	10.00	6.22

Table 1. Santa Maria, Brazil: Physical and chemical parameters analyzed in the irrigated rice field, on different days after flooding, during the 2007/08 growing season, in experimental plots in central Rio Grande do Sul. TO, treatment with the herbicide Only®; TB, treatment with the herbicide Bispyribac-sodium; TQ, treatment with the herbicide Quinclorac; TC, control treatment. Similar letters indicate statistical similarity.

40 mg. L<sup>-1</sup>, decreasing from day 42 on. Water turbidity ranged from 2.53 NTU on day 84 to 28.90 NTU on day 56 (Table 1).

3.2 Agrochemical persistence

Quinclorac showed the highest persistence (Table 2), and was detected until 84 days after the plots were flooded. On this date, however, its concentration was low and it was not detected on day 90, the day of harvest (on this day, the water sample was collected in the refuge). The herbicide Only® showed the lowest persistence, and was detected until day 21 (Table 2).

Treatments/ Days	Pesticides Concentration µg. L <sup>-1</sup>												
	0	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	7 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>	28 <sup>th</sup>	42 <sup>nd</sup>	56 <sup>th</sup>	77 <sup>th</sup>	84 <sup>th</sup>	90 <sup>th</sup>
TO	12.9	22.2	16.0	11.7	7.1	3.7	2.4	-	-	-	-	-	-
TB	8.7	20.8	12.9	10.5	8.9	4.3	1.8	1.2	0.3	0.2	-	-	-
TQ	138.8	296.7	173.3	107.6	47.0	39.2	38.0	28.2	20.6	9.8	4.9	3.8	-

Table 2. Santa Maria, Brazil: Mean concentrations of agrochemicals in irrigated rice plots during the 2007/08 growing season. TO, treatment with the herbicide Only®; TB, treatment with the herbicide Bispyribac-sodium; TQ, treatment with the herbicide Quinclorac. Day 0, flooding of the experimental plots, and then successive dates.

3.3 Benthic macroinvertebrates

A total of 3763 animals were identified, 1799 in the first collection (day 28) and 1964 in the second one (day 84). All the taxa identified in the first collection were present in the second. In the latter, some taxa were identified that were not present in the first collection (Table 3). In both collections the control treatment (TC) showed the highest density of organisms (no. ind. m<sup>-2</sup>), followed by TO, TB, and TQ (Table 4). However, regarding the abundance of taxa recorded in the second collection, the treatment with the most abundance was TO.

In relation to fauna composition (abundance and diversity), in the first collection the ANOSIM indicated a similarity in the community composition only between TC and TB and between TB and TO. In the second collection, all the treatments diffed among themselves and also differed from the first collection.

The ANOSIM, used to assess if there was similarity in the fauna composition (abundance and diversity) in each trophic guild when comparing the treatments, showed that some were similar. The treatment showing the highest differences was TQ. The data for the day 28 collection are given in Figure 1A, and the data from day 84 in Figure 1B. The results for each guild in the treatments also differed between collections; i.e., the guilds in TC in the first treatment showed a different community composition in the second, and the same occurred for TO, TB, and TQ.

The PRC showed that, in general, all the herbicides caused an initial stress on the benthic community, and this stress decreased with time. At the end of the cultivation, the community was structured again, except the community that received TQ which continued to be affected by this herbicide until the end of the cultivation.

4. Discussion

The results showed that the pesticides did not significantly alter the abiotic factors of the water used in the experiment, considering that there were no significant differences among



treatments, in the same collection. These results differ from those obtained by Faria et al. (2007), who reported that localities with high rates of contamination by pesticides and heavy metals, showed pH and O<sub>2</sub> values different from contamination-free areas or areas with low contamination, and attributed this difference to the presence of chemicals in the environment. Similarly, Schulz & Liess (1999), in a study of streams that supply water for the field, separated into low, medium, and high impact, also attributed to pesticides the differences found in the water quality. The influence of pesticides in reducing water quality was also reported by Molozzi et al. (2006), who evaluated irrigation and drainage water from rice fields.

In areas of rice cultivation, the oxygen concentration tends to be low, because the water depth is between 5 and 15 cm. In experiments carried out in the same area of the present study, in previous years the oxygen concentration was 0.6 to 2.2 mg. L<sup>-1</sup> (Golombieski et al., 2008) and between 2.4 – 4.6 mg.L<sup>-1</sup> (Reimche et al., 2008). In this experiment, the variation in the level of oxygen was 2.4 – 11.9 mg.L<sup>-1</sup>, and most days it was higher than 6 mg. L<sup>-1</sup>, a high level for the area. This may have favored an increase in the abundance obtained in the last collection, since some taxa are sensitive to low levels of dissolved oxygen.

The values of hardness and alkalinity were higher than in the present study compared to data from Golombieski et al. (2008) and from Reimche et al. (2008). A difference in the dissociation of ions of the compounds used in the experiments could explain this disparity. A factor that may also explain these results is that during the rice cultivation the temperature was decreasing, and the highest temperature occurred at the beginning of the study in January 2008, and the lowest temperatures in the last collection, in April. In the above-cited studies, the temperatures were lower than those recorded in this study, 16.4-23.9 °C (Reimche et al., 2008) and 17.6-25.7°C (Golombieski et al., 2008).

Another factor to consider is the relatively low persistence of the compounds used in this study. All the herbicides except Quinclorac showed lower persistence than found in some studies (USEPA, 1996; Stevens et al., 1998; Mesléard et al., 2005; Mize et al., 2008; Reimche et al., 2008). However, Quinclorac was at least 2.5 times more persistent than observed by Reimche et al. (2008).

An evaluation of the persistence of pesticides in water of rivers in the region found that the compounds forming the commercial formulation Only (Imazapic + Imazethapyr) were present in all the sampling periods (Silva et al., 2009b). These data differ from those obtained in this study, where the herbicide Only® was measurable until day 21. These results are similar to those found in the water of rivers in the region of Pelotas, Rio Grande do Sul, in a study carried out in 2007, where Quinclorac also persisted during the entire period of rice cultivation (Grutzmacher et al., 2008). It is possible that these differences in persistence are due to the management of the rice field. It may be advisable to maintain water in the field as long as possible (Marchesan et al., 2005; Grutzmacher et al., 2008). It is important to note that the studies cited here assessed the persistence of pesticides in the field water. If this persistence were analyzed in the sediment, the values might be different.

During the period of cultivation, the absolute abundance and diversity of the aquatic community tended to increase. Similar increases were found by other researchers (Schulz and Liess, 1999; Suhling et al., 2000). The most coherent explanation for this is that, as time passes, the pesticides become diluted and dissipate, thus decreasing their toxicity to the aquatic organisms (Schulz & Liess, 1999). However, Molozzi et al. (2007) did not record temporal differences in the benthic community in relation to the stages of the rice seedling.

The benthic macroinvertebrate community is composed of many taxa, among which one of the most studied is the insect family Chironomidae. Some genera of this family are considered plagues in rice fields, and because of this pesticides have been developed to minimize their effects. In the present study, all the herbicides tested, although they do not target these organisms, negatively affected the chironomid population. This influence was perceptible for the herbicide Only® in the first collection and Bispyribac-sodium in the second, and for Quinclorac in both. This influence was also evident in other taxa of the benthic community, mainly in the first collection when the agrochemicals were present in higher concentrations in the water.

The coleopterans Curculionidae, Dytiscidae, and Hydrophilidae, the dipterans Ceratopogonidae and Chironomidae, the hemipteran Corixidae, the odonate Aeshnidae, the trichopteran Odontoceridae, as well as the Collembola, Ostracoda, Annelidae, Nematoda, and the mollusc Ampullaridae could be classified as persistent. Only the mollusc family Planorbidae were classified as resilient. The other taxa must be considered occasional, since they either were not collected in TC or were collected only in the second sampling.

Quinclorac showed a different dynamic than that studied in the laboratory by Crosby (2003), where it showed low persistence. In the study by Reimche et al. (2008), this pesticide was detected until the 31st day, with its highest concentration observed on the 7th day with  $102 \mu\text{g.L}^{-1}$ . In the present study, its persistence was higher than the other pesticides investigated, being detected until day 84 in a concentration of  $3.8 \mu\text{g.L}^{-1}$  concentration. Its highest value was on the first day, with  $296.7 \mu\text{g.L}^{-1}$ . The negative influence of this pesticide was perceptible in this study, since in both collections the treated plot had the lowest abundance and diversity of taxa compared to the other treatments. This possible influence was also recorded by Reimche et al. (2008), where the cladoceran assemblage showed a density variation caused by the stress of the application of this herbicide.

The use of pesticides reduces the availability of food for the benthic macroinvertebrates and changes the structure of the algae community, and the toxic effect on these food resources may also be associated with this (Gagneten, 2002). Some studies have suggested the possibility that the toxic agents to non-target organisms, in the case of herbicides, are the adjuvants and the surfactants used in the commercial formulation of each pesticide (Tatum 2004; Kitulagodage et al., 2007). However, more studies taking these compounds into consideration separately are necessary.

In relation to the community composition, it was clear that the pesticides cause alterations in their composition. This effect was most apparent over the long term, even with the community recovery. According to Berezen et al. (2005), this potential relationship between contamination by pesticides and the community structure is based mainly on physiological differences that affect the life cycle and the species' mobility. Therefore, each pesticide has a different effect on the organisms.

In studies in rice fields in France, no significant difference was found between a conventional area of cultivation and an organic one for the family Chironomidae (Mesléard et al., 2005). This may have occurred due to a decrease of predators of this group. A decrease in the numbers of predators was also observed in the present study, and may have been responsible for the alterations recorded in the proportions among the trophic guilds analyzed. This may have made possible an increase in the density of detritivores, for instance.

The guild most injured by pesticides was the scrapers, which play an important role in this ecosystem since they feed on algae, bacteria, fungi and dead organic matter adsorbed on the substrate surface (Merrit & Cummins, 1996). This result is probably due to the action of the herbicides on the algae, which serve as food for these animals. Detritivores, which start their



feeding process after the action of microorganisms that make this food more palatable (Cummins et al., 1989), were recorded in low densities in the first collection; however, at the end of the culture their densities increased. Detritivores remained in low densities even at the end of the culture only in the TQ-treated plot, probably because of the effect of this chemical on the microorganisms needed to increase the palatability of the food. The data from this study, carried out in area of rice cultivation, reinforce the role of bioindicators performed by the benthic macroinvertebrates. In addition, as observed here, abiotic and biotic data not always give similar results when environmental matters are investigated. However, the combination of these two parameters of analyses can generate more substantial information on environmental impacts.

Taxa	Trophic guild	Sampling	TC	TO	TB	TQ
Artropoda						
Insecta						
Coleoptera						
Curculionidae	HR	28	X	X	X	X
		84	X	X	-	-
Ditiscidae	P	28	X	X	X	-
		84	X	X	-	-
Hidrophilidae	P	28	X	X	-	X
		84			X	-
Psephenidae	HR	28	-	-	-	-
		84	-	-	X	-
Diptera						
Ceratopogonidae	CF/ CC/ P	28	X	X	X	X
		84	X	X	X	X
Chironomidae	CF/ CC/ P	28	X	X	X	X
		84	X	X	X	X
Tabanidae	P	28		-		
		84	-	X	-	X
Tipulidae	P/ D	28	-	-	-	-
		84	-	-	X	-
Ephemeroptera						
Baetidae	CF/ CC	28	-	-	-	-
		84	-	-	-	X
Caenidae	CF/ CC	28	-	-	-	-
		84	X	-	-	-
Leptophlebiidae	CF/ CJ	28	-	-	-	-
		84	-	-	X	-
Leptohyphidae	CF/ CC	28	-	-	-	-
		84	-	X	X	-
Hemiptera						
Belostomatidae	P	28	-	-	-	-
		84	X	-	-	-
Corixidae	HR	28	-	-	-	-
		84	X	X	-	-

Pentatomidae	P	28	-	-	-	-
		84	-	X	-	-
Odonata						
Aeshnidae	P	28	X	-	-	X
		84	X	-	X	X
Coenagrionidae	P	28	-	-	-	-
		84	X	X	-	-
Lestidae	P	28	-	-	X	-
		84	-	-	X	-
Libellulidae	P	28	-	-	-	-
		84	X	-	-	-
Perilestidae	P	28	-	-	-	-
		84	-	X	-	-
Plecoptera						
Perlidae	P	28	-	-	-	-
		84	-	X	-	-
Trichoptera						
Odontoceridae	D	28	X	X	X	-
		84	X	X	X	-
Collembola	D	28	X	X	X	X
		84	X	X	X	X
Crustacea						
Ostracoda	D	28	X	X	X	X
		84	X	X	X	-
Arachnidae						
Aranae	P	28	-	-	-	-
		84	-	X	-	-
Hidracarina	P	28	X	-	-	-
		84	-	X	-	-
Annelida						
Hirudinea	P	28	X	X	X	-
		84	X	X	X	X
Oligochaeta	D	28	X	X	X	X
		84	X	X	X	X
Nematoda	P	28	X	X	X	X
		84	X	X	X	X
Mollusca						
Ampullaridae	HR	28	X	X	X	X
		84	X	X	X	X
Planorbidae	HR	28	X	-	-	X
		84	X	X	X	X

Table 3. Santa Maria, Brazil, 2007/08 growing season: Abundance of taxa identified in the area of irrigated rice cultivation with different treatments. HR: herbivore scraper, P: predator, CC: collector catcher, CF: filter collector, D: detritivore, TC: control treatment, TO: treatment with Only®, TB: treatment with Bispyribac-sodium, TQ: treatment with Quinclorac. “X” indicates the presence of a taxon in the plot, and “-” indicates its absence.

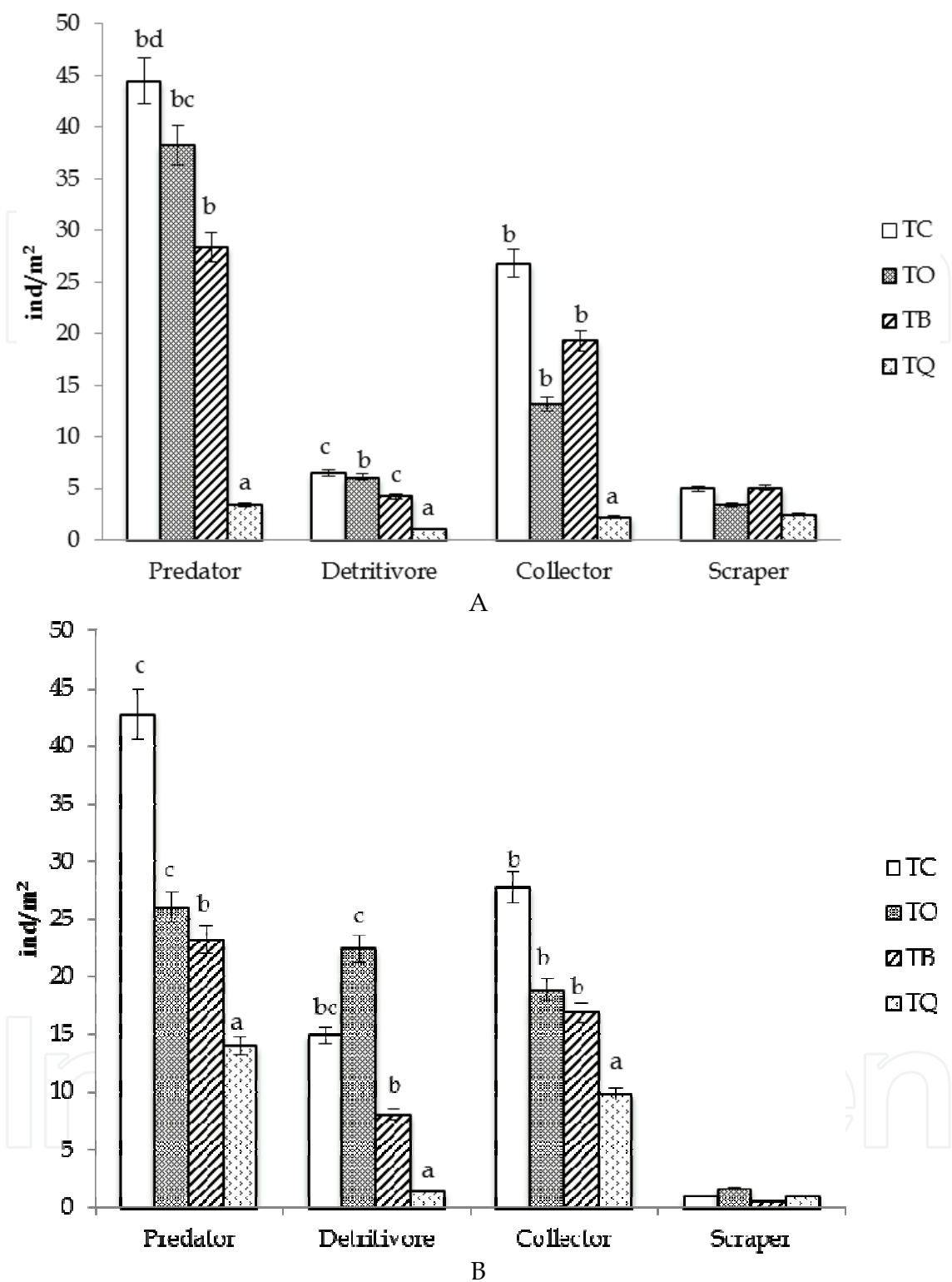


Fig. 1. Santa Maria, Brazil, 2007/08 growing season: Differences in the faunal composition in each trophic guild, evaluated by ANOSIM ( $p < 0.05$ ). Different letters indicate a statistical difference. A: collection 28 days after the experimental plots were flooded, B: collection 84 days after flooding. TC: control treatment, TO: treatment with the herbicide Only®, TB: treatment with the herbicide Bispyribac-sodium, TQ: treatment with the herbicide Quinclorac.

	Org. average density.m <sup>-2</sup>		Abundance of the taxa collected	
	28ºd	84ºd	28ºd	84ºd
TC	147.81	157.24	15	18
TO	126.54	134.87	12	21
TB	100.66	86.84	12	17
TQ	18.42	43.20	11	11

Table 4. Santa Maria, Brazil, 2007/08 growing season: mean densities (no. ind.m<sup>-2</sup>) and abundances of taxa identified in the experimental plots, by treatment.

5. Acknowledgments

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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