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Disturbance accumulation hampers fish assemblage recovery long after the worst mining spill in the Iberian Peninsula

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**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

De Miguel, RJ, Galvez-Bravo, L, Oliva-Paterna, FJ and Fernandez-Delgado, C (2016) Disturbance accumulation hampers fish assemblage recovery long after the worst mining spill in the Iberian Peninsula. JOURNAL OF APPLIED ICHTHYOLOGY. 32 (1). pp. 180-189. ISSN 0175-8659

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#### **TABLES**

**Table 1**. Selected environmental variables and regression model results for native (-na) and exotic (-ex) fish species richness (S) and Shannon's diversity (H) at the catchment (\_C) and site (\_S) scales at the Guadiamar River Basin eight years after a toxic spill (2006-2007). The first column shows the variables selected after variable selection. S.E. = standard error, p of model = probability, Adjusted R<sup>2</sup> = coefficient of determination, p = significance. RLCRU = River length covered by reservoirs upstream. \* = p < 0.05, \*\* = p < 0.01, \*\*\* = p < 0.001.

**Table 2.** Native (S-na) and exotic (S-ex) fish species richness and Shannon's diversity (H) values in each sampling site within the Guadiamar River Basin eight years after a toxic spill (2006-2007). The last two columns show fish species composition. Native species codes: *Luciobarbus sclateri* (L.s.), *Cobitis paludica* (C.p.), *Pseudochondrostoma willkommii* (P.w.), *Iberochondrostoma lemmingii* (I.1.), *Squalius alburnoides* (S.a.), *Squalius pyrenaicus* (S.p.), *Liza ramada* (L.r.), *Mugil cephalus* (M.c.). Exotic species codes: *Carassius gibelio* (C.g.), *Cyprinus carpio* (C.c.), *Gambusia holbrooki* (G.h.), *Lepomis gibbosus* (L.g.), *Micropterus salmoides* (M.s.).

**Table 3**. Mean and standard deviation (SD) values for native (-na) and exotic (-ex) fish species richness (S) and Shannon's diversity (H) sampled between 2006 and 2009 in the reach downstream from the a) the Agrio reservoir (Guadiamar River, SW Spain) and b)-g) six other reaches downstream from reservoirs in

watersheds within the northern Gualadquivir River Basin: Cala (Rivera de Cala River), Pintado (Viar River), Huesna (Rivera del Huesna River), Montoro (Montoro River), Rumblar (Rumblar River) and Fernandina (Guarrizas River). The geographic coordinates of each site are shown. n = number of available sites downstream from the reservoirs. \* = significant differences (p < 0.05) between the reach downstream from the reservoir and the selected Guadiamar reach according to post-hoc t-tests.

## Table 1

	Selected variables for the model	Significant variables selected by models and percentage of variance explained after variance partitioning	Adjusted R <sup>2</sup>	p of model	Estimate (coefficient)	S.E.	Student t_value	р
S-na	Catchment uses, No. reservoirs upstream, Drainage area, Habitat, Distance from mouth, Riparian vegetation width	Catchment uses (33%) No. reservoirs upstream. (28%) Drainage area (20%) Habitat (19%)	0.70	0.0001	0.67 -1.98 0.01 0.38	0.1614 0.3455 0.0013 0.1516	4.17 -5.74 4.99 2.53	0.0006 *** 0.0001 *** 0.0001 *** 0.0215 *
S-ex	Catchment uses , Drainage area, Habitat, Riparian vegetation width, Mean valley width, RLCRU	Mean valley width	0.42	0.0007	0.01	0.0001	3.99	0.0007 ***
H-na	Catchment uses , No. reservoirs upstream, Drainage area, Habitat, Distance from mouth, Riparian vegetation width	Catchment uses (46%) No. reservoirs upstream (31%) Drainage area (23%)	0.52	0.0009	0.21 -0.52 0.01	0.0474 0.1198 0.0004	4.59 -4.36 3.79	0.0002 *** 0.0003 *** 0.0013 **
S-na_C	Catchment uses, Drainage area	Catchment uses	0.23	0.0129	0.47	0.1757	2.72	0.0129 *
H-na_C	Catchment uses, Drainage area	Catchment uses	0.11	0.0688	0.10	0.0522	1.92	0.0688 .
S-na_S	No. reservoirs upstream, Habitat, Distance from mouth, Riparian vegetation width, Site uses, Distance to source	Habitat (45%) Distance to source (23%) No. reservoirs upstream (21%) Site uses (11%)	0.53	0.0015	0.66 0.01 -1.46 -0.41	0.1481 0.0001 0.3837 0.1879	4.48 3.94 -3.81 -2.19	0.0003 *** 0.0010 ** 0.0013 ** 0.0421 *
S-ex_S	Habitat, Riparian vegetation width, Mean valley width, RLCRU	Mean valley width (55%) RLCRU (45%)	0.53	0.0001	0.01 0.01	0.0001 0.0001	2.91 2.43	0.0089 ** 0.0248 *
H-na_S	No. reservoirs upstream, Habitat, Distance from mouth, Riparian vegetation width, Site uses, Distance to source	Distance to source (54%) No. reservoirs upstream (46%)	0.35	0.0462	0.01 -0.43	0.0001 0.1263	3.62 -3.41	0.0025 ** 0.0038 **

Table 2	2
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Sampling sites	S-na	S-ex	Η	Native Species	Exotic Species
1	2	1	0,11	L.s., C.p.	G.h.
2	0	0	0,00		
3	3	0	0,30	L.s, C.p., P.w.	
4	1	0	0,00	L.s	
5	5	0	0,97	L.s, I.a., C.p., P.w., S.p.	
6	5	0	0,94	L.s, I.a., C.p., P.w., S.p.	
7	6	2	1,60	L.s, I.a., C.p., P.w., S.p., I.l.	G.h., M.s.
8	4	1	1,30	L.s, I.a., C.p., P.w.	L.g.
9	1	2	0,00	L.s,	L.g., C.g.
10	0	0	0,00		
11	3	3	0,29	L.s, I.a., P.w.	L.g., M.s., C.c
12	1	1	0,00	I.a.	G.h.
13	1	0	0,00	C.p.	
14	1	0	0,00	L.s.	
15	0	0	0,00		
16	0	0	0,00		
17	0	0	0,00		
18	1	0	0,00	C.p.	
19	1	1	0,00	L.s	C.g.
20	1	0	0,00	L.s	
21	0	1	0,00		G.h.
22	3	4	1,10	L.s., L.r., M.c.	L.g., C.g., C.c., C

## Table 3

	a) Guadiamar-Agrio (n = 5) (37°31'N, 6°17'W) Mean+SD	b) Cala (n = 2) (37°43'N, 6°05'W) Mean+SD	c) Pintado (n = 4) (37°59'N, 5°57'W) Mean+SD	d) Huesna (n = 3) (37°46'N, 5°41'W) Mean+SD	e)Montoro (n = 8) (38°31'N, 4°05'W) Mean+SD	f) Rumblar (n = 2) (38°09'N, 3°48'W) Mean+SD	g) Fernandina (n = 2) (38°10'N, 3°34'W) Mean+SD
S-na	1.20±1.09	3.00±0.00*	3.75±0.95*	3.00±1.00	1.50±1.19	0.50±0.71*	0.00±0.00
H-na	$0.28 \pm 0.47$	0.29±0.19	0.83±0.35	0.53±0.46	0.22±0.27	$0.00 \pm 0.00$	$0.00 \pm 0.00$
S-ex	$2.00{\pm}1.58$	$1.00 \pm 0.00$	$0.25 \pm 0.50$	$1.00 \pm 1.00$	$2.37 \pm 1.85$	$1.50 \pm 2.12$	$1.00{\pm}1.41$
H-ex	$0.43 \pm 0.46$	$0.00 \pm 0.00$	$0.00 \pm 0.00$	$0.23 \pm 0.40$	0.49±0.53	$0.24 \pm 0.34$	0.16±0.22

#### FIGURE CAPTION

**Figure 1**. Location of the Guadiamar River Basin (SW Spain) and the 22 sampling sites were fish species richness and diversity (Shannon's index H) were sampled in 2006-2007, eight years after a toxic spill.



Figure 1

### Appendix

Appendix 1. Environmental variables (62) registered at the site and catchment scale at the Guadiamar River Basin eight years after a toxic spill (2006-2007). (†) variables taken in situ and (‡) variables registered by GIS. The first column at each scale shows the variables not summarized in PCAs. \*Water quality: 1 (low organic and inorganic pollution) – 4 (high organic and inorganic pollution). \*\* Index based on slope, vegetation cover, height and substrate of the river bank.

# Appendix 1

	CATCHMENT SCALE				
	Included in PCA1	Included in PCA2	Included in PCA3	Not included in	Included in PCA4
Not included in PCAs	(Habitat)	(Bank stability)	(Site uses)	PCAs	(Catchment uses)
Mean channel width (m)†	Surface occupied by each type of mesohabitat (m <sup>2</sup> ) <sup>†</sup>	Runoff (0-1)†	% Native forests †	Drainage area (km <sup>2</sup> )‡	% Native forests‡
Reach length (m)†	% each type of inorganic substrate in riffle, run or pool †	Livestock access (0-1)†	% Low disturb Runoff natural areas †	Mean catchment slope (%)‡	% Native shrub or grassland‡
Mean valley width (m) <sup>‡</sup>	% organic substrate in runs and pools †	Human access (0-1)†	% Non-irrigated crops †		% Grazed fields‡
Riparian vegetation width (m)‡	% of the reach occupied by riffles, run or pool †	Ford, culvert or bridge (0-1) <sup>†</sup>	% Irrigated crops †		% Wetlands‡
Canopy cover (m <sup>2</sup> )†	Surface area occupied by riffles and runs (m <sup>2</sup> )†	Bank deforestation (0-1)†	% Forestry †		% Recreational areas‡
Distance to source (m) <sup>‡</sup>	Number of riffles †	Water extraction (0-1) <sup>†</sup>	% Recreational areas †		% Non-irrigated crops‡
River length covered by reservoirs upstream (m)‡	Number of runs †	Gravel or sand extraction (0-1) <sup>†</sup>	% Urban, industrial, intensive agriculture †		% Irrigated crops‡
No. obstacles downstream ‡	Number of pools †	Drain pipes (0-1)†			% Urban areas‡
No. reservoirs upstream ‡	Mean depth in runs (m)†	Other erodibility factors (0-1) <sup>†</sup>			% Industrial‡
Distance from mouth (m)‡	Mean pool depth (m)†	% Cobbles embeddedness in rifles †			% Mining‡
Distance to the nearest reservoir upstream (m)‡	Emerged aquatic vegetation (%)†	Bank stability Index † **			
No. reservoirs downstream ‡	Black underside in cobbles (0- 1)†				
Distance to the nearest reservoir	Submerged aquatic vegetation				
downstream (m)‡	(%)†				
Altitude (m.a.s.l.)‡	Floating aquatic vegetation <sup>†</sup>				
Upstream order (Strahler)‡	Conductivity ( $\mu$ S cm <sup>-1</sup> )†				
Water quality(1-4)	Cover of wood or rock shelters $(m^2)$ <sup>+</sup>				