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The paradox of the conservation of an endangered fish species in a Mediterranean region under agricultural intensification

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ABSTRACT

We studied the relative value of natural habitats, river and coastal wetlands, and artificial habitats, irrigation canal and ponds, for the conservation of an endangered fish, Iberian toothcarp, in its southernmost area of distribution, characterised by agricultural intensification. Our results show that the bulk of the population of the Iberian toothcarp is concentrated in irrigation ponds. Natural habitats sustained null or impoverished subpopulations, and individuals showed signs of low metabolic activity. This coincided with the relatively high habitat quality observed in ponds, particularly those with submerged aquatic vegetation, in contrast with the chronic eutrophication of the coastal lagoons. In spite of a generalised aggressive management in the irrigation system, featured by periodic vegetation clearance, desiccation and biocide treatment, the subpopulation of the Iberian toothcarp thrives in it probably thanks to adequate water quality and to an active path dynamics maintained by connectivity through the canal. Agro-environmental measures are discussed for the improvement of this species conservation in natural and artificial habitats.

1. Introduction

Continental aquatic ecosystems are experiencing declines in biodiversity far greater than those in the most affected terrestrial ecosystems (Ricciardi and Rasmussen, 1999; Sala et al., 2000; Xenopoulos et al., 2005; Dudgeon et al., 2006). As pointed out by Dudgeon et al. (2006), conservation of biodiversity in rivers, lakes and wetlands is complicated by the landscape position of these as 'receivers' of land-use effluents, and the problems posed by endemism and thus non-substitutability. Initiatives of legal protection of aquatic ecosystems have failed because of the complexity of changing or correcting human activities at the catchment-wide scale, particularly in semiarid regions where water is subject to severe competition among multiple stakeholders (e.g. Amezaga and Santamaria, 2000).

In Mediterranean regions wetland loss – in excess of 50% (Hollis, 1992) – and impairment have occurred primarily due to the expansion of irrigated agriculture, which in turn gave rise to the construction of thousands of irrigation facilities (e.g. Casas et al., 2010). Frequently, canals and ponds for irrigation receive water of good quality (e.g. Bonachela et al., 2007) that might generate valuable habitats for biodiversity conservation. As a matter of fact, in agricultural landscapes of Britain, Williams et al. (2003) found that ponds contributed most to macroinvertebrate biodiversity, supporting considerably more species, more unique species and more scarce species than other water body types on the regional scale. However, recent studies carried out in intensive agriculture irrigation ponds of South-eastern Spain revealed that the potential to harbour biodiversity of these water bodies may be substantially constrained by an altered hydrological regime, the type of construction and the incidence of aggressive management to which ponds may be subjected (e.g. Abellán et al., 2006; Sebastian-González et al., 2010; Casas et al., 2010). Therefore, a common conclusion of these studies, also in other regions (e.g. Bellio et al., 2009), is that irrigation structures are not adequate replacements for loss of natural wetlands.

The Iberian toothcarp, *Aphanius iberus* (Cuvier and Valenciennes, 1846), is a cyprinodontid fish endemic to the Mediterranean coast

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of Spain. At present, *A. iberus* is considered in danger of extinction by the Spanish National Catalogue of Endangered Species, by the Convention on the Conservation of European Wildlife and Natural Habitats (Doadrio, 2002) and by the IUCN red list (IUCN, 2008). This is due to habitat loss and impairment as well as to the introduction of exotic fish species which out-compete *A. iberus* (e.g. Oliva-Paternaa et al., 2006). This study is designed to test whether irrigation systems may serve as valuable habitats, compared to natural ones, for the conservation of this species in its southernmost area of distribution, the lower River Adra (Almería). This is one of Europe's driest regions which has, paradoxically, become one of the continent's most important horticultural areas. For the last 30 years it has endured a net water abstraction overdraft leading to serious reservoir depletion, groundwater imbalances, and wetland loss and impairment primarily due to an exponential increase in agricultural demands (Casas et al., 2003; Downward and Taylor, 2007). Our specific objectives were to: (1) Document habitat quality in irrigation facilities compared to natural habitats, coastal lagoons and river, (2) Determine whether differences in quality between habitats affect population parameters and individual metabolic stage of *A. iberus*, and in the light of these results, (3) Provide recommendations for agro-environmental schemes to allow compatibility between the conservation of *A. iberus* and agricultural activities.

2. Materials and methods

2.1. Study area

Our study focused on the southernmost distribution area of *A. iberus* (Doadrio, 2002), the lower basin of the River Adra, below the Benínar Dam (Fig. 1). The climate is semiarid Mediterranean, with low annual rainfall (250 mm) and warm (mean annual temperature 18 °C). The river drains a basin of 742 km^2, with headwaters located around 2500 m a.s.l. in the Sierra Nevada. In 1982 the Benínar Dam was built for irrigation and urban supply purposes. Below the dam the river bed dries-up, but 6 km downstream, due to a spring (Fuentes de Marbella) the river reappears. This spring maintains a permanent flow along a reach of ca. 8 km, at the end of which river discharge is completely impounded. This permanent reach, at present fully invaded by *Arundo donax,* was included in the Natura Network 2000 in Spain (Habitat Directive 92/43 EEC), because it was considered crucial for the conservation of *A. iberus* in Andalusia (Southern Spain).

The Albufera de Adra is made up of three coastal lagoons located in the river delta (Fig. 1). Due to its ecological importance for the conservation of endangered waterfowl and *A. iberus*, the Andalusian Regional Government declared it a Natural Reserve in 1989. Since 1994 it has been included on the Ramsar Convention list of Protected Areas. However, land reclamation for agriculture in this delta became critical as highly profitable greenhouse horticulture developed rapidly during the 1970–1980s. This reclamation programme reduced the size of the lagoons and impaired water quality due to eutrophication (Cruz-Pizarro et al., 2002; de Vicente et al., 2003).

Irrigation ponds in our study area were mainly fed by water impounded from the River Adra distributed along several canals. However, at present most of these canals has been replaced by piped waterways. Only the San Fernando Canal, on the eastern side, is still in use (Fig. 1), however, due to increasing water demands and shortages, the canal is rarely fed by the river and mainly receives groundwater pumped up from the detrital aquifer of the lower reach of the river. All studied ponds are made of concrete and are above-ground level, which prevent the reception of agricultural drainage directly. Several morphometric characteristics of the studied ponds, canal, river and coastal lagoons are given in Table 1.

2.2. Habitat characterisation

We sampled those habitats where *A. iberus* was reported to be distributed in a qualitative study carried out one decade ago (Nevado and Paracuellos, 1999). Natural habitats were the permanent reach of the lower River Adra, downstream of Benínar Reservoir, and the three coastal lagoons of Albuferas de Adra. Eight and nine sites were sampled in the river and the lagoons, respectively. In the river, sampling sites were regularly distributed along its permanent reach (Fig. 1), and in the lagoons we assigned the number of sites surveyed per lagoon depending on size (Table 1): four sites in Laguna Nueva, three sites in Laguna Honda and two sites in Laguna Cuadrada (Fig. 1). Artificial habitats were classified in three types: the irrigation canal where seven sites were studied, six irrigation ponds with a submerged aquatic vegetation (SAV) cover of over 50%, and 11 irrigation ponds free of SAV (Fig. 1). All ponds were fed with water from the studied canal.

During the period July–August 2008, habitat quality was characterised by measuring the following abiotic and biotic parameters at each site. Water pH and electric conductivity were measured using specific field probes (WTW® model 340i, Germany). Dissolved oxygen was measured using an optical field probe (Hach® model HQ-30d, USA) in the morning (7–9 h) and afternoon (15–17 h), averaging measurements at depths intervals of 0.25 m whenever possible. Planktonic chlorophyll-*a* was determined by the trichromatic method using alkaline acetone extracts (Wetzel and Likens, 1991). Water samples were filtered (Whatman® GF/F) to measure alkalinity (titration to a pH of 4.5) and nutrients. Total dissolved phosphorous (TDP) and total dissolved nitrogen (TDN) were measured on potassium persulphate digested water samples, using the molibdene-blue (Wetzel and Likens, 1991) and the ultraviolet (APHA, 1992) methods, respectively. Sediment samples, obtained integrating three sub-samples randomly collected, were dried at room temperature, sieved (1 mm), grinded and emulsified before being analysed using X-ray fluorescence for heavy metals, Cu and Mn, contained in biocides used in ponds and canal (see below). Percentage cover of submerged aquatic vegetation (SAV) and % perimeter covered by emergent marginal vegetation (EMV) was determined using quadrat frames and optical distance meters, respectively.

2.3. Growers interviews to characterize artificial habitat management

Interviews were organized with each of the growers to gain knowledge about the management of their ponds, and with managers of the canal. All questionnaires were conducted by a common interviewer (MJ), ensuring a standardized approach to data collection. Because the interviewer was present while the questionnaire was completed, any ambiguous question was carefully explained and all questions were answered. The interviewer was also able to further explore answers that lacked precision (Benthan and Moseley, 1982). The interviews were structured mainly around questions on management topics established from preliminary interviews which determined that the common management practices were biocide treatment and periodic cleaning. Therefore, the final questionnaire contained the following questions: water body age, reasons for management processes; type, frequency and biocide dosage; type, frequency and time elapsed since the cleaning.

2.4. Fish sampling and population characterisation

Fish sampling was carried out using plastic minnow traps made out of 2 L plastic soda bottles. This trap type is recommended as the most appropriate method for sampling small fish populations

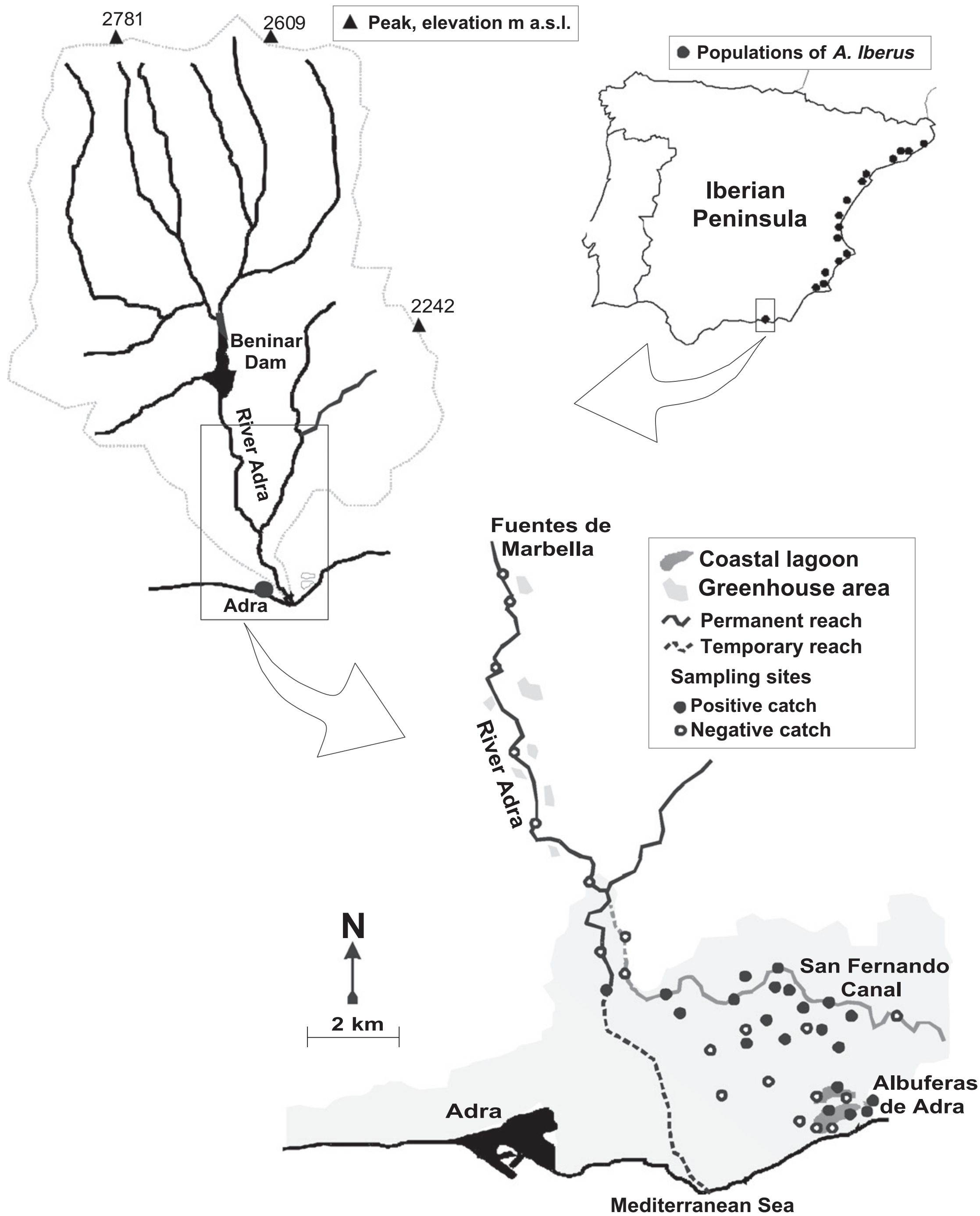

Fig. 1. Map of the study area in South-eastern Spain, the southernmost distribution area of *A. iberus*. Location of sampling sites is shown, with indication if positive or negative catches were obtained.

compared to large-meshed traps (Clavero et al., 2006). At each site and sampling date, four traps were set on the bottom, separated between 4 and 6 m, and anchored to the bank. Traps were always set in pools of the river and the canal, in order to maintain comparative flow conditions with other habitats. All traps were baited with tinned cat food. Each site survey included at least one daytime and one night-time period. The mean trapping time was 2.6 h (±1.6 SD). Due to the low yield of fish traps in natural habitats and in the canal we increased the sampling effort in these habitats, carrying out on average 3.3 (±2.1 SD) and 1.5 (±1.1 SD) surveys per site, respectively. Captured fish in each trap were deposited separately on a scaled white tray, where a digital image was taken for later counting, sex identification and length measurement using the software SigmaScan Pro 5.0 (SPSS Inc., 1999), and released. An aliquot (⩽10% total sample, agreed on with the Andalusian Environmental Protection Agency) of both sexes and different size-classes was retained, frozen in liquid nitrogen and preserved at −80 °C until subsequent biochemical analyses.

2.5. Biochemical analyses

Biochemical analyses were only carried out on individuals from lagoons and ponds, due to the insufficient material obtained in the river and canal. Samples were homogenized in ice-cold buffer

Table 1
Selected morphometrical characteristics of habitats (mean ± 1 SEM, range between parentheses). Surface area is given for each of the three lagoons. Data of lagoon depth correspond to the littoral stratum where samples were taken.

Parameter	Coastal lagoons			River	Canal	Ponds with SAV	Ponds without SAV
	Nueva	Honda	Cuadrada				
Surface (m^2)	$10^4 \times 26$	$10^4 \times 8$	$10^3 \times 2$			278 ± 59 (140–530)	208 ± 32 (121–361)
Channel width (m)				3.9 ± 0.8 (0.5–6.9)	1.3 ± 0.3 (0.5–3.3)		
Depth (m)	1.5 ± 0.2 (0.8–1.7)	1.2 ± 0.1 (0.7–1.4)	1.1 ± 0.2 (0.8–1.3)	0.5 ± 0.1 (0.1–0.7)	0.4 ± 0.3 (0.1–0.9)	1.6 ± 0.3 (0.8–2.4)	1.9 ± 0.2 (1.0–2.9)
Age (years)					71	21 ± 2 (15–24)	20 ± 2 (11–27)

(100 mM Tris–HCl, 0.1 mM EDTA and 0.1% triton X-100 (v/v), pH 7.8) at a ratio of 1:4 (w/v). Homogenates were centrifuged at 30,000*g* for 30 min, and the supernatant was collected for analyses. All enzymatic assays were carried out at 25 ± 0.5 °C using a PowerWave$_x$ microplate scanning spectrophotometer (Bio-Tek Instruments, USA) in duplicate in 96-well microplates (UVStar®, Greiner Bio-One, Germany). The enzymatic reactions were started with the addition of the tissue extract, except for SOD where xanthine oxidase was used.

Catalase (CAT; EC 1.11.1.6) activity was determined according to Aebi (1984) with modifications (Trenzado et al., 2006). Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured following the procedure of McCord and Fridovich (1969) with modifications (Trenzado et al., 2006). Glutathione peroxidase (GPX; EC 1.11.1.9) activity was measured according to Flohe and Günzler (1984) with some modifications (Trenzado et al., 2006). Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as described by Calberg and Mannervik (1975) with some modifications (Trenzado et al., 2006). Glutathione transpherase (GST; EC 2.5.1.18) activity was determined by the method of Habig et al. (1974) adapted to microplate. DT-diaphorase (DTD; EC 1.6.99.2) activity was measured as described by Sturve et al. (2005). The reaction mixture contained 50 mM Tris–HCl (pH 7.6), 50 μM 2,6-dichlorophenol indophenol (DCPIP) and 0.49 mM NADH. A control reaction (three measures average) with distilled water was subtracted to tissue sample reactions to determine DTD activity. One unit of activity (U) was defined as the amount of enzyme necessary to produce 1 μmol of substrate min^{-1}. For SOD was defined as the quantity of enzyme necessary to produce 50% inhibition of the ferricytochrome *c* reduction. Tissue extracts protein content was determined according to Bradford (1976) using bovine serum albumin as the standard. Lipid-peroxidation levels were determined according to Buege and Aust (1978). Total lipids were measured gravimetrically after two extractions by homogenisation in a warring blender for 3 min with chloroform/methanol (2:1, v/v) and later purification.

All biochemicals, including substrates, coenzymes, and purified enzymes, were obtained from Roche (Mannheim, Germany) or Sigma Chemical Co. (USA).

2.6. Statistical analysis

To explore multivariate environmental relationships among habitats, we carried out a standardized PCA-analysis on the entire set of measured environmental variables. Analysis of variance (ANOVAs) was used to test for significant differences of environmental variables among habitat types. Tukey HSD tests for unequal sample size were carried out for post-hoc comparisons between pairs of habitats. Forward stepwise discriminant function analysis (DA) was used to test the consistency of site groups. Squared Mahalanobis distances of each site from each group centroid were computed, and the environmental variables – of those with the highest load on any of the principal components extracted by PCA – which were the best predictors for habitat types were determined. ANOVAs and DA were performed on transformed variables, except pH, to make the variances homocedastic, using ln $(x + 1)$, and arcsin $\sqrt{x}$ for percentages. The variables %SAV and %EMV were not included in DA since they showed zero variance for several groups.

The size structure of *A. iberus*, females and males separately, was compared between pairs of habitat using the Kolmogorov–Smirnov two-sample test, which is sensitive to differences in the general shapes of the distributions in two samples (Sokal and Rohlf, 1981). Sex ratio (♂:♀) in each habitat type was compared with a balanced sex ratio (50:50) using chi-square (χ^2) test. Level of significance was accepted at $p < 0.05$.

Results of mean catch per unit effort (CPUE), and biochemical species were analysed by a one-way analysis of variance (ANOVA). Significant differences between means of habitat types ($p < 0.05$) in the same variable were determined by post-hoc Tukeys' tests for unequal sample size.

The statistical analyses were performed in STATISTICA 7.1 (StatSoft, 2005).

3. Results

3.1. Habitat characterisation

Principal Component Analysis (Fig. 2) and ANOVAs (all $p < 0.01$), followed by Tukey's post-hoc test (Table 2), revealed conspicuous environmental differences among habitat types. PCA extracted four PCs which accounted for 82.2% of total variance. PC1 (45% expl. var.) showed high positive loading and important covariation of %SAV cover and dissolved oxygen (morning and afternoon measurements), distinguishing between ponds, canal, and river sites with SAV and oversaturated oxygen concentrations compared to sites without SAV, particularly lagoon sites, which always showed the lowest oxygen concentrations (Table 2). Trophic level variables, Chl *a* and TDP, heavily loaded on positive PC2 (17% expl. var.), where most sites of coastal lagoons were clustered as eutrophicated (Table 2). Copper concentration in sediment loaded on the positive PC3 (11.8% expl. var.) dimension, where ponds without SAV were particularly segregated. On average, all artificial habitats showed significantly higher mean copper concentration in sediment than natural ones (Table 2). PC4 (8.7% expl. var.) was defined mostly by TDN in its positive dimension, where most artificial-habitat sites were arranged. The lagoons showed significantly lower TDN concentrations compared to pond and canal sites (Table 2), despite receiving nitrogen lixiviates from the surrounding greenhouses. This suggests that substantial denitrification may occur in the lagoons.

The model provided by DA included the four variables with the highest load on each one of the PCs extracted by PCA, as significantly discriminating habitat types (global Wilks' $\lambda = 0.013$; $F = 19.33$, $p < 0.00001$). In order of decreasing contribution to the

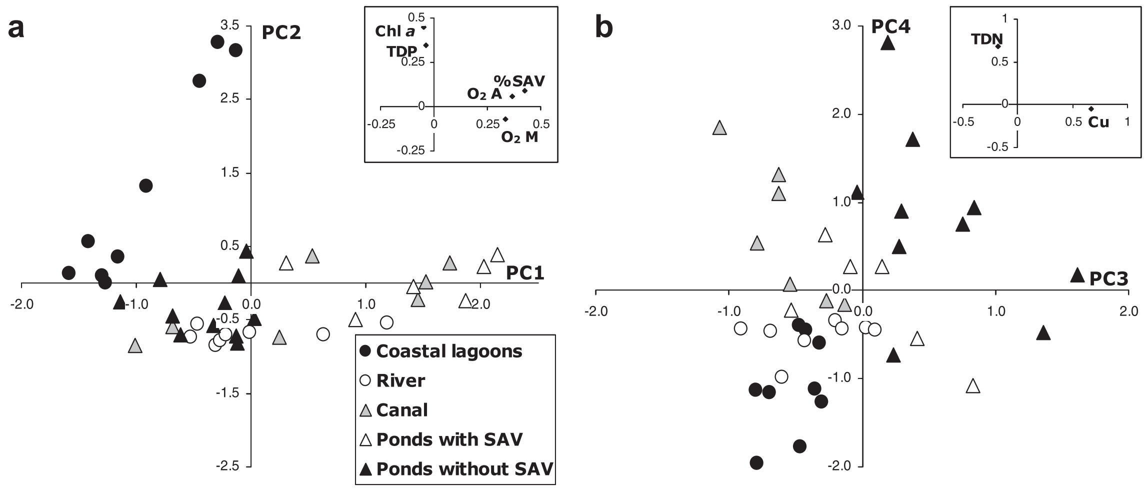

Fig. 2. Results of a standardized Principal Components Analysis on 12 environmental variables; (a) biplot PC1 × PC2, and (b) biplot PC3 × PC4. Symbols in the large plots represent scores of sampling sites of habitat types. Small plots represent scores of environmental variables with loading > 0.8 in any PC. PC1 – eigenvalue = 4.92; PC2 – eigenvalue = 1.87; PC3 – eigenvalue = 1.29; PC4 – eigenvalue = 0.96. O_2 M: Morning dissolved oxygen; O_2 A: Afternoon dissolved oxygen; %SAV: %Coverage of submerged vegetation; TDP: total dissolved phosphorus; Chl *a*: Chlorophyll concentration of phytoplankton; Cu: copper concentration of sediment; TDN: total dissolved nitrogen.

Table 2
Observed variation (mean ± 1 SEM, range between parentheses) in environmental variables among the 5 habitats of *A. iberus* studied in the lower basin of River Adra (Almería, Southern Spain). Repeated sampling occasions indicated after variable. Different letter indicates statistically significant differences ($p < 0.05$) after ANOVAs and Tukey's post-hoc tests.

Parameter	Coastal lagoons	River	Canal	Ponds with SAV	Ponds without SAV
pH (3×)	8.3 ± 0.1^a (7.8–8.9)	7.8 ± 0.1^a (7.5–8.1)	7.9 ± 0.1^a (7.3–8.5)	8.4 ± 0.2^a (8.0–9.2)	8.3 ± 0.1^a (7.8–9.1)
EC (3×) (mS cm^{-1})	7.1 ± 1.6^a (2.1–11.3)	2.5 ± 0.0^b (2.4–2.5)	2.1 ± 0.1^b (1.3–2.5)	2.0 ± 0.1^b (1.8–2.2)	2.4 ± 0.1^b (2.0–3.2)
Alkalinity (2×) (meq l^{-1})	8.9 ± 1.2^a (4.6–10.1)	4.8 ± 0.1^b (4.6–4.9)	4.3 ± 0.2^b (3.7–4.5)	4.1 ± 0.2^b (3.8–4.4)	4.7 ± 0.2^b (4.1–5.2)
TDP (2×) (μg l^{-1})	53.7 ± 6.9^a (30–82)	12.7 ± 0.8^b (10–16)	17.8 ± 3.1^b (13–36)	14.1 ± 1.9^b (9–21)	22.3 ± 4.3^b (8–53)
TDN (2×) (mg l^{-1})	2.0 ± 0.1b^c (1.3–2.3)	0.9 ± 0.1^c (0.6–1.2)	5.2 ± 0.7^a (2.1–7.3)	3.1 ± 0.6^{ab} (1.1–4.5)	3.9 ± 0.8^{ab} (1.0–10.9)
O_2 M (2×) (mg l^{-1})	5.5 ± 0.4^b (3.0–6.5)	8.7 ± 0.2^a (7.9–9.5)	8.7 ± 0.1^a (8.4–9.2)	10.2 ± 0.2^a (9.7–10.8)	8.5 ± 0.2^a (7.1–10.1)
O_2 A (2×) (mg l^{-1})	6.8 ± 0.4^c (4.8–8.4)	10.3 ± 0.6^b (8.7–13.3)	14.3 ± 1.2^a (9.7–17.9)	14.8 ± 0.7^a (13.0–17.1)	10.3 ± 0.4^b (7.7–12.7)
Chl *a* (2×) (μg l^{-1})	65.3 ± 20.4^a (20–172)	0.7 ± 0.2^c (0–2)	1.5 ± 0.5^c (0–4)	4.9 ± 1.1^{cb} (2–8)	9.0 ± 2.2^b (3–25)
%SAV	0.0 ± 0.0^c (0–0)	16.9 ± 10.8^b (0–80)	47.0 ± 15.4^{ab} (0–95)	80.2 ± 8.0^a (55–100)	0.0 ± 0.0^c (0–0)
%EMV	95.1 ± 2.4^a (76–100)	33.2 ± 11.8^b (0–98)	7.0 ± 3.4^c (0–27)	1.0 ± 1.0^c (0–6)	0.0 ± 0.0^c (0–0)
Cu (mg kg^{-1})	82 ± 21^b (19–216)	39 ± 1^b (34–43)	542 ± 195^a (113–1630)	795 ± 303^a (362–1989)	4737 ± 1925^a (100–24,630)
Mn (mg kg^{-1})	801 ± 74^a (495–1120)	462 ± 30^{ab} (341–566)	340 ± 36^b (204–521)	91 ± 17^c (53–150)	190 ± 51^c (38–658)

discriminant function – the lower Wilks' λ the higher the contribution – the predictor variables included in the model were: TDN ($\lambda = 0.024$), Cu ($\lambda = 0.033$), DO afternoon ($\lambda = 0.038$) and Chl *a* ($\lambda = 0.052$). The squared Mahalanobis distances indicated that all sites were correctly classified in its assigned, a priori, habitat type, except for one pond site in each one of the two groups differentiated, which instead were clustered in the "canal" group.

3.2. Management of ponds and the canal

Canal managers and pond owners focus their management on the control of SAV, with periodical, mechanical removal and biocide treatment. The canal was managed evenly along its length, with 1 application of copper sulphate per year and frequent mechanical removal of SAV (Table 3), due to its small flow capacity and rapid saturation with biomass of fast growing forms (fennel pond weed and filamentous algae). Pond cleaning was less frequent compared to the canal, and the common method used by growers entailed pond drying and vegetation-sediment removal. Ponds without SAV were cleared and dredged more frequently and more recently, compared to ponds with SAV (Table 3). Biocide dosage per pond volume was significantly higher in ponds without SAV compared to ponds with SAV, whereas biocide dosage per pond habitat did not differ significantly between pond types

Table 3
Characterisation of management regime of the canal and ponds (means ± 1 SEM, range between parentheses, except for the canal that was evenly managed all over its extension preventing differentiation among sites in the interviews). Different letter indicates significant differences at $p < 0.05$ after one-way ANOVA. Biocide dosage refers to amount of copper, or manganese, accounting for the chemical composition and hydration of the salts used, copper sulphate or potassium permanganate. Biocide dosage per volume of water was estimated for full pond volume when application occurs. The lack of discharge data for the canal impedes this estimation.

Parameter	Canal	Pond with SAV	Ponds without SAV
Frequency of cleaning (times year^{-1})	21	0.1 ± 0.1^{b} (0.0–0.3)	0.5 ± 0.1^{a} (0.2–1.0)
Elapsed time from last cleaning (years)	0.04	9.2 ± 3.5^{a} (1.6–25.0)	3.9 ± 1.3^{b} (0.7–8.0)
Frequency of biocide treatment (applications year^{-1})	1	0.7 ± 0.4^{a} (0–3)	4.7 ± 1.7^{a} (0–15)
Biocide dosage per pond volume (mg l^{-1} year^{-1})		1.0 ± 0.6^{b} (0.0–4.5)	4.4 ± 1.1^{a} (0.0–11.8)
Biocide dosage per habitat (kg year^{-1})	18.4	1.2 ± 1.0^{a} (0.0–7.3)	2.1 ± 0.9^{a} (0.0–11.0)

(Table 3). Copper sulphate was generally applied in most treated ponds, except for one pond of each type where potassium permanganate was used.

3.3. Population structure

Catch per unit effort (CPUE) of *A. iberus*, estimated as the number of individuals per sampling unit and hour of exposition, was significantly different among habitat types (ANOVA: $df = 4$, $F = 8.48$, $p < 0.0001$). Ponds with SAV showed by far the highest-mean CPUE followed by ponds without SAV and canal, whereas natural habitats showed extremely low CPUE values, particularly the river where just one positive catch was obtained (Table 4). This prevented further population analyses in this habitat.

Sex ratio in ponds did not differ significantly from a balanced sex ratio ($p > 0.05$). However, in coastal lagoons and, particularly, in the canal, sex ratio was significantly biased towards a higher proportion of small-size females ($p < 0.05$) (Table 4; Fig. 3).

Female size distribution differed significantly among habitats (Table 4). Average size of females was higher in pond with SAV followed by ponds without SAV, lagoons and canal. Male size distribution did not differ between pond types nor between lagoons and canal, but significantly higher size of males was observed in the first compared with the second two habitat types (Table 4). Overall, pooling females and males, size distribution in the four habitats was unimodal (Fig. 3), which suggests that most individuals belong to one cohort with low winter survival of previous-year cohort. However, size distribution in lagoons and canal were clearly more leptokurtic and right-skewed compared to ponds, suggesting lower growth rates and/or survival of individuals in the first two habitats (Fig. 3).

3.4. Biochemical analyses

Generally, the highest enzymatic activity of the antioxidant system was measured in individuals from ponds without SAV and the lowest in those from lagoons, whereas ponds with SAV showed intermediate values (Fig. 4). Particularly, activities of the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX) and DT-diaphorase (DTD) were significantly higher in ponds without SAV, followed by ponds with SAV and lagoons. Activities of glutathione reductase (GR), catalase (CAT) and glutathione transpherase (GST) were significantly higher in ponds compared with lagoons (Fig. 4). Average lipid-peroxidation (nmol MDA g^{-1} tissue) was significantly higher in ponds (ponds with SAV: 26.2 ± 2.3 SEM; ponds without SAV: 24.5 ± 1.2 SEM), compared to lagoons (16.3 ± 1.6 SEM). The average percentage of total lipids of individuals from ponds with SAV (16.7 ± 2.6 SEM) and lagoons (15.8 ± 1.5 SEM) was significantly higher compared to individuals from ponds without SAV (13.2 ± 2.2 SEM).

4. Discussion

Our results show that the bulk of the population of *A. iberus* in the lower basin of River Adra is concentrated in irrigation ponds, whereas natural habitats sustain impoverished subpopulations. This situation seems to be critical in the river habitat, where a virtual lack of *A. iberus* was detected whereas in a study carried out nearly a decade ago (Nevado and Paracuellos, 1999) the species was detected in all the present study sites (Fig. 1). We are unable to precisely define the factors responsible for this subpopulation depletion in the river area, since our data revealed relatively good habitat quality, at the local and time scales used, compared to other habitats. However, we can hypothesize that several disturbances, and their combinations, operating at larger temporal-spatial scales, may have impaired the river subpopulation. For instance, the occurrence of flash floods caused by discharges during winter to reduce pressure on the dam due to dam instability (García-López et al., 2009) might further contribute to the natural high winter mortality rates of the species (e.g. García-Berthou and Moreno-Amich, 1992). This impact may be burdened by the loss of lateral connectivity (absence of marginal pool refuges) that seems to occur due to the extreme encroachment of the active channel

Table 4
Characterisation of population structure of *A. iberus* in five habitats studied in the lower basin of River Adra (Almería, Southern Spain). N_t = Total number of sampling sites in a habitat, N_p = Number of sites with detected presence of *A. iberus*. CPUE = Catch per unit effort, as number of individuals per sampling unit per hour. ♂:♀ = sex ratio, with χ^2 comparisons with a balance sex ratio: * significant differences at $p < 0.05$; ns, no significant difference. Data on CPUE and size are means ± 1 SEM with range between parentheses, with different letter indicating significant differences at $p < 0.05$, after ANOVA and post-hoc Tukey's tests, and Kolmogorov–Smirnov tests, respectively. Extremely low catches in the river prevented further population analyses.

Parameter	Coastal lagoons	River	Canal	Ponds with SAV	Ponds without SAV
N_t:N_p	9:5	8:1	7:4	6:6	11:5
CPUE (# SU^{-1} h^{-1})	0.03 ± 0.02^{c} (0.00–0.19)	0.001 ± 0.001^{c} (0.00–0.004)	3.82 ± 2.95^{bc} (0.00–21.00)	54.08 ± 21.06^{a} (6.50–144.37)	29.97 ± 14.34^{b} (0.00–140.00)
♂:♀	0.81*		0.45*	0.87 ns	0.88 ns
♀ size (cm)	3.0 ± 0.1^{c} (2.1–4.9)		2.1 ± 0.1^{d} (1.5–3.8)	3.4 ± 0.0^{a} (1.5–5.5)	3.2 ± 0.0^{b} (2.0–5.5)
♂ size (cm)	2.4 ± 0.0^{b} (1.9–3.1)		2.3 ± 0.0^{b} (1.9–3.0)	2.9 ± 0.0^{a} (1.8–5.3)	2.9 ± 0.0^{a} (1.7–4.3)

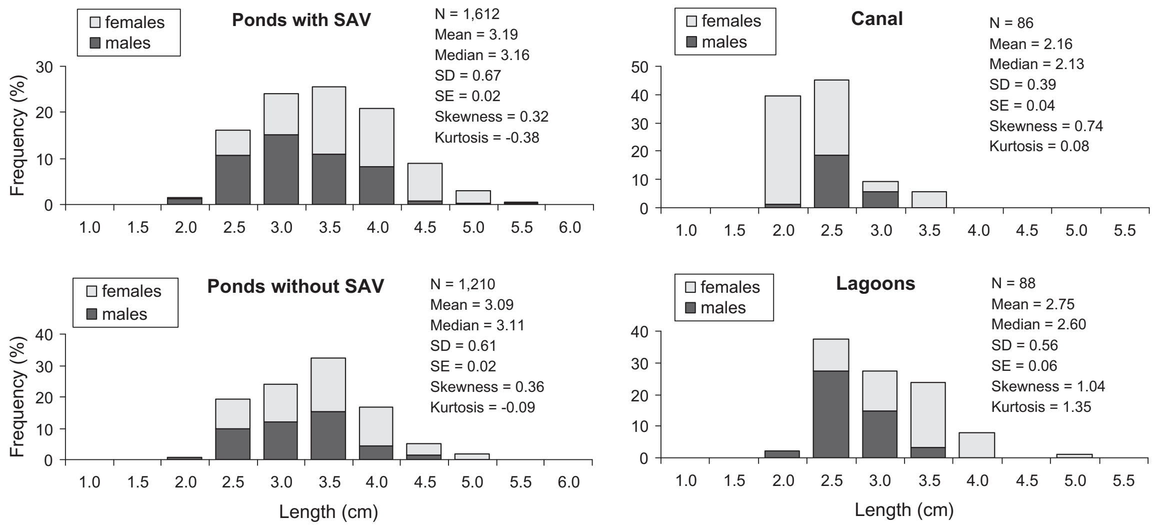

Fig. 3. Size-frequency distribution, differentiating females and males, of subpopulations in ponds, canal and coastal lagoons.

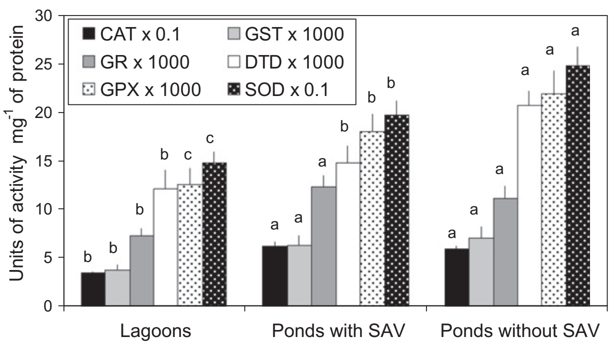

Fig. 4. Mean (+1 SEM) activity of antioxidant system enzymes of individuals from coastal lagoons and the two pond types studied. Different letter indicates significant differences at $p < 0.05$ after one-way ANOVA and post-hoc Tukey's test. CAT: catalase; GST: glutathione transpherase; GR: glutathione reductase; DTD: DT-diaphorase; GPX: glutathione peroxidase; SOD: superoxide dismutase.

and substrate stabilisation associated to the invasion of the riparian corridor by *A. donax* (Bell, 1997).

Although *A. iberus* was recorded in the three lagoons of Albuferas de Adra, this subpopulation show clear symptoms of impairment and low antioxidant system response of individuals, the latter likely related to low metabolic activity (Trenzado et al., 2006). This can be attributed to the intense eutrophication process these lagoons have endured for decades (Cruz-Pizarro et al., 2002; de Vicente et al., 2003). Hypoxia is a singular trait of these lagoons which probably determines low activity in fish living in this habitat, with consequent effects on the population level. Despite the shallowness of these lagoons, with mean depth of around 2 m, and the high exposure to wind that impedes thermal stratification, their high internal and external phosphorous loads (de Vicente et al., 2003) maintain the system in a highly persistent and plankton-productive turbid water-phase, featured by large filamentous cyanobacteria governing the phytoplankton community, absence of SAV, and anoxia or hypoxia in sediments and deep layers (Cruz-Pizarro et al., 2002; Bayo et al., 2003; Moreno-Ostos et al., 2007). Apart from the direct effect of hypoxia, indirect effects may also impair the activity of *A. iberus* in these lagoons. This fish strongly depends on benthic invertebrates as its major protein and fat source, particularly during reproduction (Alcaraz and García-Berthou, 2007). However, extremely low density of benthic invertebrates was reported in the Albuferas de Adra over a two-year study, under a turbid water-phase, that was associated to a generalised anoxia in sediments (Bayo et al., 2003). Both, better oxygen conditions and food availability in the shallower littoral belt may force the fish to inhabit it, where the risk of predation by waterbirds may by higher than in deeper zones, since the more frequent potential avian predators of *A. iberus* in these lagoons usually hunt in shallow peripheral areas (Paracuellos, 2006).

Although infrequent and sporadic, lasting less than 4 months, clear water-phases were detected in the two larger lagoons of Albuferas de Adra during 2002 and 2003. Studies of these clear water-phases (Moreno-Ostos et al., 2007, 2009) highlighted the major consequences that alternative ecosystem states, typical of shallow lakes (Scheffer, 1998), have on population dynamics of species inhabiting the Albuferas de Adra. These clear water-phases were unleashed by heavy rains or wind events, favouring a dominance of edible algae in the phytoplankton community, which allowed a *Daphnia magna* population to grow exponentially and induce the algae collapse by grazing (Moreno-Ostos et al., 2007). Consequently, marked increases in abundance and brood recruitment of waterbirds were recorded, attributed to the increase of SAV and associated fauna that attracted and stimulated reproduction (Moreno-Ostos et al., 2009).

Similar consequences, to those reported for birds, of SAV-dominated clear water-phases, might be forecast for the lagoon subpopulations of *A. iberus*, as deduced from the comparable situation reported in our SAV ponds. This pond type harboured the subpopulation with higher density, larger individual size and intermediate activity of the antioxidant system, which may be attributed to the suitability of habitat provided by SAV (provision of shelter, food, high dissolved oxygen). The circumstances that promote greater population density and, ultimately, greater survival and reproduction, involve highly active animals, and consequently increased metabolic activity leading to high levels of antioxidant protection system activity. Other studies have pointed out the crucial role of SAV for the conservation of fish species (e.g. de Nie, 1987). As a matter of fact, *A. iberus* was absent in a great number of ponds without SAV and average density, fat content and size of females was lower in this pond type than in SAV ponds. The most consistent difference detected in the management of both pond types is related to physical management, which almost certainly substantiates the consolidation of vegetation stands in SAV ponds.

Somewhat unexpectedly, there were no clear-cut differences between pond types for biocide treatments. This was corroborated by the Cu content in pond sediment. Even untreated ponds showed relatively high Cu concentration in sediment, probably due to the reception of the biocide added in the canal. Despite the reported high toxicity of this trace metal, when present in excess, to different aquatic organisms (e.g. Bossuyt and Janssen, 2005; Parra et al., 2005), the treatment with Cu seems not to be a major constraint to the pond subpopulation of *A. iberus*, probably due to the high pH and alkalinity in our study area, which lessen Cu bioavailability. Studies on Cu toxicity demonstrate that at high pH the formation of copper hydroxides is favoured, and increasing alkalinity enhances the formation of Cu carbonate complexes, both determining a reduction of cupric ion (Cu^{2+}) concentration in water, the chemical species responsible for Cu toxicity for freshwater fish (Erickson et al., 1996; MacRae et al., 1999). Moreover, the accumulation of Cu^{2+} at surface active binding sites at the gill is inhibited by the presence of elevated Ca^{2+} concentrations, resulting in reduced Cu toxicity (Playle et al., 1993; Meyer et al., 1998).

Notwithstanding the above consideration, we cannot discard any deleterious effect of biocide treatment in artificial habitats. In fact, lipid-peroxidation was significantly higher in ponds compared to lagoons, and the activity of three antioxidant enzymes was significantly higher in ponds without SAV, despite lower oxygen concentrations, but somewhat higher levels of Cu, compared to SAV ponds. Several studies demonstrate that Cu generates excessive free radicals leading to the accumulation of reactive oxygen species (ROS), which react with cellular components resulting in peroxidation of lipids, oxidation of proteins, and changes in the cellular redox status (Florence et al., 2002), to which the antioxidant system may respond with higher activity. For instance, Sturve et al., 2005 have shown increased DT-diaphorase activity in trout liver in response to diverse water pollutants with peroxidation potential. It is also well known that superoxide dismutase is the first enzymatic defence against ROS (Trenzado et al., 2006; Furné et al., 2009).

Compared to ponds, individuals seem unable to thrive in the canal. This is not surprising bearing in mind the high frequency of physical disturbance in the canal due to frequent vegetation clearance, and fluctuations of discharge caused by the turn system used for the distribution of irrigation water. However, a key function might be attributed to this structure, since it is likely to provide the species with a means of dispersion among ponds. In fact, many ponds have drainage and/or overflow outlets leading into the canal, therefore guaranteeing connectivity and possibly articulating a path dynamics among ponds that might be crucial for the subsistence of the species in the entire irrigation system. Uchida and Inoue (2010) demonstrated that the conservation of fish diversity in seasonal spring ponds connected to a river depends more on the maintenance of connectivity and conservation of source habitats, than on local habitat conditions. If ponds are the main habitat for survival and reproduction of *A. iberus*, this would be completely useless if each pond were to function as a fully watertight compartment, since, sooner or later, it would be desiccated and/or treated with biocides leading to the loss of the corresponding subpopulation, playing the sole role of a population sink. A long-term survey on the irrigation system would be needed to test this patch-dynamics metapopulation hypothesis.

In conclusion we showed that, paradoxically, natural habitats under legal protection support null or impoverished subpopulations of this endangered species as opposed to a healthier subpopulation status observed in the irrigation system. The degradation of natural habitats; eutrophication of the coastal lagoons, dam regulation and riparian invasion by *A. donax* in the river, is a common syndrome of the widespread agricultural intensification, especially accentuated in semiarid Mediterranean regions (Alvarez-Cobelas et al., 2005; Casas et al., 2006; Salinas and Casas, 2007). Finding a compromise between maintaining agriculture and human food supply and conserving aquatic ecosystems, is far from easy and not attainable by cosmetic and local measures (Moss, 2008). In Spain there are further examples of aquatic ecosystems that despite being under the highest level of legal protection (National Parks), the wetlands of Tablas de Daimiel and Doñana, are suffering increasing degradation due to encroachment by heavily used agricultural areas (Amezaga and Santamaria, 2000; Serrano et al., 2006). Both ecosystems studied here seem to have engaged in a persistent impaired alternative state, highly resilient due to strong positive feedback, which, to be reversed, would need radical restoration measures (Sunding et al., 2004; Gordon et al., 2008). The improvement of habitat quality for *A. iberus* in the permanent reach of River Adra seems to rest on actions to mitigate the effect of flash floods from the dam and/or to improve lateral connectivity of the river. The second would probably depend on the naturalisation of riparian vegetation by eradicating *A. donax*, a difficult task (Bell, 1997). Improvement of habitat quality of the coastal lagoons of Albuferas de Adra is complicated due to the high internal and external P and N load originating from surrounding greenhouses (de Vicente et al., 2003, 2006). In this area, there has been little implementation of agro-environmental measures on nutrient lixiviates reduction (Thompson et al., 2007). The improvement of crop nutrient management practices and the demarcation of substantial buffer areas to permit absorption of phosphorus and denitrification of nitrate, are urgent requirements. Wetland buffer areas are effective at removing nitrogen but not phosphorus; drier semi-natural areas are more effective for the latter (Moss, 2008).

The conservation of *A. iberus* in the irrigation system of the San Fernando Canal appears to depend mostly on the presence of a number of under-managed ponds that allow SAV to develop, and on active patch dynamics articulated by connectivity thorough the canal. However, the conservation of this subpopulation is not totally guaranteed. On the contrary, the recent transformation of nearby irrigation structures into pipeline waterways foretells a complicated future, as deduced from the significant declines in biodiversity observed in other regions and attributed to the replacement of traditional structures by pipelines, among other structural changes associated to modernisation (e.g. Kadoya et al., 2009). Irrigation modernisation in Mediterranean regions is generally understood to target four main goals: Water loss reduction, localized irrigation efficiency improvement, management promotion in irrigation communities and compatibility of all actions with environmental conservation (Bernal-Fontes, 2008). However, the ruling paradigm of irrigation system modernisation actually focuses on the minimisation of water loss, with environmental conservation issues, if considered, being much less of a priority. Environmentally friendly construction and management strategies to preserve biodiversity in irrigation systems are nonexistent in Spain, and they are urgently needed, particularly in systems such as that in the present study with a high value for the conservation of endangered species.

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References

Abellán, P., Sanchéz-Fernández, D., Millán, A., Botella, F., Sánchez-Zapata, J.A., Giménez, A., 2006. Irrigation pools as macroinvertebrate habitat in a semi-arid agricultural landscape (SE Spain). Journal of Arid Environments 67, 255–269.
Aebi, H., 1984. Catalase in vitro. In: Parcker, L. (Ed.), Methods in Enzymology, vol. 105. Academic Press, Orlando, FL, pp. 121–126.

Alcaraz, C., García-Berthou, E., 2007. Food of an endangered cyprinodont (*Aphanius iberus*): ontogenetic diet shift and prey electivity. Environmental Biology of Fishes 78, 193–207.
Alvarez-Cobelas, M., Rojo, C., Angeler, D.G., 2005. Mediterranean limnology: current status, gaps and the future. Journal of Limnology 64, 13–29.
American Public Health Association, 1992. Standard Methods for the Examination of the Water and Wastewater, 18th ed. APHA, Washington, DC.
Amezaga, J.M., Santamaria, L., 2000. Wetland connectedness and policy fragmentation: steps towards a sustainable European wetland policy. Physics and Chemistry of the Earth 25, 635–640.
Bayo, M., Casas, J.J., Langton, P.H., Vivas, S., López, D., 2003. Las comunidades de invertebrados bentónicos de las Albuferas de Adra (Almería, Sureste Ibérico). In: Paracuellos, M. (Ed.), Ecología, Manejo y Conservación de los Humedales. Conservación de los Humedales. Instituto de Estudios Almerienses, Diputación de Almería, Almería, Spain, pp. 85–97.
Bell, G.P., 1997. Ecology and management of *Arundo donax*, and approaches to riparian habitat restoration in southern California. In: Brock, J.H., Wade, M., Pysek, P., Green, D. (Eds.), Plant invasions: Studies from North America and Europe. Backhuys Publishers, Leiden, The Netherlands, pp. 102–113.
Bellio, M.G., Kingsford, R.T., Kotagama, S.W., 2009. Natural versus artificial wetlands and their waterbirds in Sri Lanka. Biological Conservation 142, 3076–3085.
Benthan, C.G., Moseley, M.J., 1982. Social surveys. In: Haynes, R. (Ed.), Environmental Sciences Methods. Chapman & Hall, London, pp. 355–361.
Bernal-Fontes, J., 2008. Sostenibilidad del regadío, Fundación Foro Agrario, Madrid.
Bonachela, S., Acuña, R.A., Casas, J.J., 2007. Environmental factors and management practices controlling oxygen dynamics in agricultural irrigation ponds in a semiarid Mediterranean region: implications for pond agricultural functions. Water Research 41, 1225–1234.
Bossuyt, B.T.A., Janssen, C.R., 2005. Copper toxicity to different field collected cladoceran species: intra- and inter-species sensitivity. Environmental Pollution 136, 145–154.
Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye-binding. Analytical Biochemistry 72, 248–254.
Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. In: Fleischer, S., Packer, L. (Eds.), Methods in Enzymology, vol. 52. Academic Press, New York, pp. 302–310.
Carlberg, I., Mannervik, B., 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. The Journal of Biological Chemistry 250, 5475–5480.
Casas, J., Calvache, F., Delgado, S., García-Mayoral, J., Vivas, S., Bayo, M.M., López, D., Ortega, M., 2003. Inventario abierto de los humedales de la región semiárida almeriense: consideraciones sobre su tipificación. In: Paracuellos, M. (Ed.), Ecología, Manejo y Conservación de Humedales. Instituto de Estudios Almerienses, Diputación de Almería, Almería, Spain, pp. 171–186.
Casas, J.J., Gessner, M.O., Langton, P.H., Calle, D., Descals, E., Salinas, M.J., 2006. Diversity of patterns and processes in rivers of eastern Andalusia. Limnetica 25, 155–170.
Casas, J.J., Toja, J., Bonachela, S., Fuentes, F., Gallego, I., Juan, M., León, D., Peñalver, P., Pérez, C., Sánchez, P., 2010. Artificial ponds in a Mediterranean region (Andalusia, Southern Spain): agricultural and environmental issues. Water and Environment Journal. doi:10.1111/j.1747-6593.2010.00221.x.
Clavero, M., Blanco-Garrido, F., Prenda, J., 2006. Monitoring small fish populations in streams: a comparison of four passive methods. Fisheries Research 78, 243–251.
Cruz-Pizarro, L., Benavente, J., Casas, J., Amores, M.V., May, L., Fabián, D., Rodríguez, M., El Mabrouki, K., Rodríguez, I., de Vicente, I., Moreno-Ostos, E., Rodrigues da Silva, S.L., Bayo, M., Moñino, A., Paracuellos, M., 2002. Control de la eutrofización en las lagunas de la Albufera de Adra. Diagnóstico, evaluación y propuesta de recuperación. Informe final del proyecto UE-LIFE B4-3200/98/458.
de Nie, H.W., 1987. The Decrease in Aquatic Vegetation in Europe and its Consequences for Fish Populations. EIFAC/CECPI Occasional paper No.19. Food and Agriculture Organization of the United Nations, Rome.
de Vicente, I., Moreno-Ostos, E., Amores, V., Rueda, F., Cruz-Pizarro, L., 2006. Low predictability in the dynamics of shallow lakes: implications for their management and restoration. Wetlands 26, 928–938.
de Vicente, I., Serrano, L., Amores, V., Clavero, V., Cruz-Pizarro, L., 2003. Sediment phosphate fractionation and interstitial water phosphate concentration in two coastal lagoons (Albuferas de Adra, SE Spain). Hydrobiologia 492, 95–105.
Doadrio, I., 2002. Atlas y Libro Rojo de los Peces Continentales de España. CSIC y Ministerio de Medio Ambiente, Madrid.
Downward, S.R., Taylor, R., 2007. An assessment of Spain's Programa AGUA and its implications for sustainable water management in the province of Almería, southeast Spain. Journal of Environmental Management 82, 277–289.
Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81, 163–182.
Erickson, R.J., Benoit, D.A., Mattson, V.R., Nelson Jr., H.P., Leonard, E.N., 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Environmental Toxicology and Chemistry 15, 181–193.
Flohe, L., Günzler, W.A., 1984. Assay of glutathione peroxidase. In: Parcker, L. (Ed.), Methods in Enzymology, vol. 105. Academic Press, Orlando, FL, pp. 115–121.
Florence, G., Serafim, A., Barreira, L., Bebianno, M.J., 2002. Response of antioxidant systems to copper in the gills of the clam *Ruditapes decussates*. Marine Environmental Research 54, 413–417.
Furné, M., García-Gallego, M., Hidalgo, M.C., Morales, A.E., Domezain, J., Domezain, A., Sanz, A., 2009. Oxidative stress parameters during starvation and refeeding periods in Adriatic sturgeon (*Acipenser naccarii*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition 15, 587–595.
García-Berthou, E., Moreno-Amich, R., 1992. Age and growth of an Iberian cyprinodont, *Aphanius iberus* (Cuv. & Val.), in its most northerly population. Journal of Fish Biology 40, 929–937.
García-López, S., Benavente, J., Cruz-Sanjulián, J.J., Olías, M., 2009. Conjunctive use of water resources as an alternative to a leaky reservoir in a mountainous, semiarid area (Adra River basin, SE Spain). Hydrogeology Journal 17, 1779–1790.
Gordon, L.J., Peterson, G.D., Bennett, E.M., 2008. Agricultural modifications of hydrological flows create ecological surprises. Trends in Ecology and Evolution 23, 211–219.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases – the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 249, 7130–7139.
Hollis, G.E., 1992. The causes of wetland loss and degradation in the Mediterranean. In: Finlayson, C.M., Hollis, G.E., Davis, T.J. (Eds.), Managing Mediterranean Wetlands and their Birds. Slimbridge, UK, p. 285 (IWRB special publication No. 20).
IUCN, 2008. 2008 IUCN Red List of Threatened Species. IUCNRedList.org.
Kadoya, T., Suda, S., Washitani, I., 2009. Dragonfly crisis in Japan: a likely consequence of recent agricultural habitat degradation. Biological Conservation 142, 1899–1905.
MacRae, R.K., Smith, D.E., Swoboda-Colberg, N., Meyer, J.S., Bergman, H.L., 1999. Copper binding affinity of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) gills. Environmental Toxicology and Chemistry 18, 1180–1189.
McCord, J.M., Fridovich, I., 1969. Superoxide dismutase: an enzymatic function for erythrocuprein. The Journal of Biological Chemistry 244, 6049–6055.
Meyer, J.S., Santore, R.C., Bobbitt, J.P., DeBrey, L.D., Boese, C.J., Paquin, P.R., Allen, H.E., Bergman, H.L., Di Toro, D.M., 1998. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free ion activity does not. Environmental Science and Technology 33, 913–916.
Moreno-Ostos, E., Paracuellos, M., de Vicente, I., Nevado, J.C., Cruz-Pizarro, L., 2009. Response of waterbirds to alternating clear and turbid water phase in two shallow Mediterranean lakes. Aquatic Ecology 42, 701–706.
Moreno-Ostos, E., Rodrigues da Silva, S.L., de Vicente, I., Cruz-Pizarro, L., 2007. Interannual and between-site variability in the occurrence of clear water phases in two shallow Mediterranean lakes. Aquatic Ecology 41, 285–297.
Moss, B., 2008. Water pollution by agriculture. Philosophical Transactions of The Royal Society of Britain 363, 659–666.
Nevado, J.C., Paracuellos, M., 1999. El fartet en Almería. Una estrategia de conservación. In: Planelles-Gomis, M. (Ed.), Monografía sobre los peces Ciprinodóntidos Ibéricos Fartet y Samaruc. Conselleria de Medio Ambiente, Generalitat Valenciana, Valencia, pp. 163–168.
Oliva-Paternaa, F.J., Torralva, M., Fernández Delgado, C., 2006. Threatened fishes of the world: *Aphanius iberus* (Cuvier & Valenciennes, 1846) (Cyprinodontidae). Environmental Biology of Fishes 75, 307–309.
Paracuellos, M., 2006. How can habitat selection affect the use of a wetland complex by waterbirds? Biodiversity and Conservation 15, 4569–4582.
Parra, G., Jiménez-Melero, R., Guerrero, F., 2005. Agricultural impacts on Mediterranean wetlands: the effect of pesticides on survival and hatching rates in copepods. Annals Limnologie – International Journal of Limnology 41, 161–167.
Playle, R.C., Dixon, D.G., Burnison, K., 1993. Copper and cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands. Canadian Journal of Fisheries and Aquatic Sciences 50, 1–11.
Ricciardi, A., Rasmussen, J.B., 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13, 1220–1222.
Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.
Salinas, M.J., Casas, J.J., 2007. Riparian vegetation of two semi-arid Mediterranean rivers: basin-scale responses of woody and herbaceous plants to environmental gradients. Wetlands 27, 831–845.
Scheffer, M., 1998. Ecology of Shallow Lakes. Chapman and Hall, London.
Sebastian-González, E., Sánchez-Zapata, J.A., Botella, F., 2010. Agricultural ponds as alternative habitat for waterbirds: spatial and temporal patterns of abundance and management strategies. European Journal of Wildlife Research 56, 11–20.
Serrano, L., Reina, M., Marín, G., Reyes, I., Arechederra, A., León, D., Toja, J., 2006. The aquatic systems of Doñana (SW, Spain): watersheds and frontiers. Limnetica 25, 11–32.
Sokal, R.R., Rohlf, F.J., 1981. Biometry. Freeman, New York.
SPSS Inc., 1999. Sigma Scan Pro 5.0, SPSS Science Marketing Department, Chicago, IL.
StatSoft, I., 2005. STATISTICA (data analysis software system) version 7.1. <http://www.statsoft.com>.
Sturve, J., Stephensen, E., Forlin, L., 2005. Effects of redox cycling compounds on DT diaphorase activity in the liver of rainbow trout (*Oncorhynchus mykiss*). Comparative Hepatology 4, 4.
Sunding, K.N., Gross, K.L., Housman, D.R., 2004. Alternative states and positive feedbacks in restoration ecology. Trends in Ecology and Evolution 19, 46–53.
Trenzado, C., Hidalgo, M.C., García-Gallego, M., Morales, A.E., Furné, M., Domezain, A., Domezain, J., Sanz, A., 2006. Antioxidant enzymes and lipid peroxidation in

sturgeon *Acipenser naccarii* and trout *Oncorhynchus mykiss.* A comparative study. Aquaculture 254, 758–767.
Thompson, R.B., Martínez-Gaitan, C., Gallardo, M., Giménez, C., Fernández, M.D., 2007. Identification of irrigation and N management practices that contribute to nitrate leaching loss from an intensive vegetable production system by use of a comprehensive survey. Agricultural Water Management 89, 261–274.
Uchida, Y., Inoue, M., 2010. Fish species richness in spring-fed ponds: effects of habitat size versus isolation in temporally variable environments. Freshwater Biology. doi:10.1111/j.1365-2427.2009.02335.x.
Wetzel, R.G., Likens, G., 1991. Limnological Analyses, second ed. Springer-Verlag, New York, USA.
Williams, P., Whitfield, M., Biggs, J., Bray, S., Fox, G., Nicolet, P., Sear, D., 2003. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in southern England. Biological Conservation 115, 329–341.
Xenopoulos, M.A., Lodge, D.M., Alcamo, J., Marker, M., Schulze, K., van Vuuren, D.P., 2005. Scenarios of freshwater fish extinctions from climate change and water withdrawal. Global Change Biology 11, 1557–1564.