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## A standardised analytical framework for sampling fish communities: relevance to Turkish riverine ecosystems

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**Abstract:** Fish communities are important indicators of the health of riverine ecosystems. Fish monitoring for The Living Murray (TLM) initiative, one of Australia's most ambitious restoration undertakings for the degraded river-floodplain biota of the River Murray system (south-eastern Australia), was carried out annually from 2005 to 2011 across six 'Icon Sites', including the habitats and reaches therein. These spatial and temporal components of variation in fish community abundance and composition must be taken explicitly into account for consistent assessment of riverine ecosystem health. To address this requirement, a standardised analytical framework, consisting of a sampling design and appropriate statistical methods, is outlined in this study. Based on an extensive dataset, multivariate patterns and univariate trends in fish community structure were analysed to show the flexibility, modularity and ability of the framework. The applicability of the framework to Turkish riverine ecosystems is then discussed with emphasis on the need for participatory discussion between researchers and environmental managers. The possibility to incorporate other measures of fish health such as biomass and condition, and to extend the proposed framework to the quantitative assessment of vegetation and bird communities also exists.

**Key words:** Experimental design, spatial variation, temporal variation, permutational multivariate analysis of variance (PERMANOVA), generalised estimating equations (GEE), Murray-Darling Basin

### 1. Introduction

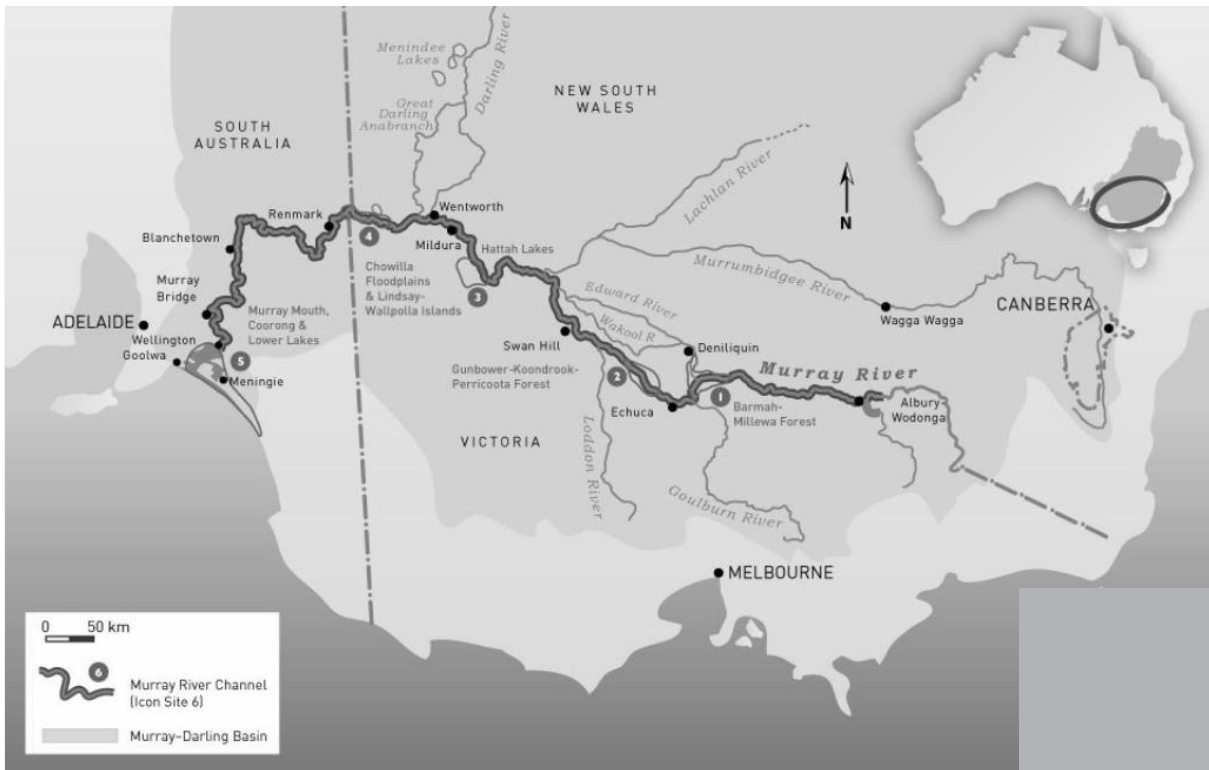
Globally, the ecosystem health of several water bodies, and especially those acting as biodiversity hotspots, has been subjected to increasing human-induced pressure. These stress factors, which include degradation of the natural flood plain, deforestation, pollution, habitat fragmentation and disruption of the hydrological regime, have led to substantial changes in the physico-chemical parameters and hydromorphological characteristics of the affected water bodies, thereby impacting on the abundance and composition of their communities (e.g., Fausch et al., 1990; Jekel, 2005; Gallardo et al., 2016). Continuous monitoring of aquatic ecosystems including riverine ones is therefore of utmost importance to assist managers and decision makers in the formulation and implementation of proper regulations and management measures aimed at mitigating impacts (Boulton, 1999). However, restoration projects for river-floodplain ecosystems are known to represent long-term undertakings (e.g., Buijse et al., 2002;

Deffne and Haase, 2018; Haase and Pilotto, 2019; Pilotto et al., 2019).

Development of a standardised analytical framework (Keith, 2000; Souter et al., 2010) capable of synthesising (medium-long term) quantitative information on riverine fish communities is therefore a key requirement to ensure consistency in the reporting of monitoring outcomes. In Turkey, large-scale fish monitoring studies are scarce and poorly implemented, and recent proposals to develop a fish-based index for monitoring ecosystem health have been either in compliance with the Water Framework Directive (Ergönül et al., 2018, 2020) or in a regionally-adjusted form (Yerli et al., 2016), but they have not been backed up by an appropriate analytical framework. Recently, an open document concerned with the monitoring of the biological quality components of surface waters (official gazette of the Turkish Ministry of Agriculture and Forestry)<sup>1</sup> was published, demanding a standardised protocol to be implemented with regard to

<sup>1</sup>T.C. Cumhurbaşkanlığı Resmi Gazete (2021). Biyolojik İzleme Tebliği (in Turkish) [online]. Website [www.resmigazete.gov.tr/eskiler/2019/06/20190621-9.htm](http://www.resmigazete.gov.tr/eskiler/2019/06/20190621-9.htm) [accessed 11 March 2021].

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**Figure 1.** Map of the River Murray system in south-eastern Australia with indication of the six Icon Sites part of The Living Murray initiative (see Table 1).

the biological sampling of aquatic ecosystems including fish. However, despite its availability, this document has been so far unable to provide consistent directions for hypothesis-based biological monitoring.

To fill this knowledge gap, the aim of the present study is to outline and implement a standardised analytical framework for fish monitoring consisting of a sampling design coupled with appropriate statistical methods. Specifically: (i) the structure of the sampling design is first outlined with emphasis on its flexibility, modularity and ability to distinguish between spatial and temporal components of variation; and (ii) the design is then implemented using an extensive dataset from a case study in Australia to illustrate both multivariate patterns and univariate trends in fish community abundance and composition. Assumptions relative to the identification of spatial components of variation and computation of fish abundance are discussed, and suggestions for improvement are provided with emphasis on the need for participatory discussion between researchers and environmental managers. The outcomes of the findings are then addressed in terms of their prospective application to the monitoring of riverine fish communities in Turkey.

## 2. Methods

### 2.1. Background, experimental design, and data sourcing

In south-eastern Australia, human-induced degradation of the river-floodplain ecosystem of the Murray Darling Basin (MDB) has caused significant declines in the diversity, abundance and distribution of the native biota, including fish (Koehn and Lintermans, 2012). In response to evidence for deteriorating conditions throughout the River Murray system resulting from extensive regulation and the introduction of invasive alien species, The Living Murray (TLM) initiative<sup>2</sup> was established in 2002 as one of Australia's most ambitious, long-term river restoration undertakings with the main objective to improve habitat condition at six selected 'Icon Sites' (Figure 1). The River Murray is Australia's longest river at 2508 km in length, and its tributaries include five of the next six longest rivers of Australia (i.e. Murrumbidgee, Darling, Lachlan, Warrego and Paroo rivers), whose catchment area ( $1073 \times 10^6 \text{ km}^2$ ) together form the MDB. The River Murray is highly regulated through the construction of weirs, which has resulted in most parts of the river channel to be converted into a series of impoundments ('locks') with profound consequences on the hydrological regime (e.g., Walker and Thoms, 1993; Maheshwari et al., 1995).

<sup>2</sup> Government of South Australia Department for Environment and Water (2021). The Living Murray Program [online]. Website [www.environment.sa.gov.au/topics/river-murray/improving-river-health/the-living-murray-program](http://www.environment.sa.gov.au/topics/river-murray/improving-river-health/the-living-murray-program) [accessed 11 March 2021].

Environmental monitoring of fish is one of the programmes through which TLM initiative was implemented. From 2005 to 2011, fish monitoring occurred annually across the six Icon Sites, yielding a large amount of data on fish community abundance and composition. Despite this regular sampling, analytical methods for reporting on the status and composition of fish communities at the Icon Site level proved inconsistent. This was due both to the involvement of different jurisdictional (state-based) agencies responsible for Icon Site-specific monitoring/reporting and to differences between the range of habitats encountered at the Icon Sites themselves.

Systematic sampling of the Icon Sites (including the habitats and reaches therein) over time lies at the core of TLM fish monitoring and implies an experimental design that must be accounted for in the quantitative assessment of spatial and temporal variation in fish community abundance and composition. The sampling design underlying TLM fish monitoring (hereafter, 'TLM design') implemented throughout this study consists of the following factors: (i) Icon Site crossed with Habitat (as the same habitat may occur across the different Icon Sites); (ii) Reach nested within each Habitat by Icon Site combination (as reaches are unique to the habitats encountered at a certain Icon Site); and (iii) Time, which accounts for the temporal replication of the sampling events (usually, annual sampling), crossed with Icon Site, Habitat and Reach (as reaches are repeatedly sampled, i.e. annually, at a certain Habitat and Icon Site). Overall, the TLM design consists of two main components of variation: (i) spatial, including Icon Site, Habitat, their interaction Icon Site  $\times$  Habitat, and Reach nested within Icon Site  $\times$  Habitat; and (ii) temporal, including Time and the interaction terms Time  $\times$  Icon Site, Time  $\times$  Habitat, and Time  $\times$  Icon Site  $\times$  Habitat.

Statistically, the TLM design is a repeated measures 'subject-by-trials' design based on a split-plot factorial (SPF) template (sensu Kirk, 1995). Accordingly, the reaches represent the 'subjects' (or 'blocks') and the sampling events the 'trials' (terminology inherits from usage in the social sciences: Kirk, 1995). Icon Site and Habitat are the two between-reach factors and Time is the within-reach factor. Reaches are the experimental units (see Hurlbert, 1984; Mead, 1988). In formal notation, the design is denoted as SPF- $pr$ - $q$  (Vilizzi, 2005), that is a split-plot factorial with  $p$  ( $j = 1, \dots, p$ ) Icon Sites,  $r$  ( $l = 1, \dots, r_p$ ) habitats,  $q$  ( $k = 1, \dots, q$ ) times of sampling, and  $s$  ( $i = 1, \dots, spr_p$ ) reaches. Due to the different number of habitats available at any Icon Site, the different number of reaches sampled in any habitat within a certain Icon Site, and the different times of sampling for any Icon Site  $\times$  Habitat combination, the TLM design is unbalanced (sensu Milliken and Johnson, 2008).

Data on fish abundance were obtained from published reports and available datasets (see Vilizzi, 2012a and references therein) for each of the six Icon Sites: Barmah-Millewa Forest (BMF), Chowilla Floodplain and Lindsay-Wallpolla Island (CFLWI), Gunbower-Kondrook-Perricoota Forest (GKPF), Hattah Lakes (HL), Lower Lakes, Coorong and Murray Mouth (LLCMM), and River Murray Channel (RMC). For consistency purposes, four habitats were identified: Creek, Lake, Riverine and Wetland (noting that, by definition, only the Riverine habitat is identified in the RMC, for which data were collated from the River Murray reaches sampled across the other Icon Sites) (Table 1).

## 2.2. Statistical analysis

Fish abundance data were only available at the reach level for BMF, CF (including the RMC reaches therein) and LLCMM. Therefore, implementation of the TLM design, i.e. over all the Icon Sites, habitats and years of sampling (= fish 'biological seasons', taken to last from July to June: Cattaneo, 2005), was on presence/absence data. Conversely, the availability of raw data for GKPF, HL and LWI (including the RMC reaches therein) at the site-within-reach level allowed implementation of a subset of the TLM design on catch per unit effort (CPUE) abundance. This was computed as an 'integrated measure' expressed as number of fish per hour (fish  $h^{-1}$ ) and obtained as the time-adjusted sum of the total number of fish sampled at certain site within a reach and in a certain year with any combination of sampling method. Notably, this approach is similar to that of Vilizzi (2012b), who combined abundance of larvae sampled in the lower River Murray (South Australia) into an 'integrated catch index' due to inherent differences in efficiency for each sampling method.

Raw abundance data for a total of 467,947 fish across 29 fish taxa (Table 2) recorded from 2005 to 2011 across the six Icon Sites were converted into presence/absence; whereas, CPUE abundance data for the fish groups (Table 2) identified at the GKPF, HL, LWI and RMC Icon Sites were fourth-root transformed. This resulted in two data matrices to which a Bray-Curtis dissimilarity measure was applied to obtain the corresponding distance matrices for multivariate analysis. Permutational multivariate analysis of variance (PERMANOVA) was used as the hypothesis-based statistical method for testing differences in fish community structure based on the TLM design. Icon Site (for species presence/absence: BMF, CFLWI, GKPF, HL, LLCMM and RMC; for group CPUE abundance: GKPF, HL, LWI, RMC) and Habitat (for species presence/absence: Creek, Lake, Riverine, Wetland; for group abundance: Creek, Riverine, Wetland) were the spatial factors, Year (2005–06, 2006–07, 2007–08, 2008–09, 2009–10, 2010–11) the temporal factor, and Reach the

**Table 1.** Icon Sites and habitats in the sample implementation of the TLM (The Living Murray) design. Year(s) are fish 'biological seasons'. C = Creek; L = Lake; R = Riverine; W = Wetland. In parentheses next to each habitat the total number of reaches sampled.

Icon Site			
Name	Code	Habitat(s)	Year(s)
Barmah-Millewa Forest	BMF	C (6), L (3), R (2), W (3)	2007–08, 2008–09, 2009–10, 2010–11
Chowilla Floodplain and Lindsay-Wallpolla Island (including Mulcra)	CFLWI (CF, LWI)*	C (22), W (5)	2005–06†, 2006–07, 2007–08, 2008–09, 2009–10, 2010–11
Gunbower-Kondrook-Perricoota Forest	GKPF	C (7), W (17)	2008–09, 2009–10, 2010–11
Hattah Lakes	HL	C (1), W (8)	2005–06, 2006–07, 2007–08, 2008–09, 2009–10, 2010–11
Lower Lakes, Coorong and Murray Mouth	LLCMM	C (20), L (8)	2008–09
River Murray Channel	RMC	R (17)	2005–06, 2006–07, 2007–08, 2008–09, 2009–10, 2010–11

\* Chowilla Floodplain (CF) and Lindsay-Wallpolla Island (including Mulcra) (LWI) 'sub-Icon Sites'.

† CF only.

experimental unit. Factors Icon Site, Habitat and Year were fixed, factor Reach was random. Canonical (discriminant) analysis of the principal coordinates (CAP) was then used to display patterns in species presence/absence and group CPUE abundance for the statistically significant effects ( $\alpha = 0.05$ ). All multivariate statistical analyses were carried out in PERMANOVA+ for PRIMER v6 (Anderson et al., 2008), with 9999 permutations for both PERMANOVA and CAP.

Temporal trends in mean CPUE abundance of all species and in species richness (total, nativeness and nonnativeness) were analysed across Icon Sites and habitats. Since the lack of sampling in several years for some reaches would have resulted in too many missing values, only reaches with no more than two missing years of sampling were included in the analyses. Total richness was computed as the total number of species recorded; nativeness and nonnativeness as the proportion of native and nonnative species, respectively, out of the total number of species. Generalised estimating equations (GEE; Zuur et al., 2009) were used as the hypothesis-based approach for univariate trend analysis. GEE are linear models suited to the TLM design, in which reaches are nested within Icon Sites and habitats and sampling is carried out over years. GEE were implemented with the free software package R (R Development Core Team, 2019)<sup>3</sup> using library geepack, with a Poisson distribution and an autoregressive correlation structure of order 1. The latter was under the assumption that native/nonnative CPUE abundance and species richness in a certain year would be more closely

correlated to those in the previous year than two years or more before.

### 3. Results

There were differences in species presence/absence both amongst Icon Sites and habitats (spatial variation) and amongst years at the habitat level depending on Icon Site (temporal variation) (Table 3). Spatially, at the Icon Site level apart from the clear segregation of LLCMM from the other icon Sites, there was a higher occurrence of oriental weatherloach *Misgurnus anguillicaudatus* at BMF, golden perch *Macquaria ambigua* and bony herring *Nematalosa erebi* at CFLWI, flathead gudgeon *Philypnodon grandiceps* at GKPF, European perch *Perca fluviatilis* at LLCMM, as well as common carp *Cyprinus carpio* (also ubiquitous at BMF and CFLWI) and Murray cod *Maccullochella peelii* in the RMC (Figure 2a). At the habitat level, Lake differed from all other habitats due to lower occurrence of all species, and Creek differed from Wetland due to higher occurrence of silver perch *Bidyanus bidyanus*, *Macquaria ambigua*, *Maccullochella peelii*, unspecked hardyhead *Craterocephalus stercusmuscarum fulvus* and Murray-Darling rainbowfish *Melanotaenia fluviatilis* in the former and of carp gudgeon *Hypseleotris* spp. in the latter (Figure 2b). Temporally, in the Creek habitat of CFLWI there was a higher occurrence of *Hypseleotris* spp. in 2007–08, *Bidyanus bidyanus*, *Macquaria ambigua*, *Craterocephalus stercusmuscarum fulvus* and flathead gudgeon in 2008–09, and eastern mosquitofish *Gambusia holbrooki* in 2010–11 (Figure 2c). At RMC, there was a higher occurrence

<sup>3</sup> The R Foundation (2021). The R Project for Statistical Computing [online]. Website <http://www.R-project.org> [accessed 11 March 2021].

**Table 2.** Fish taxa recorded at the six TLM Icon Sites in the River Murray system from 2006 to 2011. Groups are (modified) after Ralph et al. (2010): ES = Estuarine (new group); FS = Flood spawners; LFWO = Low-flow specialists and wetland opportunists; MCGWO = Main channel generalists and wetland opportunists; MCS = Main channel specialists; NN = Nonnatives (new group).

Taxon name	Common name	Code	Group
<i>Arenigobius bifrenatus</i>	Bridled goby	Are.bif	ES
<i>Arripis trutta</i>	Australian salmon	Arr.tru	ES
<i>Atherinosoma microstoma</i>	Small-mouthed hardyhead	Ath.mic	ES
<i>Bidyanus bidyanus</i>	Silver perch	Bid.bid	FS
<i>Carassius auratus</i>	Goldfish	Car.aur	NN
<i>Craterocephalus fluviatilis</i>	Murray hardyhead	Cra.flu	LFWO
<i>Craterocephalus stercusmuscarum fulvus</i>	Unspecked hardyhead	Cra.ste	LFWO
<i>Cyprinus carpio</i>	Common carp	Cyp.car	NN
<i>Cyprinus carpio</i> × <i>Carassius auratus</i>	Common carp × goldfish (hybrid)	Cyp.hyb	NN
<i>Galaxias maculatus</i>	Common galaxias	Gal.mac	ES
<i>Gambusia holbrooki</i>	Eastern mosquitofish	Gam.hol	NN
<i>Hyperlophus vittatus</i>	Sandy sprat	Hyp.vit	ES
<i>Hypseleotris</i> spp.	Carp gudgeon	Hyp.spp	LFWO
<i>Leiopotherapon unicolor</i>	Spangled perch	Lei.uni	FS
<i>Maccullochella macquariensis</i>	Trout cod	Mac.mac	MCS
<i>Maccullochella peelii</i>	Murray cod	Mac.pel	MCS
<i>Macquaria ambigua</i>	Golden perch	Mac.amb	FS
<i>Melanotaenia fluviatilis</i>	Murray-Darling rainbowfish	Mel.flu	LFWO
<i>Misgurnus anguillicaudatus</i>	Oriental weatherloach	Mis.ang	NN
<i>Nannoperca australis</i>	Southern pygmy perch	Nan.aus	MCGWO
<i>Nematalosa erebi</i>	Bony herring	Nem.ere	MCGWO
<i>Perca fluviatilis</i>	European perch	Per.flu	NN
<i>Philypnodon grandiceps</i>	Flathead gudgeon	Phi.gra	MCGWO
<i>Philypnodon macrostomus</i>	Dwarf flathead gudgeon	Phi.mac	MCGWO
<i>Pseudaphritis urvillii</i>	Congolli	Pse.uvi	ES
<i>Pseudogobius olorum</i>	Western blue-spot goby	Pse.olo	ES
<i>Retropinna semoni</i>	Australian smelt	Ret.sem	MCGWO
<i>Tandanus tandanus</i>	Freshwater catfish	Tan.tan	ES
<i>Tasmanogobius lasti</i>	Lagoon goby	Tas.las	NN

of trout cod *Maccullochella macquariensis* in 2007–08, *Philypnodon grandiceps* in 2008–09, *Nematalosa erebi* in 2009–10, and *Hypseleotris* spp. and *Gambusia holbrooki* in 2010–11 (Figure 2d).

Apart from differences in group CPUE abundance among (sub-)Icon sites (spatial variation), which was mainly attributable to higher abundances of all groups in the RMC (except for NN at GKPF), there were differences amongst years both at the (sub-)Icon Site and at the habitat level (temporal variation) (Table 4). Overall, temporal differences were due to an increase in the abundance of

the NN group across all (sub-)Icon Sites and habitats in 2010–11. In addition, LFWO were also more abundant in 2010–11 at GKPF (Figure 3a), whereas at LWI and RMC this was true in the previous years (especially 2007–08 to 2009–10) also for the MCGWO (Figures 3b and 3c). In the Creek habitat, all groups except the FS were responsible for the above temporal patterns (Figure 3d); whereas this was true for the LFWO and the MCGWO in the riverine habitat (Figure 3e) and for the FS and LFWO in the wetland habitat (Figure 3f), the latter group being more abundant in 2010–11 along with the NN.

**Table 3.** Permutational multivariate analysis of variance (PERMANOVA) differences in presence/absence of 29 fish species (see Table 2) recorded during six years of monitoring in four habitats at the six TLM Icon Sites (sampling at the reach level, with data pooled over sites). For simplicity, only statistically significant ( $\alpha = 0.05$ ) a posteriori pair-wise comparisons are given.  $F^{\#}$  = Permutational  $F$  value;  $t^{\#}$  = Permutational  $t$ -test value;  $P^{\#}$  = Permutational  $P$  value (9999 permutations). Icon site codes as in Table 1. See also Figure 2.

Component of variation	df	MS	$F^{\#}/t^{\#}$	$P^{\#}$
Spatial				
Icon Site	5	19955.0	17.95	< 0.001
BMF vs. CFLWI			4.29	< 0.001
BMF vs. GKPF			1.57	0.049
BMF vs. HL			2.69	< 0.001
BMF vs. LLCMM			6.41	< 0.001
CFLWI vs. GKPF			5.14	< 0.001
CFLWI vs. HL			3.27	< 0.001
CFLWI vs. LLCMM			9.05	< 0.001
GKPF vs. HL			2.90	< 0.001
GKPF vs. LLCMM			5.04	< 0.001
HL vs. LLCMM			2.20	0.025
Habitat	3	5186.6	4.57	0.002
Creek vs. Lake			2.31	0.001
Creek vs. Wetland			2.16	0.012
Lake vs. Wetland			2.05	0.008
Riverine vs. Wetland			2.36	0.001
Icon Site $\times$ Habitat	4	1313.6	1.28	0.226
Reach (Icon Site $\times$ Habitat)	110	1099.6	2.36	< 0.001
Residual	254	465.8		
Temporal				
Year	5	1273.1	3.98	< 0.001
Year $\times$ Icon Site	15	997.1	3.12	< 0.001
Year $\times$ Habitat	13	603.2	1.89	0.015
Year $\times$ Icon Site $\times$ Habitat	8	850.0	2.66	0.002
CFLWI				
Creek				
2005–06 vs. 2006–07			–	–
2005–06 vs. 2007–08			2.73	0.004
2005–06 vs. 2008–09			16.92	< 0.001
2005–06 vs. 2009–10			5.79	< 0.001
2006–07 vs. 2008–09			3.48	0.003
2006–07 vs. 2009–10			2.13	0.032
2006–07 vs. 2010–11			2.38	0.010
2007–08 vs. 2008–09			3.57	0.002
2007–08 vs. 2009–10			2.18	0.014
2007–08 vs. 2010–11			2.74	0.003

Table 3. (Continued).

2008–09 vs. 2009–10			2.14	0.032
2008–09 vs. 2010–11			3.85	< 0.001
2009–10 vs. 2010–11			4.09	< 0.001
RMC				
(Riverine)				
2006–07 vs. 2009–10			3.08	0.002
2008–09 vs. 2010–11			2.81	0.002
2009–10 vs. 2010–11			1.88	0.025
Residual	212	850.0		

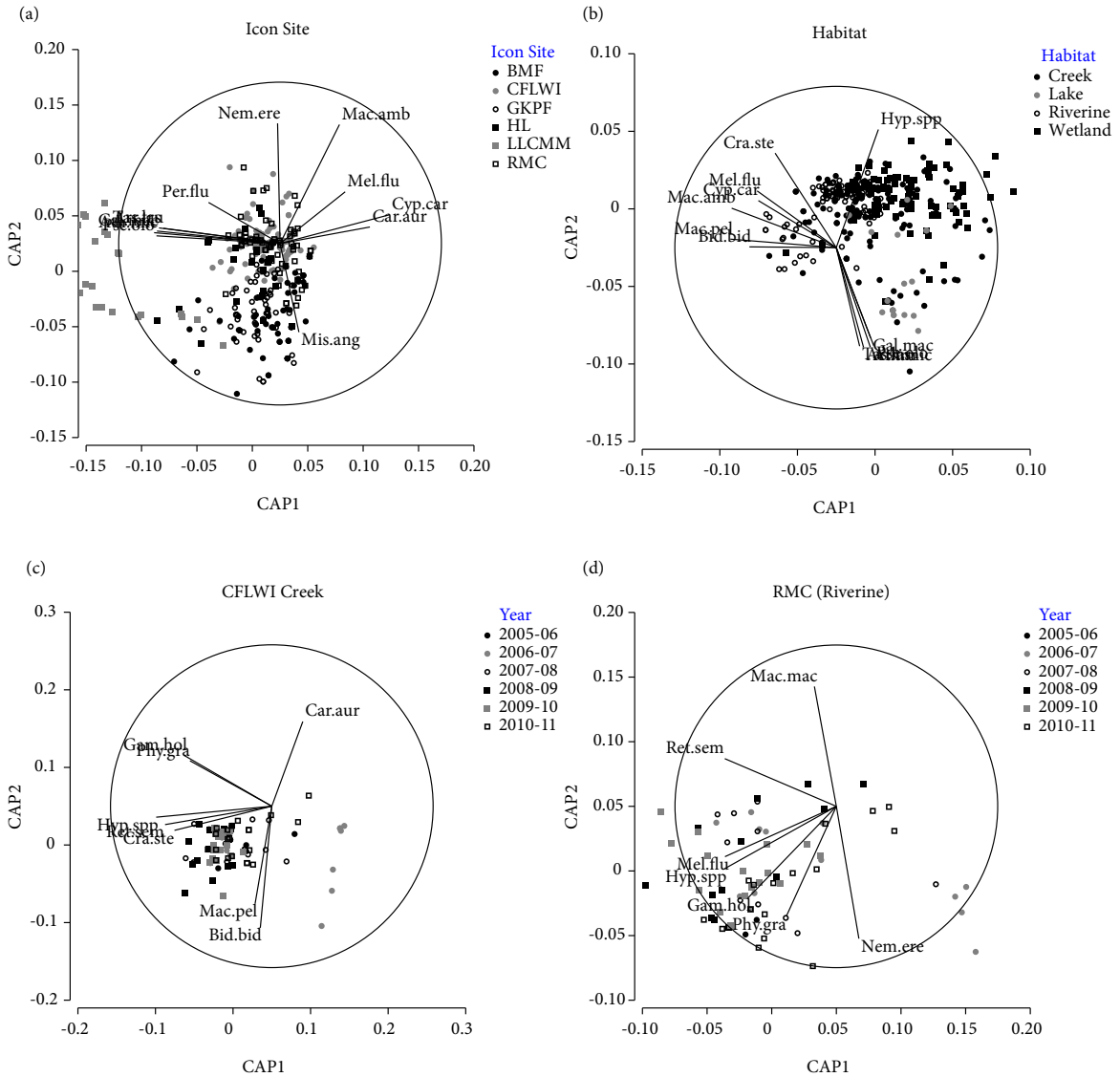


Figure 2. Canonical (discriminant) analysis of the principal coordinates (CAP) ordination plots for the statistically significant components of variation (see Table 3) in presence/absence of the fish species (codes in Table 2) recorded over six years of TLM monitoring in four habitats at the six Icon Sites (codes in Table 1) of the River Murray system. The fish species responsible for the patterns (Spearman rank correlation coefficient with the first CAP axis  $|\rho| \geq 0.5$ ) are indicated.



**Table 4.** PERMANOVA differences in catch-per-unit effort (fish h<sup>-1</sup>) abundance of six fish groups (see Table 2) recorded during six years of monitoring in three habitats at GKPF, HL, LWI and the RMC reaches therein (sampling at the reach level, with data pooled over sites) (codes in Table 3). For simplicity, only statistically significant ( $\alpha = 0.05$ ) a posteriori pair-wise comparisons are given.  $F^*$  = Permutational  $F$  value;  $t$  =  $t$ -test value;  $P^*$  = Permutational  $P$  value (9999 permutations). (Sub-)Icon site codes as in Table 1. See also Figure 3.

Component of variation	df	MS	$F^*/t$	$P^*$
Spatial				
(Sub-)Icon Site	2	8843.3	8.52	< 0.001
GKPF vs. HL			2.97	< 0.001
GKPF vs. LWI			1.95	0.010
HL vs. LWI			2.66	0.002
Habitat	1	2421.9	2.50	0.097
(Sub-)Icon Site $\times$ Habitat	1	1263.2	1.35	0.225
Reach((Sub-)Icon Site $\times$ Habitat)	45	871.1	1.39	0.010
Residual	108	625.4		
Temporal				
Year	2	2630.9	4.12	< 0.001
Year $\times$ (Sub-)Icon Site	5	1410.2	4.76	< 0.001
GKPF				
2008–09 vs. 2009–10			2.37	0.005
2008–09 vs. 2010–11			3.00	< 0.001
LWI				
2006–07 vs. 2007–08			8.50	< 0.001
2006–07 vs. 2008–09			4.99	0.001
2006–07 vs. 2009–10			7.28	0.001
2006–07 vs. 2010–11			5.53	< 0.001
2007–08 vs. 2008–09			2.67	0.014
2007–08 vs. 2009–10			2.43	0.031
2007–08 vs. 2010–11			2.38	0.011
2008–09 vs. 2009–10			5.03	0.002
2008–09 vs. 2010–11			3.48	0.001
2009–10 vs. 2010–11			2.15	0.024
RMC				
2008–09 vs. 2009–10			2.72	0.013
2008–09 vs. 2010–11			2.07	0.040
2009–10 vs. 2010–11			3.27	0.002
Year $\times$ Habitat*	4	1314.0	4.43	< 0.001
Creek				
2006–07 vs. 2007–08			8.50	0.001
2006–07 vs. 2008–09			4.99	0.002
2006–07 vs. 2009–10			2.70	0.039
2006–07 vs. 2010–11			2.46	0.021
2007–08 vs. 2008–09			2.67	0.010
2007–08 vs. 2009–10			2.43	0.022
2007–08 vs. 2010–11			2.38	0.008

**Table 4.** (Continued).

2008–09 vs. 2009–10			4.99	< 0.001
2008–09 vs. 2010–11			3.78	< 0.001
Riverine				
2006–07 vs. 2008–09			3.06	0.027
2006–07 vs. 2009–10			5.04	0.005
2006–07 vs. 2010–11			3.93	0.007
2007–08 vs. 2009–10			3.28	0.020
2008–09 vs. 2009–10			2.72	0.013
2008–09 vs. 2010–11			2.07	0.044
2009–10 vs. 2010–11			3.27	0.003
Wetland				
2006–07 vs. 2010–11			2.30	0.011
2008–09 vs. 2009–10			2.50	0.004
2009–10 vs. 2010–11			2.35	0.008
Year × (Sub-)Icon Site × Habitat	1	482.2	1.63	0.196
Residual	88			

Mean abundance (CPUE) for the native and nonnative species varied among habitats at the Icon Site level (Table 5, Figure 4). At HL, there was a sharp increase in abundance in 2010–11 in the Creek relative to the Wetland habitat for both native and nonnative species. In the Creek habitat of LWI, there was an increase in the abundance of the nonnative species especially in 2010–11, contrary to the native species, which showed a decline. At RMC, there was a sharp increase in the abundance of both native and nonnative species from 2005–06 until 2009–10, and this was followed by a decrease in 2010–11.

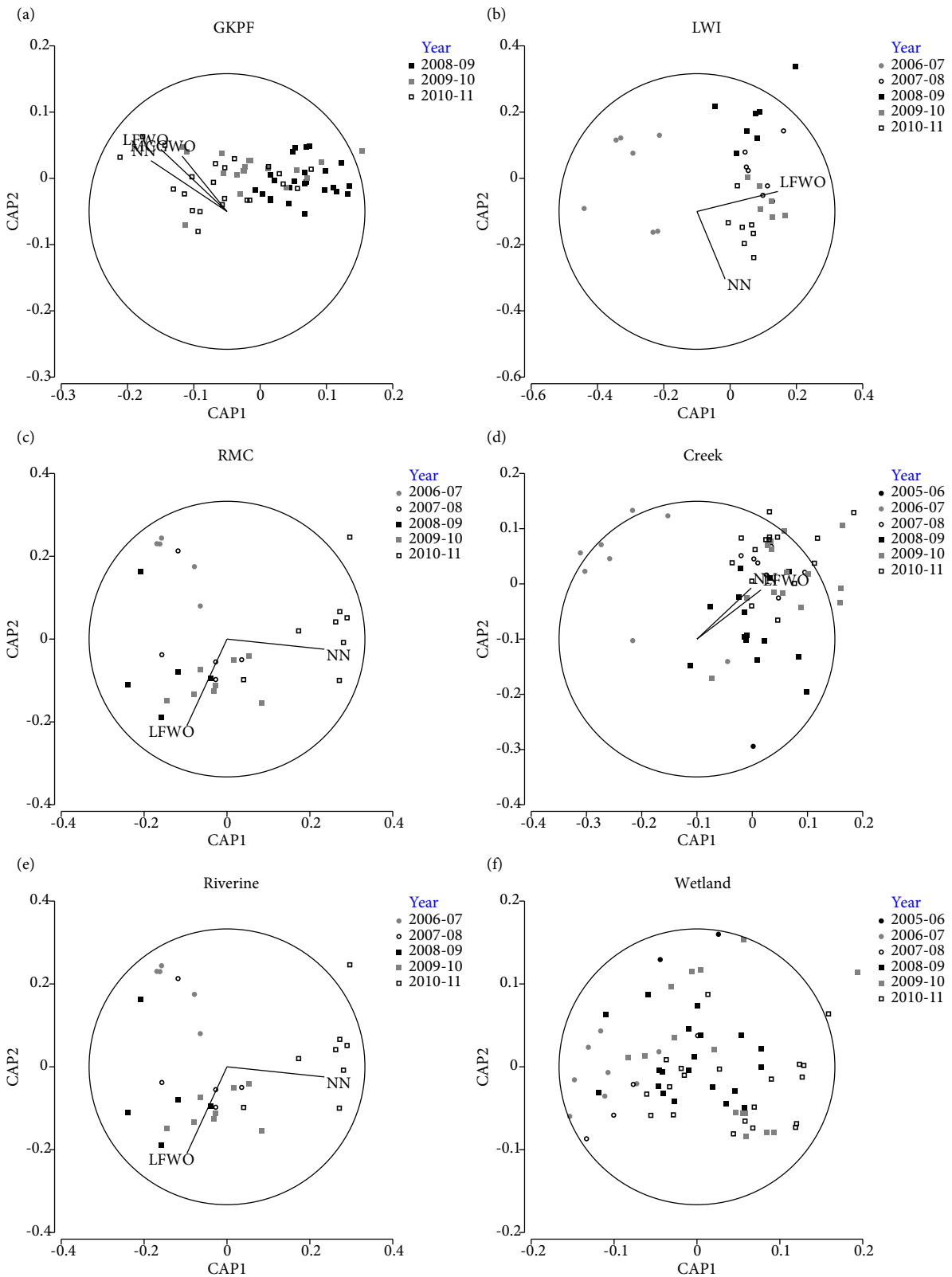
Total richness varied among habitats at the Icon Site level, nativeness varied only among Icon Sites, and no significant differences were detected for nonnativeness either among sites or habitats (Table 5). At BMF total richness was higher in the Riverine compared to the other habitats from 2006–07 to 2009–10 and decreased in 2010–11 (Figure 5a). This was contrary to the other habitats, in which an increase was recorded relative to the previous years. Thus, at CFLWI, total richness increased in the Creek habitat until 2009–10 and varied more widely during the study period in the Wetland habitat (Figure 5b); at GKPF, total richness slightly decreased from 2008–09 to 2010–11 in both the Creek and Wetland habitats (Figure 5c); at HL, richness increased sharply in 2010–11 in the Wetland habitat (Figure 5d); finally, at RMC (i.e. Riverine habitat), richness increased progressively over the study period (Figure 5e). Nativeness was higher at CFLWI and RMC relative to the other Icon Sites throughout the study period, even though in 2010–11 at HL it became similar to that of the above Icon Sites (Figure 5f).

## 4. Discussion

### 4.1. Overall outcomes

Multivariate patterns in species presence/absence and group CPUE abundance consistently pointed to the ubiquity of nonnative species across most of the Icon Sites as well as to an increase in their abundance in 2010–11. Spatially, the higher occurrence of *Cyprinus carpio* in the RMC supports the use by this highly invasive species of the main river channel as a pathway for movement and dispersion (Stuart and Jones, 2006); whereas, the importance of *Misgurnus anguillicaudatus* at BMF and *Perca fluviatilis* at LLCMM in structuring the corresponding fish communities is a further indication of the constant threat posed by nonnative species across the MDB (e.g., Lintermans, 2007). Temporally, the overall increase in CPUE abundance of the nonnative species in 2010–11 is an indication that higher flows in the River Murray system as a result of flood events following drought conditions (Jensen and Walker, 2012) favoured their dispersal, as observed at Hattah Lakes (Vilizzi et al., 2013). And the higher abundances of the LFWO and MCGWO groups under low flows is in accordance with the ‘low flow recruitment hypothesis’ of Humphries et al. (1999). Finally, univariate trends in total abundance and species richness overall mirrored the multivariate patterns.

The repeated sampling of Icon Sites and habitats over time introduces a ‘longitudinal’ (= temporal) aspect in TLM fish monitoring that cannot be discounted. In this respect, the ability of the TLM design to ‘tease apart’ both spatial and temporal components of variation overcome the risk of obtaining spurious results using ‘ordinary’ (i.e.



**Figure 3.** CAP plots for the statistically significant components of variation (see Table 4) in CPUE abundance of the fish groups (cf. Table 2) recorded over six years of TLM monitoring in three habitats at the four (sub-)Icon Sites (codes in Table 1) of the River Murray system. The fish groups responsible for the patterns ( $|\rho| \geq 0.5$ ) are indicated.

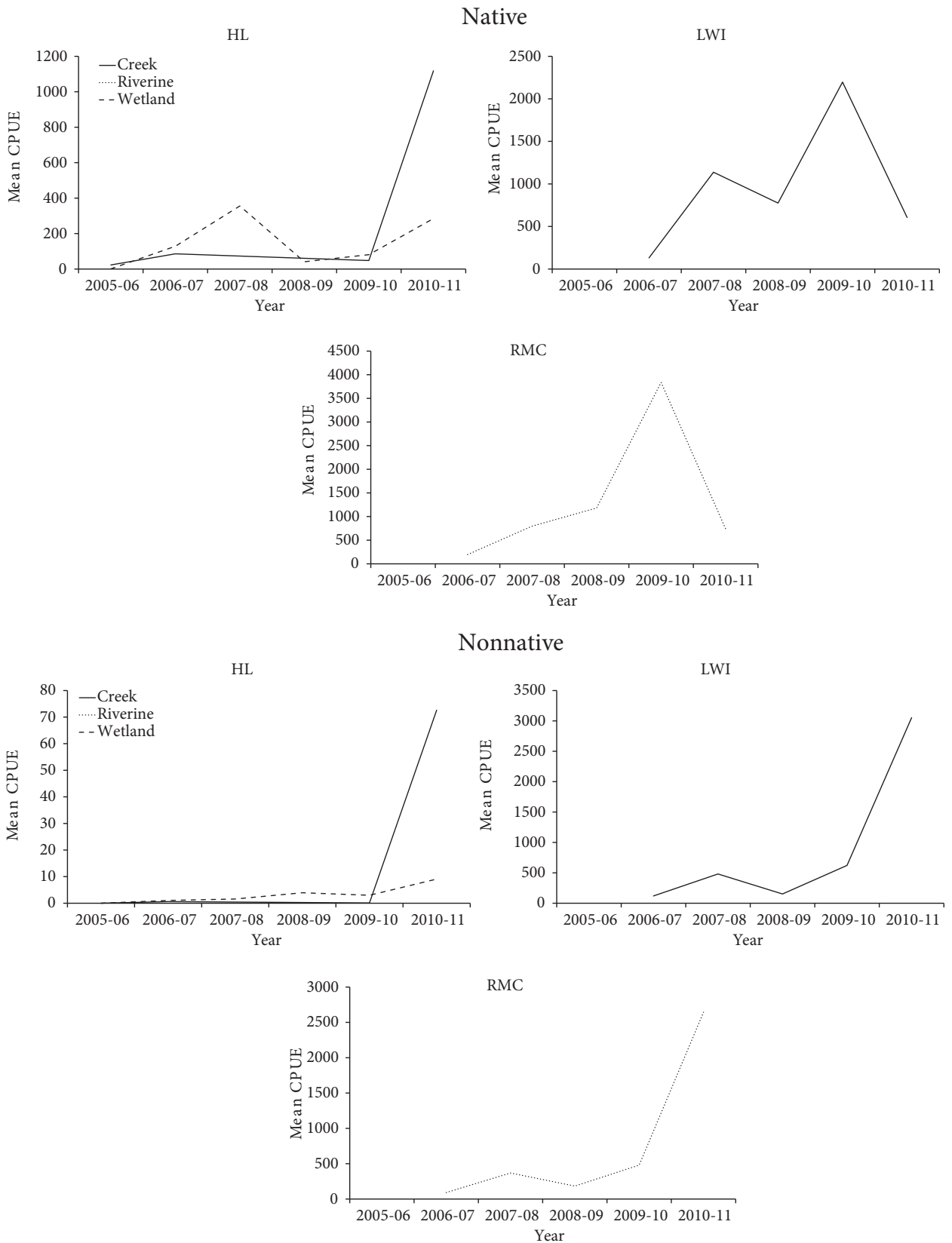
**Table 5.** Generalised estimating equations results for total abundance (CPUE: natives and nonnatives) and species richness (total, nativeness and nonnativeness) of the fish sampled respectively at the four TLM (sub-)Icon Sites and six TLM Icon Site and in four habitats (i.e. Creek, Lake, Riverine, Wetland) from 2006 to 2011. SE = Standard error of the estimate. Wald = Wald statistic. Statistically significant values of interest ( $\alpha = 0.05$ ) in bold. See also Figures 4 and 5.

Effect	Estimate	SE	Wald	P
Total CPUE abundance				
Natives				
Intercept	10.53	0.53	397.4	< 0.001
(Sub-)Icon Site	-1.04	0.12	69.3	< 0.001
Habitat	-2.84	0.30	88.3	< 0.001
(Sub-)Icon Site $\times$ Habitat	0.64	0.06	105.1	< 0.001
Nonnatives				
Intercept	19.46	0.83	547.0	< 0.001
(Sub-)Icon Site	-3.90	0.20	366.0	< 0.001
Habitat	-9.00	0.39	540.0	< 0.001
(Sub-)Icon Site $\times$ Habitat	2.09	0.09	565.0	< 0.001
Species richness				
Total richness				
Intercept	1.81	0.17	119.1	< 0.001
Icon Site	0.22	0.07	8.7	0.003
Habitat	0.04	0.07	0.4	0.549
Icon Site $\times$ Habitat	-0.06	0.03	4.9	0.026
Nativeness				
Intercept	1.36	0.22	38.3	< 0.001
Icon Site	0.25	0.09	7.6	0.006
Habitat	0.00	0.09	0.1	0.973
Icon Site $\times$ Habitat	-0.06	0.03	3.2	0.076
Nonnativeness				
Intercept	0.84	0.17	23.7	< 0.001
Icon Site	0.12	0.09	2.1	0.148
Habitat	0.09	0.07	1.5	0.213
Icon Site $\times$ Habitat	-0.05	0.03	2.9	0.091

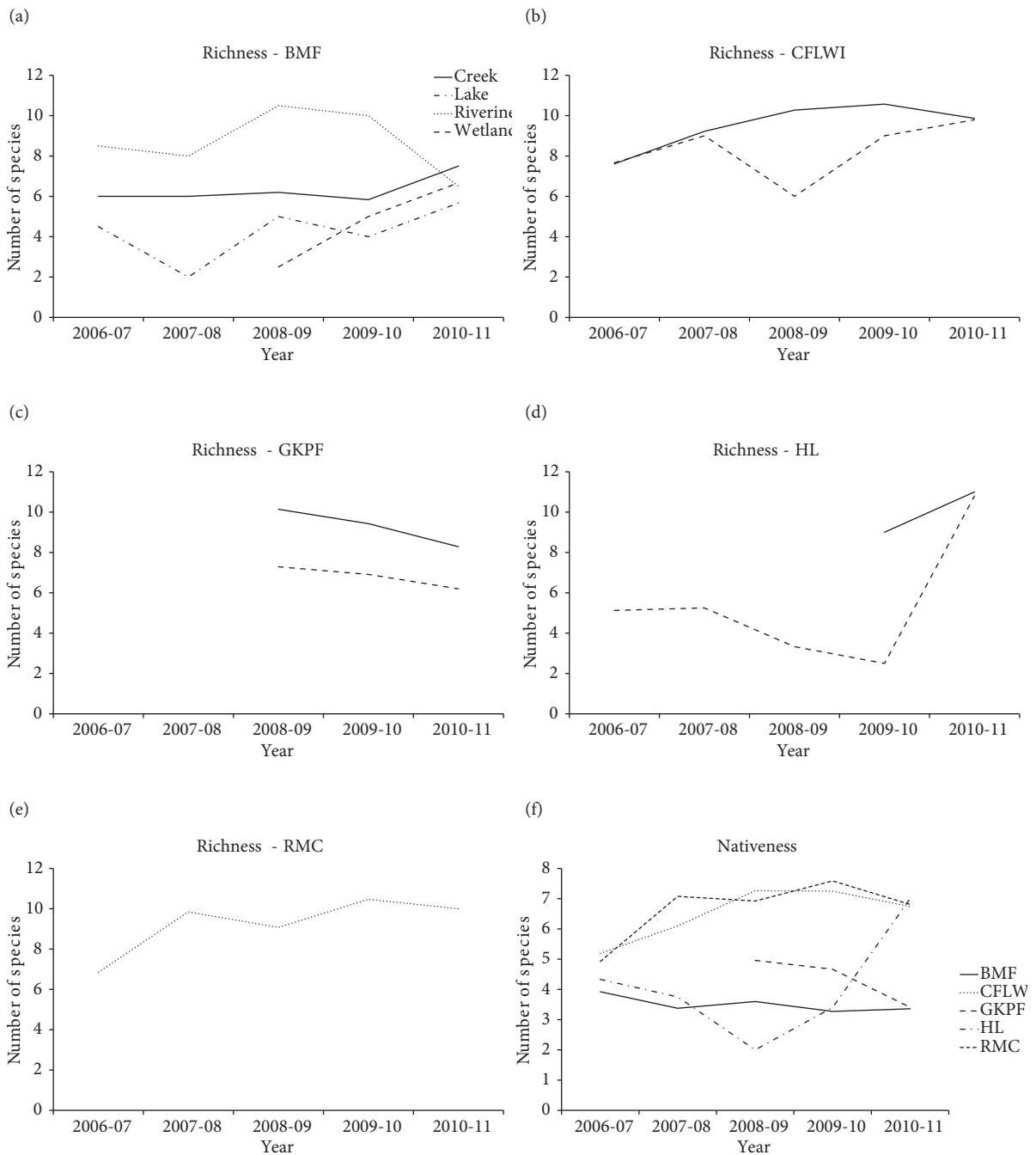
simple factorial) designs (Von Ende, 2001). Also, because of its 'modularity' the TLM design can accommodate the analysis of subsets of its main components of variation, such as conditional upon testable hypotheses, data availability, reporting needs and/or management-specific requirements. For example, description of temporal and spatial changes in fish community structure at a specific Icon Site can be achieved by excluding the Icon Site factor, thereby limiting the spatial extent to reaches and habitats only within the Icon Site of interest. Alternatively, comparisons could be made across Icon Sites and habitats at 'one point in time', hence excluding the Year (temporal)

factor from the design, which would then become a simple 'two-way factorial' (cf. Quinn and Keough, 2002)—the only case in which such design would be statistically justified.

This study has relied on several assumptions that might need to be relaxed in view of future implementations of the proposed standardised analytical framework. Notably, the above suggestions for improvement should be subject to participatory discussion and reviewing amongst both researchers and environmental managers, so that consensus can be achieved and appropriate decisions for further monitoring made (Burgman, 2005). The identification



**Figure 4.** Trend profiles in mean CPUE abundance (fish h<sup>-1</sup>) for the native and nonnative fish species recorded over six years of TLM monitoring in three habitats at the four (sub-)Icon Sites in the River Murray system (codes in Table 1). See also Table 5.



**Figure 5.** Trend profiles in total richness (a–e) and nativeness (f) recorded over six years of TLM monitoring in four habitats at the six Icon Sites in the River Murray system (codes in Table 1). See also Table 5.

of four habitats (i.e. Creek, Lake, Riverine and Wetland) in this study was a ‘judicious’ choice to ensure both consistency across Icon Sites and meaningful replication at the reach level. Although inclusion of additional habitats into the TLM design may be possible, a potential drawback is a reduction in the number of replicates at the reach level, which may ultimately affect the robustness of the statistical methods.

The integrated CPUE measure used in this study has ‘made the most’ of all available data collected with different sampling methods. However, sampling inconsistencies related to effort, precision and accuracy (e.g., Copp, 2010) may have introduced a bias. On the other hand, inclusion of fish data collected only with identical sampling gear and effort (if possible) is likely to reduce severely the size of the dataset, with resulting loss of information. Use

of presence/absence data may represent a satisfactory compromise between the two options, as indicated by the overall similar findings in this study based on fish presence/absence and CPUE abundance.

Although this sample implementation of the analytical framework has been limited to abundance and richness, other measures of 'fish health' such as biomass and condition (i.e. Fulton's *K*, defined as fish weight/length ratio: Nash et al., 2006) could be easily incorporated into the framework, as length and weight are routinely measured as part of fish monitoring surveys. Further, although development and implementation of the analytical framework in this study has been limited to fish, extension to the analysis of patterns in species abundance and composition for e.g. vegetation and birds (the other biota monitored under TLM initiative) would be possible.

#### 4.2. Relevance of the framework to Turkish riverine ecosystems

Freshwater fish richness is remarkably high in Turkey, yet the greatest part of its freshwater habitats has been exposed to high human-induced pressure that has increasingly challenged efforts to mitigate the drastic changes occurring in ecosystem health and integrity (Sekercioglu et al., 2011). This has slowly, albeit distinctly, altered the standpoint of governmental agencies towards an increase in the allocation of resources for monitoring riverine species and populations (including those of fish). For example, long-term monitoring studies have been planned countrywide on the aftermath of a broad reconnaissance project conducted between 2013 and 2019 by the General Directorate of Nature Conservation and National Parks of the Turkish Ministry of Agriculture and Forestry.<sup>4</sup> Therefore, increasing attention has been recently paid to the importance of standardising both data acquisition and monitoring methods. Apart from the open document issued by the Turkish Ministry of Agriculture and Forestry (see Introduction), other published proposals for monitoring environmental quality based on fish community health are all based on metric selection and scoring (Yerli et al., 2016; Çiçek et al. 2018; Ergönül et al. 2018, 2020). Yet, so far all of these

studies have ignored to analyse multivariate patterns of variation in both space and time.

The Murray-Darling basin shows considerable similarity with many of the Turkish river systems because of its human-induced degradation, high regulation, and invasion by nonnative fishes. Therefore, significant declines in the diversity and abundance of native fishes cannot be ruled out for Turkey. A key requirement for halting this decline is the availability of spatial and temporal information on the status and trends of fish occurrence and/or abundance. However, the inherent heterogeneity and limited availability of raw biodiversity data has represented a major hurdle in benefiting from the results of previous large-scale faunal studies. Within the last decade, several studies have documented long term fish occurrence data covering relatively wide geographical ranges including multiple river basins at once. These include the ichthyofauna of the Aras and Kura river basins (Çiçek and Birecikligil, 2016; Kaya et al., 2020) of the western Black Sea and upper Kızılırmak basins (Yoğurtçuoğlu et al., 2020) and of the Seyhan, Ceyhan and Orontes river basins (Bayçelebi, 2020). The TLM design outlined in the present study offers therefore great potential to harmonise the results of these monitoring studies by teasing out the main components of both spatial and temporal variation. This would be possible using presence/absence data (as in the current implementation of the TLM design), with the possibility to incorporate abundance data whenever available. Clearly, there might be some difficulties in implementing the proposed analytical approach to Turkish rivers at the temporal scale due to the lack of repeated (annual) sampling in some river basins. However, this may also represent an opportunity to identify existing knowledge gaps in biodiversity data as a base for future monitoring studies. In turn, this will also facilitate the assessment of outcomes from the framework as well as align the country's profile to international quality standards on conservation of biodiversity (e.g., European Commission, 2014)<sup>5</sup> by documenting status and trends of fish biodiversity in space and time.

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<sup>5</sup> European Commission (2014). Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. Official Journal of the European Union [online]. Website [http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:JOL\\_2014\\_317\\_R\\_0003](http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:JOL_2014_317_R_0003) [accessed 11 March 2021].

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