EFFECTS OF FLOW REGULATION AND NON-NATIVE SPECIES ON FEEDING HABITS OF EURASIAN OTTER *LUTRA LUTRA* IN MEDITERRANEAN TEMPORARY RIVERS

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ABSTRACT

In Mediterranean temporary rivers, ecological resources greatly fluctuate due to the high hydrological variability throughout the year. However, flow regulation prevents this natural regime and commonly entails associated non-native species, which change the structure of aquatic communities. Nonetheless few studies have tested the interaction of these two disruptive factors (flow regulation and non-native species) and their synergistic effects on the Eurasian otter (*Lutra lutra*) diet at the river-scale. The aim of this study was to compare the seasonal feeding habits of the otter between a temporary non-regulated stretch and two regulated stretches invaded by non-native species in a Mediterranean water course. The Bullaque River (Guadiana River basin, central Spain) was seasonally sampled for otter spraints and prey abundance assessed from December 2009 to November 2010. Three stretches were considered: High (source, non-regulated), Medium (transition, regulated) and Low (confluence, regulated). Diet varied from native prey in the High stretch (amphibians, insects and endemic cyprinids) to non-native species in the Low stretch (red-swamp crayfish *Procambarus clarkii* and pumpkinseed sunfish *Lepomis gibbosus*). Seasonally, ingested biomass of native prey increased in spring. Diet was more diverse in the High stretch. Otter neutrally selected native cyprinids in the high stretch throughout the year; whereas crayfish was selected in the other two stretches. Overall results showed flow regulation and non-native species have increased prey availability for the otter; however this paper highlights the importance of maintaining natural regimes in Mediterranean temporary rivers to conserve native communities and thus least-impacted food webs in Iberian freshwaters.

KEY WORDS: bioinvasion; diet; Iberian Peninsula; *Lutra lutra*; prey selection; temporary rivers

SHORT TITLE: Diet of Eurasian otters in Mediterranean temporary rivers

INTRODUCTION

Temporary rivers are highly represented in Mediterranean regions because of the influence of climate, characterized by cool, wet winters and summer droughts (LeHouórou, 1990). The discharge regime generally follows this rainfall pattern, with torrential floods usually occurring in autumn and winter and minimum flow and severe droughts in summer (Gasith and Resh, 1999). As a consequence, ecological resources greatly fluctuate within the year in Mediterranean temporary rivers (Larned *et al*., 2010) and species living there are highly specialized to cope with this high hydrological and resource variability (Williams, 1996).

Flow regulation, resulting from the construction of dams, reduces the effect of droughts and prevents natural flooding (Nilsson *et al.*, 2005; Wang *et al.*, 2011). In Mediterranean temporary rivers, flow regulation changes composition and structure of aquatic communities such as fish (Godinho and Ferreira, 2000; Growns and Growns, 2001), becoming dominated by non-native species (Pedroso *et al*., 2007; Basto *et al*., 2011). In the Iberian Peninsula, non-native fishes are increasing their ranges and this promotes the decline of the endemic fish fauna, both in their ranges and abundances through a variety of biotic interactions (see Leunda, 2010 for a comprehensive review). Among non-native fishes, two centrarchids, pumpkinseed sunfish *Lepomis gibbosus* (L., 1758) and largemouth bass *Micropterus salmoides* (Lacépède, 1802), are among the most widespread species in the Iberian Peninsula (e.g. Blanco-Garrido *et al*., 2008). Also an invasive crustacean, the red-swamp crayfish *Procambarus clarkii* (Girard, 1852), is very widespread in the Iberian Peninsula and has deeply altered the Iberian freshwater ecosystems where it has been introduced (Geiger *et al*., 2005), particularly in relation to food web structure (Tablado *et al*., 2010). Nonetheless few studies have tested the interaction of these two disruptive factors (i.e. flow regulation and non-native species) and their synergistic effects on the diet of a top-predator, including seasonal and spatial variation.

The Eurasian otter *Lutra lutra* (L., 1758) is a top-predator and key-species in the aquatic community of European inland waters (Ruiz-Olmo and Jiménez, 2009; Clavero *et al*., 2010; Almeida *et al*., 2012a), that contributes to maintain the ecological balance of freshwater ecosystem (Chanin *et al.*, 2003; Miranda *et al*., 2008). In Mediterranean temporary rivers, summer droughts pose a handicap for otters (Ruiz-Olmo *et al*., 2007), since it is the most important limiting factor in their distribution and abundance, because of the great fluctuation of prey availability (Prenda *et al*., 2001; Ruiz-Olmo *et al*., 2001). As a consequence, this seasonal factor affects otter breeding, carrying capacity and mortality (Kruuk and Carss, 1996; Ruiz-Olmo and Delibes, 1998; Ruiz-Olmo and Jiménez, 2009).

The aim of the present study was to assess the effects of flow regulation and the associated non-native species on the feeding habits of a top-predator, the Eurasian otter, in Mediterranean temporary rivers. For this purpose, we studied the feeding habits of the otter throughout a year along a partially regulated Mediterranean river of the Iberian Peninsula. Specifically, we seasonally compared diet, trophic diversity, prey availability and prey selection between three stretches in the Bullaque River (central Spain). We hypothesized that flow regulation and non-native species will affect the feeding habits of the otter and predicted that: (i) otters will feed more on red-swamp crayfish in regulated stretches, because though less energetic is easier to capture; (ii) otter trophic diversity will be higher in the non-regulated stretch, due to its higher seasonal variability and (iii) otters will still select native prey over non-native.

METHODS

Study area

The field sampling was carried out in the 1019 km^2 Bullaque River catchment (altitude: 550–620 m.a.s.l.), located in the Guadiana River basin (central Spain, 39º11’N - 4º15’O, Figure 1). The area is characterised by a continental Mediterranean climate, with rainfall from late autumn to spring (500–800 mm), whereas summer is hot and dry. Annual mean temperature ranges between 9 and 14ºC. The lowest temperatures are recorded in December (–5ºC) and the highest in August (43ºC), (Almeida, 2008). Land use is mainly characterised by agricultural activity (e.g. corn and wheat crops, pastures for raising cattle and sheep). The Bullaque River (94 km length) includes a dam and a reservoir called Torre de Abraham (Figure 1). Upstream the reservoir, the river has an intermittent flow regime with seasonal flooding (autumn, winter) and severe droughts (summer); downstream the dam, the river has a regulated flow regime with weak seasonal fluctuations. Regarding biota, the particular invertebrate communities, fish assemblages and riparian vegetation of Bullaque River are well described in Almeida *et al*. (2012b, 2013).

Field sampling

We considered three stretches in the river according to their different hydrological and ecological characteristics: 1. *High stretch*: from the source of the river and its headwaters to the reservoir, included a tributary, the Milagro River (Figure 1). Both watercourses are lotic and oligotrophic temporary rivers, with narrow and medium-high speed flow; they are strongly affected by the climate conditions, becoming intermittent with a succession of pools of variable dimensions with little or no water flow in summer. Flow discharge is similar for both water courses, ranging between 0.3 m^3/s in summer and 0.9 in winter. Their bank vegetation is well preserved.

2. *Medium stretch*: it begins at the outlet of the reservoir and it includes the transition of the river. It is characterized by a wider and deeper channel, and it is less influenced by the meteorological conditions, keeping a minimum ecological flow all the year. It flows along an area submitted to an intensive agrarian and cattle exploitation, which have converted the gallery forest in isolated stains of ash trees (*Fraxinus angustifolia;* Vahl, 1804) and Mediterranean scrubland (mainly *Crataegus monogyna*, Jacq, 1775; *Rubus* sp.; *Rosa* sp. and *Cistus* sp.). Its flow discharge range between 0.9 m^3/s in autumn and 1.2 in winter. 3. *Low stretch*: which is the confluence of the river, and is influenced by the Guadiana River where it flows. The volume of flow here is maximum as it receives water from more tributaries than the medium stretch. It is highly eutrophicated, because of the agriculture runoff from the medium stretch and also because the river is naturally dammed in many parts of this stretch, creating semi-permanent floodplains where water is practically stagnant. This part of the river presents a discharge regime that oscillates between 1.1 m^3/s in autumn and 1.5 in winter. In this stretch of the river, the agrarian activity is lower but it is more urbanized and has more human presence. See Almeida *et al.*, 2013 for a more detailed description of the discharge regime profiles.

With the aim of assessing the variation of the otter diet throughout a year and between stretches, we searched monthly for otter faeces (referred to as 'spraints' hereafter), from December 2009 to November 2010. According to the methodology proposed by Ruiz-Olmo and Delibes (1998), we selected four sampling sites (600 m river length) per stretch (Figure 1) and monthly collected 5–6 spraints per site whenever it was possible; we grouped those spraints per stretch and season (3 months), resulting in 60 spraints per group for diet analysis, a sample size higher than in other studies in Mediterranean Rivers (Miranda *et al*., 2006; Marques *et al*., 2007; Novais *et al*., 2010). We did not collect more spraints per site to avoid disturbing natural sprainting behaviour of otters. Also, only fresh spraints were collected to reduce loss of prey remains after defecation and to ensure regular presence of otters in the site (Almeida *et al.*, 2012b). In total, we collected 731 spraints for diet analysis.

In order to assess prey availability, fish and crayfish biomasses (measured as kg ha^{-1}) were estimated once each season at each sampling site by using block nets and electrofishing (2000 W DC generator at 200–250 V, 2–3 A) in an upstream direction, following the removal sampling without replacement or Zippin's method (1956), with three passes made (sampling time for each pass 20–30 minutes). Fish and crayfish were immediately immersed in an innocuous solution of anaesthetic (MS-222 at 0.1g L^{-1}), identified to the species level, counted and weighed (± 0.1 g). Fish were kept in a tank and supplied with oxygen (two aerators Aera, portable battery pump) until fully recovery before releasing them. All field procedures were complied with animal use and care regulations of Europe and Spain (specific Licence Code: DGPF/MRP-2010 for Scientific Field Research in Castilla-La Mancha, Spain). Electrofishing was performed by trained personnel (i.e. the holder of the Licence, D.A.), who had already sampled for fish by electrofishing in the same study area for previous projects (e.g. Almeida *et al*. 2009; Almeida *et al.*, 2012b).

Dietary analysis

The diet of the otter was determined by analysing spraints and identifying indigestible parts of the food intake (e.g. bones, scales, hair and feathers). These analyses were done in the laboratory, following a standard methodology described by Beja (1997). Food items were identified to the lowest possible taxonomic level using a dedicated reference collection of scales and hair, as well as published literature (Day, 1966; Gállego and Alemany, 1985; Teerink, 1991; Prenda and Granado-Lorencio, 1992a; Conroy *et al*., 1993; Chinery, 1997; Prenda *et al*., 1997; Miranda and Escala, 2002). The minimum number of individuals of each prey item present in a spraint was estimated by integrating the number, position (left–right) and relative size of diagnostic hard structures (mainly vertebrae, pharyngeal arches and scales for fish, and endopodites/exopodites and telson for red-swamp crayfish).

Each identified prey item was considered as an 'occurrence', and we calculated four dietary indices commonly used in carnivore diet studies (Klare *et al*., 2011). The *Frequency of Occurrence* (FO, percentage of spraints in which a prey item was present), *Relative Frequency of Occurrence* (RFO, percentage of the total number of occurrences corresponding to a certain prey item), the *Percentage of Numbers* (%N, : total number of individuals corresponding to a certain prey item / total number of individuals) and the *Percentage of Ingested Biomass* (%Biomass, multiplying the total number of individuals corresponding to a certain prey item by their average weight in the environment). Average weight for fish and crayfish were calculated from averaging the weights of conspecifics from the electrofishing sampling. Thus, for the calculations of %Biomass in each spraint, we used the average weight of the fishes and crayfishes electro-fished in the same sampling site and season; as we saw in a previous study done in the same river (D. Almeida, pers. Observ.), that the average weights of prey in the environment were similar to those captured by the otter (Almeida *et al.*, 2012b). For the rest of prey items, we assigned the following weights: insects, 1g; amphibians, 10 g; reptilians (only one species, *Natrix maura*), 50 g; rodent species, 20 g; rest of mammals and birds, 100g (Beja, 1996). We calculated the Shannon index (*H'*) for trophic diversity. All scientific and common names of freshwater fishes have been checked according to Leunda *et al*. (2009).

Prey selection

As recommended by Lechowicz (1982), prey selection (preferences) for crayfish and the main fish species consumed was evaluated using the Vanderploeg and Scavia (1979) normalised electivity index (ε_i):

$$\varepsilon_i = [\alpha_i - (1/n)]/[\alpha_i + (1/n)], \text{ where } \alpha_i = (r_i/p_i)/\ \Sigma_{i=1}\ (r_i/p_i)$$

where r_i is the proportional abundance of prey *i* in the diet (Ingested Biomass_i/Ingested $\text{Biomass}_{\text{Total}}$), p_i is the proportional abundance of prey *i* in the environment (from electrofishing data), *n* is the number of prey types included in the analysis and α is the Manly-Chesson's alpha (Chesson, 1978). The electivity values range from –1 (negative selection) to 1 (positive selection), and zero implies neutral selection.

Statistical analyses

To simplify the analytical models, data were pooled per season: winter (December–February), spring (March–May), summer (June–August) and autumn (September–November). Thus, the statistical power of the remaining sources of variation is increased, which would otherwise be seriously compromised. We tested if our spatial and temporal (stretch and season) sub-sample size was representative of the spectrum of the otter diet in the river by plotting the cumulative curve of new resource items by number of sampled spraints after randomization (Marques *et al.*, 2007).

To assess the spatial and temporal interactions, as well as the variations in feeding habits and prey availability, we performed General Linear Models (GLMs: factorial and univariate ANOVAs) with the percentage of ingested biomass, *H'*, prey biomasses and ε_i as dependent variables, and stretch and season as factors, followed by post-hoc tests (Tukey-Kramer honestly significant difference, HSD test). To test whether electivity significantly deviated from 0, one sample Student's *t*-test was used with Bonferroni corrections. The percentage of ingested biomass was used for statistical analysis because it is the index best reflecting the relative importance of food items in carnivore diet (Klare *et al*., 2011), and the only one that could be compared with prey availability. The other diet indexes were also provided in order to enable comparisons with studies using them but not the ingested biomass.

For statistical analyses, proportions and electivity indices were arcsine transformed, whereas the remaining variables were $\log_{10}$ transformed. Assumptions of normality of distributions and homogeneity of variance were verified using Shapiro-Wilk and Levene tests, respectively. All statistical analyses were performed with STATISTICA 7.0 (Statsoft INC., Tulsa, OK, USA). The significance level was set at $\alpha = 0.05$.

RESULTS

The cumulative curves of the number of prey items in otter diet per number of analysed spraints show that the size of the sub-samples collected in each stretch (figure 2) and season, as well as in the whole river, were representative of the otter diet.

Red-swamp crayfish was the main prey consumed by the otter across the river and throughout the year, representing about 40% of the total ingested biomass, followed by fishes with 30%. Among fishes, non-native northern pike *Esox lucius* (L., 1758) contributed with the greatest biomass to the diet, followed by other non-native fishes, the pumpkinseed sunfish and the common carp *Cyprinus carpio* (L., 1758). The third prey category in importance was amphibians with 14% of the total ingested biomass. Other noticeable prey was the common moorhen *Gallinula chloropus* (L., 1758). The remaining prey items can be considered as of minor importance (any of them with <2% of ingested biomass, Table I). The diet showed significant spatial variation ($F_{30, 20}$ = 5.77, p < 0.001). The High stretch was characterized by a higher consumption of endemic cyprinids such as southern straight-mouth nase *Pseudochondrostoma willcommii* (Steindachner, 1866) and calandino *Squalius alburnoides* (Steindachner, 1866), as well as amphibians and insects (mostly diving beetles) (Table II). In the Low stretch, the otter fed on a high proportion of pumpkinseed sunfish, reptilians and red-swamp crayfish. In the Medium stretch the otter had intermediate trophic characteristics between the other two stretches. There, the consumption of calandino was higher while the intake of reptilians, amphibians and insects were not. Even the consumption of pumpkinseed sunfish was lower than in the Low stretch (Table II). Seasonally, the overall composition of the otter diet also varied significantly ($F_{45, 30.49}$ =1.87, p = 0.04), in particular the Iberian arched-mouth nase *Iberochondrostoma lemmingii* (Steindachner, 1866) was more consumed in spring than in autumn (Table II). Focusing on the seasonal diet variation in each stretch separately, there were differences between them. While in the Medium and Low stretches prey items did not vary seasonally, they did in the High stretch ($F_{24, 3.5}$ = 47.38, p < 0.01). In particular pumpkinseed sunfish and insects were more consumed in autumn than in the rest of the year.

Trophic diversity varied significantly along the river ($F_{2, 24}$ = 20.03, p < 0.001), being maximum in the High stretch in contraposition with the Medium and Low stretches, where no differences were found (Figure 3). Regarding spatial variation, trophic diversity did not significantly vary between seasons ($F_{3, 24}$ = 2.09, p = 0.13).

Results of electrofishing are shown in Table III. Total available biomass of fish and crayfish increased from the High stretch (mean = 12.79 kg ha^{-1}, SE = 5.58) to the Low stretch (mean = 28.32 kg ha^{-1}, SE = 9.85), although differences among stretches were not statistically significant ($F_{2, 45}$ = 1.12, p = 0.33). Conversely, composition of prey availability significantly differed between stretches ($F_{24, 50}$ = 2.23, p < 0.01), with significant differences of biomass of southern Iberian spined-loach *Cobitis paludica* (De Buen, 1930) along the stream, being higher in the High stretch than in the rest of the river (Table IV). Total available biomass also varied seasonally ($F_{3, 44}$ = 9.49, p < 0.001), being highest in summer (mean= 50.12 kg ha^{-1}, SE = 12.86) followed by spring (mean= 19.36 kg ha^{-1}, SE = 4.32), winter (mean= 4.91 kg ha^{-1}, SE = 3.44) and finally autumn (mean= 3.42 kg ha^{-1}, SE = 1.17) (Tukey test, p < 0.05). For particular prey categories, the introduced red-swamp crayfish was much more abundant in spring and in summer than in the rest of the year, opposite to calandino, which resulted much more abundant in autumn and winter (Table IV). Also, Iberian arched-mouth nase showed seasonal variations being more abundant in winter than in the rest of the year (Table IV). Available biomass of non-native Eastern mosquitofish *Gambusia holbrooki* (Girard, 1859) also varied seasonally, reaching a peak in summer (Table IV). The interaction between these two factors, Stretch and Season, was not statistically significant for any species ($F_{72, 141.82}$ = 0.93, p = 0.63).

Globally, the otter showed significant negative electivity for most prey items in the whole river and over the year (*t-test*s, p < 0.001) with the only exception of red-swamp crayfish, which was neutrally selected (*t-test*, p > 0.05). However, the electivity index showed differences both spatially and seasonally ($F_{18, 32}$ = 3.59, p < 0.001 and $F_{27, 47.37}$ = 4.65, p < 0.001; respectively), the interaction between these two factors also was statistically significant ($F_{54,86}$ = 2.17, p < 0.001). Within stretches, southern straight-mouth nase was neutrally selected in the High stretch while it was avoided in the rest of the river (Table V), contrary to red-swamp crayfish which was avoided in the High stretch and neutrally selected in the rest of the river (Table V). Also, calandino showed spatial variation, being more selected in the medium stretch than in the rest of the river (Table V). Seasonally, pumpkinseed sunfish was neutrally selected in summer and in autumn, in contrast to winter, when it was completely avoided (Table V). Red-swamp crayfish was highly positively selected in winter and avoided in spring and summer, being neutrally selected in autumn (Table V).

DISCUSSION

Considering the whole river and throughout the year, otter diet resembles that typical of regulated water courses and reservoirs, i.e. based on red-swamp crayfish and non-native fish species (López-Nieves and Hernando, 1984; Adrián and Moreno, 1986; Delibes and Adrián, 1987; Pedroso and Santos-Reis, 2006; Sales-Luis *et al*., 2007), instead of that typical of Mediterranean temporary rivers (Ruiz-Olmo *et al.,* 1998; Bartolomé, 2001; Clavero *et al.,* 2003). Possibly the opportunistic predator behaviour of the otter (Balestrieri *et al*., 2013), allows them to feed on the most abundant prey (Erlinge, 1969, Taastrom and Jacobsen, 1999). In our study, red-swamp crayfish, the most abundant prey in the river, was also the main otter prey. As in other places where the red-swamp crayfish has been introduced, it has become a major prey for a variety of predators (Delibes and Adrián, 1987; Peris *et al*., 1994; Tablado *et al*., 2010), and it has been hypothesized to be a key factor in the recovery of otter populations in the Iberian Peninsula (Ruiz-Olmo and Delibes, 1998). For this role as a factor of otter recovery, red-swamp crayfish should be also abundant during the critical periods of the year (i.e. winter floods and summer droughts) (Beja, 1996). In our study, red-swamp crayfish was highly consumed throughout the year, even in winter when it usually stays in burrows (Niquette and D'Abramo, 1991; Correia and Ferreira, 1995). Among fishes, the second prey category in importance, non-native species contributed with the highest proportion of biomass to the otter diet in the whole river. Non-native species are related to reservoirs and regulated flows (Godinho *et al*., 1998; Basto *et al*., 2011), where they interact with, and force the decline of native species becoming the dominant species (Leunda, 2010) and also the major prey for otters (López-Nieves and Hernando, 1984; Adrián and Moreno, 1986; Pedroso and Santos-Reis, 2006; Sales-Luis *et al*., 2007). Our results are in concordance with the preferences for prey type by otters (Ruiz-Olmo, 1995). Thus, among non-native species, the northern pike was the most important prey for the otter in terms of biomass. Also the higher abundance of pumpkinseed sunfish and red-swamp crayfish in the river may make them easier prey to capture than endemic cyprinids, considered preferred prey for the otter in Mediterranean rivers of the Iberian Peninsula (López-Nieves and Hernando, 1984; Callejo and Delibes, 1987; Ruiz-Olmo *et al*., 1989; Ruiz-Olmo, 1995; Bartolomé, 2001; Morales *et al*., 2004), despite the anti-predator body structures of the pumpkinseed sunfish (Blanco-Garrido *et al*., 2008) or the less energetic contribution of the red-swamp crayfish to otter diet (Beja, 1996).

Is well known that otters are selective in prey size (Lanszki *et al.*, 2001) and consequently %Biomass is biased according to this behavior. To mitigate this source of error, studies assessing %Biomass incorporate regression equations to accurately calculate the actual weight of the specimens consumed found from the indigestible parts in the spraints (Prenda and Granado-Lorencio, 1992b; Copp and Kováč, 2003). However we did not use such regression equations since in a previous study (Almeida *et al.*, 2012b) it was seen that the average weight of the fishes and crayfishes electro-fished were similar to those selected by the otter in this river (D. Almeida, pers. Observ.).

The spatial variation in otter diet reflects the effect of the reservoir and flow regulation. In the High stretch, hardly influenced by the reservoir, the diet of the otter is similar to that described for Mediterranean temporary rivers; based on endemic cyprinids and high amounts of invertebrates and amphibians (Adrián and Delibes 1987; Ruiz-Olmo, 1995; Bartolomé, 2001; Ruiz-Olmo *et al.*, 2002). Conversely, the diet of the otter in the regulated Medium and Low stretches resembles that described in reservoirs regarding non-native species (López-Nieves and Hernando, 1984; Adrián and Moreno, 1986; Pedroso and Santos-Reis, 2006; Sales-Luis *et al.*, 2007; Basto *et al.*, 2011). Seasonally and considering the river as a whole, otter diet did not show any significant variation; but if we focus on each stretch separately, the differences come to light. In particular, within the High stretch, diet varied seasonally whereas it did not in the rest of the river. This result highlights the temporary flow regime of the High stretch, which presents different prey types in each season due to its fluctuating ecological conditions (Gasith and Resh, 1999). This contrasts with the stability of the ecosystem downstream the reservoir, where most of the prey types are available for otters throughout the year. According with the optimal foraging theory, generalist predators change prey foraging patterns according to their profitability (Ferreras *et al.*, 2011). The high consumption of pumpkinseed sunfish and insects in autumn in the High stretch may be due to the scarcity of endemic cyprinids after the summer drought, and possibly to the use of the close reservoir as an alternative source of prey in the dry season (Basto *et al.*, 2011). Except for calandino, whose abundance significantly increased (Table IV), the availability of the rest of cyprinid species decreased in autumn (Table III), as it occurs in other Mediterranean streams (Mas-Marti *et al.*, 2010). This increase in the availability of calandino was followed by a non-significant increment in its consumption after the summer drought. However, an increase in the percentage of biomass for a particular prey type in the river is not necessarily related to an increase in its availability for the otter. Small pools where otters fish during summer in the High stretch increase their volume with the autumn rains and torrential flows drag fishes to them, which result in an increase of available biomass (according to electrofishing) in these habitats. However, an increase in depth and water volume in the pools can hinder the capture of fishes by the otter (Barrientos *et al.*, 2003; Kruuk, 2006; Almeida *et al.*, 2012b). Trophic diversity varied spatially in accordance with the negative relationship with water flow stability (Clavero *et al*., 2003). The higher values of *H'* in the High stretch than in the rest of the river are possibly due to the harsher environmental conditions, which force the otter to prey on less profitable prey such as amphibians or insects (Clavero *et al*., 2008; Román, 2011).

Our results of prey selection show that, except for the red-swamp crayfish, almost all prey items were used below their availability and no species was positively selected, similar to Almeida *et al.* (2012a). These results differ from the trophic behaviour stated for the otter; which establish that otters consume each different prey item according to their particular availability (Clavero *et al*., 2003; Remonti *et al*., 2010). Our results are unusual and may be explained by the wide availability of a variety of prey items in the river (i.e. pumpkinseed, red-swamp crayfish, calandino or southern straight-mouth nase) and also by the generalist and opportunistic trophic behaviour of the otter; which allows it to prey on most of them and as a result, the encounter rate with one prey item in particular is divided among the wide variety of available prey items; resulting in a prey selection under the particular availability of each prey item in particular.

Although with our data we could not fit a clear functional response related to red-swamp crayfish consumption, otters could be displaying a type II or III functional response (Holling, 1959) in their last steps. This would imply that crayfish availability had passed a threshold beyond which the otter searches actively for it. Nevertheless, this does not happen in the High stretch, possibly because the availability of the invasive crustacean in this part of the river is lower and otters search for other more profitable prey, mostly native cyprinids (such as the case of southern straight-mouth nase). This is due to its higher abundance or because they are easily captured by the otter in that part of the river (Barrientos *et al*., 2003). Seasonally, otters increased the selection of pumpkinseed sunfish during the warmer seasons, possibly because this species availability increased during these months (Almeida *et al.*, 2009). The highly positive selection of red-swamp crayfish in winter may be overestimated because of its burrowing behaviour in the cold months of autumn and winter (Niquette and D'Abramo, 1991; Correia and Ferreira, 1995), which makes them less vulnerable to sampling methods. However, otters seem not to have problems in their capture, according to the lack of seasonal variation in crayfish consumption. The apparent avoidance of this type of prey in spring and summer should be due to the red-swamp crayfish consumption rate by the otter has reached an asymptote which is independent of the higher density of red-swamp crayfish populations during these seasons in comparison with the rest of the year.

Even though prey biomass did not significantly differ between stretches, it increased from the source to the mouth of the river, with prey biomass doubling between the High stretch and the Low stretch considering all year (142 kg ha^{-1} and 350 kg ha^{-1} respectively, data obtained from Table III), as it happens in other rivers (Townsend *et al*., 2003; Davey and Kelly, 2007; Magalhães *et al*., 2007). This is because Medium and Low stretches maintain a minimum flow throughout the year due to the flow regulation by the dam downstream, which mitigates or even neutralize the effect of seasonal floods or droughts. This is opposite to the High stretch, where the narrow and shallow channel limits its prey carrying capacity. But the High stretch also undergoes seasonal floods which drag the biota, and seasonal droughts, which reduce habitat availability and suitability in several ways, resulting in a lower fish biomass (Gasith and Resh, 1999; Mas-Martí *et al*., 2010). The species composition slightly varied between stretches which is in concordance with other studies (Matthews and Marsh-Matthews, 2003; Aparicio and Vargas, 2004; Magalhães *et al*., 2007). Even so, a trend in its distribution can be appreciated, with the dominance of non-native species near the confluence of the river possibly because of the presence of the reservoir upstream, which acts as a source of non-native species (Godinho *et al*., 1998) and the influence of the outflow in the Guadiana River. Seasonally, the differences found in available prey biomass can be explained above all by the availability increase of red-swamp crayfish in summer and, in a lesser extent, by the peak of availability of the pumpkinseed sunfish in the warm months of the year (Table III).

Our study reveals the dual effect of both flow regulation and non-native species in natural Mediterranean temporary rivers. On the one hand flow regulation increases the water availability throughout the year, which allows the maintenance and growth of non-native population species normally at expense of the native fish species (Leunda, 2010). This eventually results beneficial for otters and other generalist predators (Tablado *et al*., 2010), since the carrying capacity of the river for them increases, due to the increase of prey availability. Otters also likely improved their breeding success and diminished their mortality rate (Kruuk and Carss, 1996; Ruiz-Olmo and Delibes, 1998; Ruiz-Olmo and Jiménez, 2009). On the other hand, these changes deeply modified the natural ecological processes of Mediterranean temporary rivers, i.e. changes in the composition and structure of aquatic communities and also in their natural trophic web; so it is detrimental in a broader conservation context. Thus, conservation efforts should give priority to preserve the Mediterranean temporary rivers, because they harbour a wider variety of species than Mediterranean regulated rivers, making them valuable habitats, and avoid disturbing natural feeding behaviours of predatory species in freshwater environments (Basto *et al*., 2011). Our non-regulated stretch maintains otter presence throughout the year, and more important, maintains the natural diet of the mustelid in this region. In view of the radical changes caused by flow regulation in temporary rivers, from species to ecosystem level, such type of actuations should be carried out only in unavoidable situations and the management should be aimed to preserve the original biota, avoiding the introduction of non-native species. Mitigating measures such as potamodromus fish-ways in the dams should be also implemented.

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Table I. Overall diet composition of the otter in Bullaque River. The total number of individuals (*n*) of each prey item is shown

Prey category	n	FO	RFO	%N	%Biomass
Pseudochondrostoma willcommii	201	1.78	11.36	2.62	0.48
Iberochondrostoma lemmingii	51	18.74	1.09	10.24	0.13
Squalius pyrenaicus	34	3.28	2.01	1.73	0.25
Squalius alburnoides	134	10.4	6.36	6.84	0.58
Luciobarbus spp.	6	0.68	0.84	0.31	0.16
Cyprinus carpio	7	0.96	0.57	0.36	2.69
Cyprinidae	433	35.84	22.23	22.09	4.29
Lepomis gibbosus	236	22.57	13.61	12.03	4.73
Micropterus salmoides	1	0.14	0.08	0.05	0.21
Centrarchidae	237	22.71	13.69	12.08	4.94
Gambusia holbrooki	1	0.14	0.08	0.05	0
Esox lucius	10	1.37	0.87	0.51	19.45
Cobitis paludica	46	5.88	3.6	2.34	3.23
Other fishes	57	7.39	4.55	2.9	22.68
FISHES	**727**	**65.94**	**40.47**	**37.07**	**31.91**
Arvicola sapidus	4	0.55	0.33	0.2	0.78
Oryctolagus cuniculus	1	0.14	0.08	0.05	0.97
Apodemus sylvaticus	1	0.14	0.08	0.05	0.19
MAMMALS	**6**	**0.83**	**0.49**	**0.31**	**1.94**
Alectoris rufa	1	0.14	0.08	0.05	0.97
Gallinula chloropus	5	0.68	0.42	0.25	4.86
Other birds	2	0.27	0.17	0.1	1.94
BIRDS	**8**	**1.09**	**0.67**	**0.41**	**7.77**
REPTILIANS (*Natrix maura*)	**4**	**0.55**	**0.33**	**0.2**	**1.94**
AMPHIBIANS (*Pelophylax perezi***)**	**29**	**3.42**	**2.17**	**1.48**	**14.1**
Procambarus clarkii	**1017**	**85.36**	**52.02**	**51.81**	**40.68**
Insects	169	6.02	3.68	8.61	1.64
Spiders	2	0.27	0.17	0.11	0.24
OTHER INVERTEBRATES	**171**	**6.29**	**3.85**	**8.72**	**1.88**
Total of preys	1962				
Number of samples	731				

Table II. Seasonal and spatial variations of otter diet (percentage of ingested biomass) in Bullaque River. Significant factors (St, stretch; S, season) for the Factorial ANOVA and subsequent univariate ANOVAs on prey categories are shown. Season: SWi, winter; SSp, spring; SSu, summer; and SAu, autumn. Stretch: StH, High; StM; Medium; and StL; Low. Average ranks of levels with different superscripts are significantly different (Tukey test, $p < 0.05$). Results are means ± SE in the same order as the average ranks of levels. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Factor	**Wilk's λ**	**d.f.**	**Prey items**	***F***	**d.f**	**Tukey test**	**%Biomass**
Season (S)	0.02*	48	*I. lemmingii*	2.87	3	SSp^{1}>SAu^{2} / SWi^{1,2}, SSu^{1,2}	1.61±0.1 > 0 / 0.24±0.026, 0.48±0.04
Stretch (St)	0.012***	50	*P. willcommii*	9.69***	2	StH^{1}>StM^{2}, StL^{2}	2.79±1.27 > 0.27±0.16, 0.12±0.06
			S. alburnoides	5.25*	2	StH^{1}, StM^{1}>StL^{2}	0.96±0.27, 1.02±0.54 > 0.06±0.03
			L. gibbosus	12.62***	2	StL^{1}>StH^{2}, StM^{2}	15.81±4.2 > 1.65±0.99, 2.64±0.85
			Reptilians	5.33**	2	StL^{1}>StH^{2}, StM^{2}	3.91±1.75 > 0, 0
			Amphibians	8.24**	2	StH^{1}>StM^{2}, StL^{2}	25.37±6.4 > 4.38±4.38, 2.72±1.84
			P. clarkii	10.10***	2	StH^{1}<StM^{2}, StL^{2}	21.65±5.12 < 66.11±8.33, 54.88±7.72
			Insects	8.86***	2	StH^{1}>StM^{2}, StL^{2}	10.28±4.64 > 0, 0

Table III: Prey availability in each stretch and season. Results are given in Biomass (kg ha^{-1}).

	Winter			**Spring**			**Summer**			**Autumn**		
	High	**Medium**	**Low**	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
Pseudochondrostoma willkommii	0	0.01	0.07	0	0	0	0	0	0	0	0.02	0
Iberochondrostoma lemmingii	0.09	0.41	0.12	0.07	0.2	0.29	0.02	0.01	0.05	0.02	0.02	0
Squalius pyrenaicus	0.13	0.02	3.2	3.7	0.41	0.86	0.86	0.01	0.17	0.52	0	0.02
Squalius alburnoides	1.2	2.98	3.04	2.25	2.14	2.54	1.18	1.99	3.11	3.09	1.15	2.72
Luciobarbus spp.	0.27	0	0.03	1.36	0	0.1	6.27	0	1	0	2.64	0.22
Cyprinus carpio	0	0.76	0.39	0	0	1982.5	0.63	12.39	1.22	0	0	2.09
Ciprinidae	1.69	3.42	6.4	7.38	2.75	3.78	8.32	2.01	4.33	3.63	3.82	2.95
Lepomis gibbosus	0.01	1.27	0.84	0.12	4.67	0	0	0.22	5.83	0	0.95	1.35
Micropterus salmoides	0	0	8.89	0	0	0.45	0	4.75	0.25	0	0	0.17
Centrarchidae	0.01	1.27	9.73	0.12	4.67	0.45	0	4.97	6.08	0	0.95	1.53
Gambusia holbrooki	0	0	0	0.05	0.46	0.2	0.1	0.1	3.96	0	0	0
Cobitis paludica	0.51	0.2	1.07	6.26	1.96	0.21	0.57	0.09	2.73	0.06	0.21	0
Ameiurus melas	0	0	0	0	0	0.14	0.01	0	0	0	0	0.03
Other fishes	0.51	0.2	1.07	6.3	2.42	0.55	0.68	0.19	6.69	0.06	0.21	0.03
FISHES	**2.21**	**4.88**	**17.19**	**13.8**	**9.84**	**4.78**	**9**	**7.16**	**17.11**	**3.69**	**4.98**	**4.5**
Procambarus clarkii	**0**	**0**	**0**	**28.32**	**104.92**	**70.28**	**84.33**	**75.26**	**235.96**	**0.5**	**0.4**	**0.15**

Table IV. Seasonal and spatial variations of prey availability (biomass, kg ha^{-1}) in Bullaque River. Significant factors (St, stretch; S, season) for the Factorial ANOVA and subsequent univariate ANOVAs on prey categories are shown. Season: SWi, winter; SSp, spring; SSu, summer; and SAu, autumn. Stretch: StH, High; StM; Medium; and StL; Low. Average ranks of levels with different superscripts are significantly different (Tukey test, $p < 0.05$). Results are means ± SE in the same order as the average ranks of levels. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Factor	**Wilk´s λ**	**d.f.**	**Prey items**	***F***	**d.f.**	**Tukey test**	**%Biomass**
Season (S)	0.059***	36	*G. holbrooki*	4.82**	3	$SSu^{1}>SWi^{2}, SAu^{2}/ SSp^{1,2}$	5.56±0.001 > 0, 0 / 1.34±0.001
			I. lemmingii	8.07***	3	$SWi^{1}>SSp^{2}, SSu^{2}, SAu^{2}$	1.22±0.003 > 0.82±0.001, 0.29±0.001, 0.17±0.001
			S. alburnoides	6.23**	3	$SAu^{1}>SSp^{2,3}, SSu^{3}/ SWi^{1,2}$	6.69±0.011 > 6.2±0.006, 3.39±0.002 / 5.72±0.008
			P. clarkii	31.68***	3	$SSp^{1}, SSu^{1}>SWi^{2}, SAu^{2}$	186.55±0.008, 497.61±0.01 > 0, 3.51±0.005
Stretch (St)	0.23**	24	*C. paludica*	5.36**	2	$StH^{1}>StM^{2}, StL^{2}$	8.12±0.0071 > 3.86±0.0014, 5.12±0.007

Table V. Seasonal and spatial variation in otter electivity index (ε_i) in Bullaque River. Significant factors (St, stretch; S, season) and interactions for the Factorial ANOVA and subsequent univariate ANOVAs on prey items are shown. Season: SWi, winter; SSp, spring; SSu, summer; and SAu, autumn. Stretch: StH, High; StM; Medium; and StL; Low. Average ranks of levels with different superscripts are significantly different (Tukey test, $p < 0.05$). Results are means ± SE in the same order as the average ranks of levels. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Factor	**Wilk´s λ**	**d.f.**	**Prey items**	***F***	**d.f**	**Tukey test**	**Mean values ± SE**
Season (S)	0.023***	27	*L. gibbosus*	4.41*	3	SWi^{1}<SSu^{2}, SAu^{2}/SSp^{1,2}	-1±0 < 0±0.22, -0.13±0.08 / -0.34±0.18
			P. clarkii	8.26***	3	SWi^{1}>SSp^{2}, SSu^{2}/ SAu^{1,2}	0.79±0.01 > -0.61±0.1, -0.53±0.09 / 0.03±0.1
Stretch (St)	0.11***	18	*P. willcommii*	7.18**	2	StH^{1}>StM^{2}, StL^{2}	0.24±0.12 > -0.54±0.17, -0.64±0.16
			S. alburnoides	3.84*	2	StM^{1}>StH^{2}, StL^{2}	-0.88±0.05 > -1±0, -1±0
			P. clarkii	4.5*	2	StH^{1}<StM^{2}/ StL^{1,2}	-0.56±0.01 < 0.3±0.13 / 0.02±0.09
S × St	0.013***	54	*L. gibbosus*	3.42*	6		
			P. clarkii	4.96**	6		

Figure 1. Map of the study area with sampling sites represented as follows: H, High stretch; M, Medium stretch and L, Low stretch

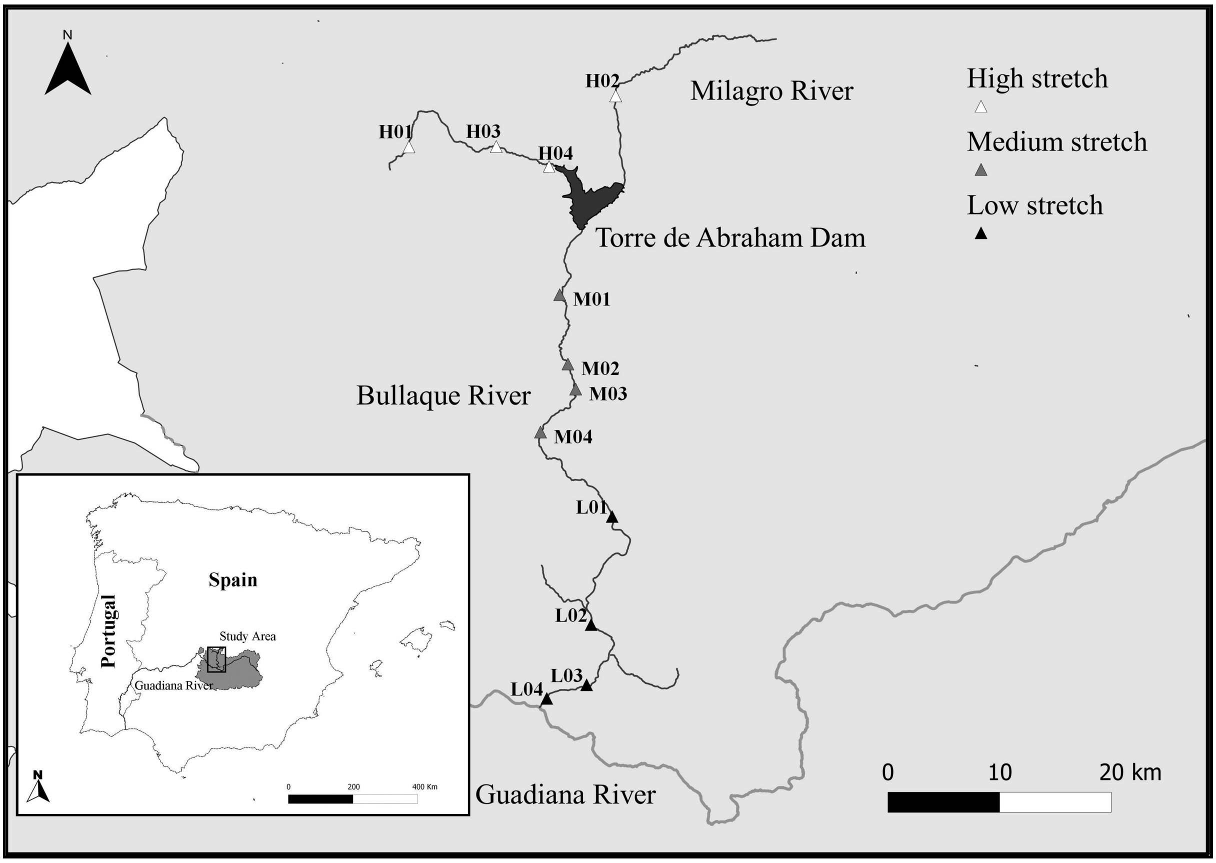
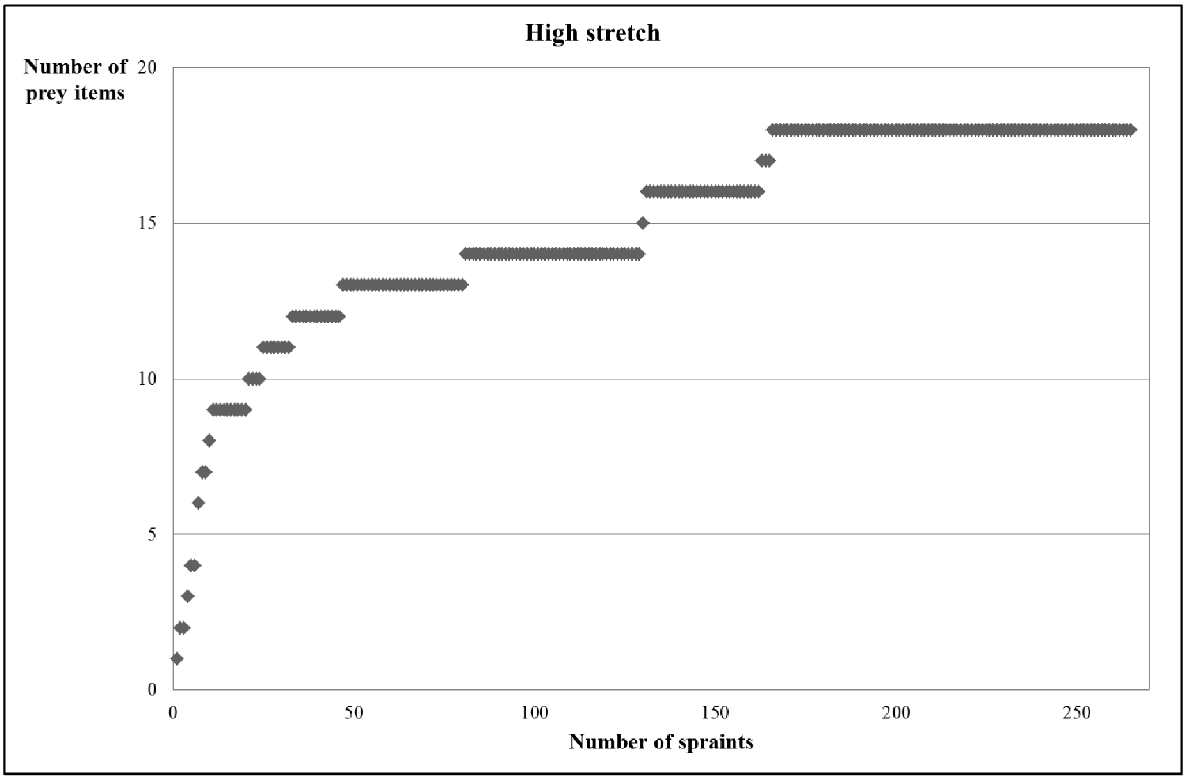
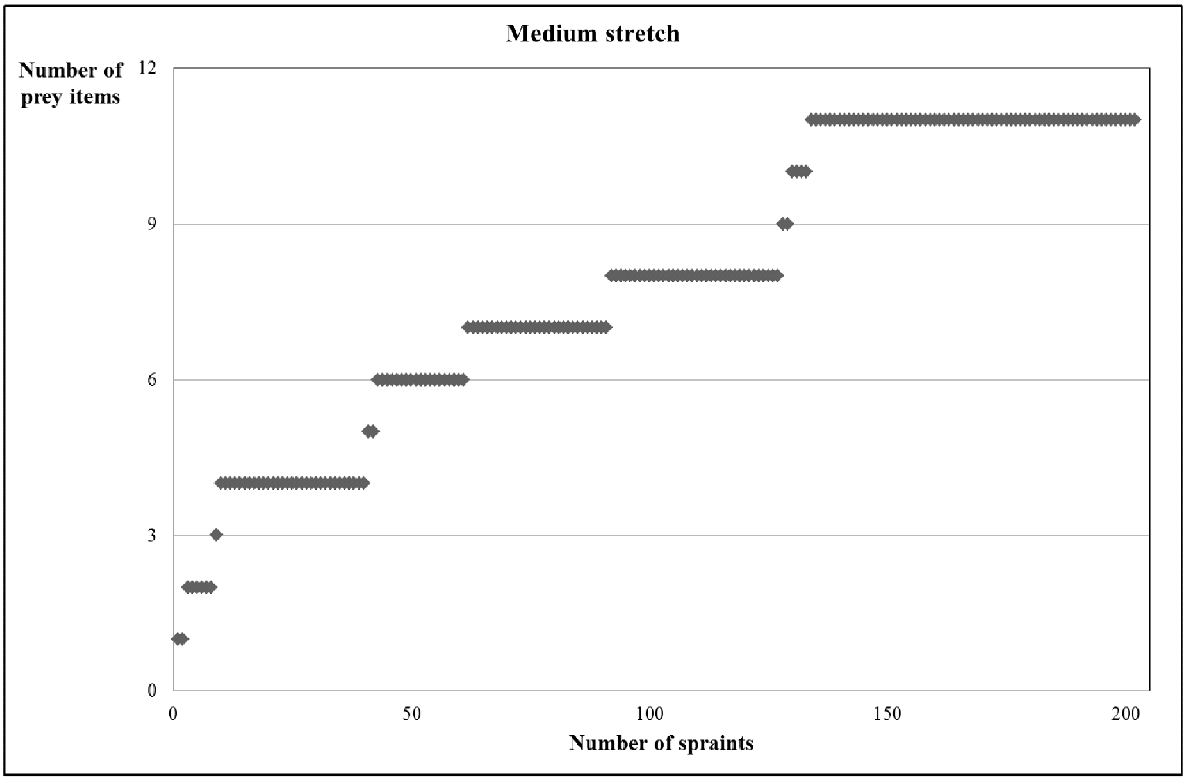
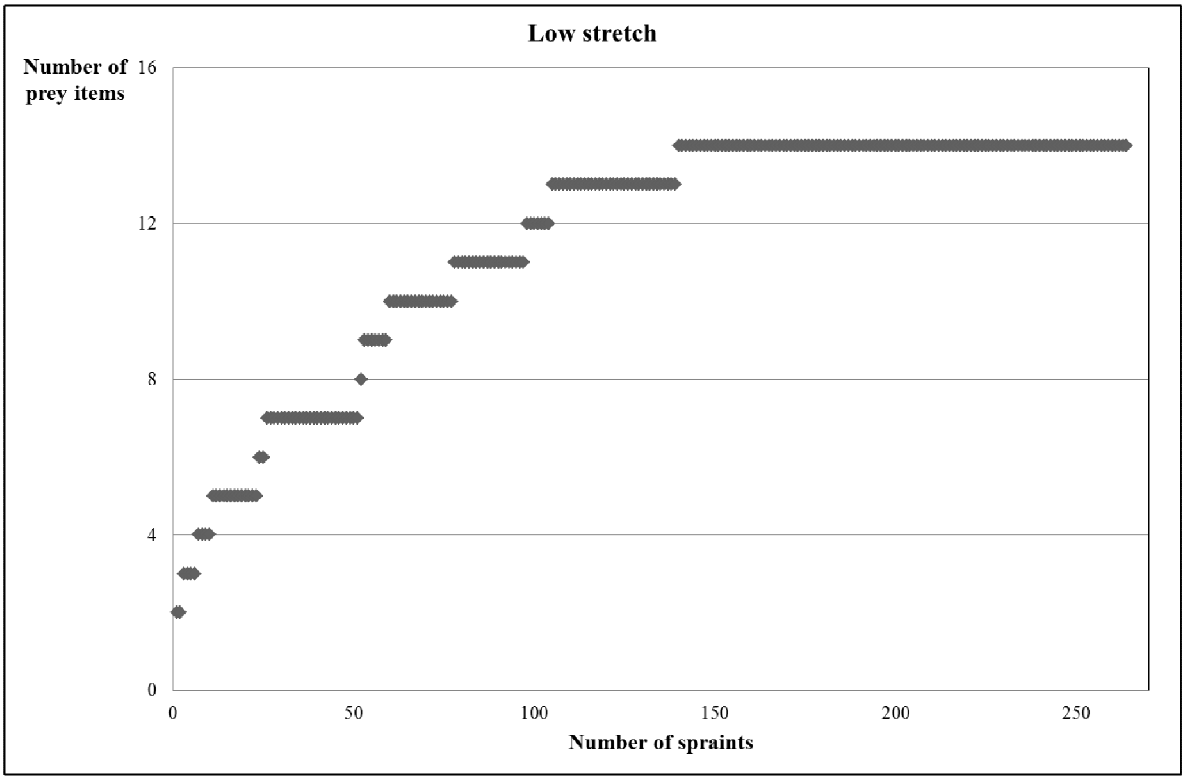


Figure 1. Cumulative frequency of resource items against increase in sample size of otter spraints in the three stretches considered and throughout the year.





Medium stretch
Number of prey items
12
9
6
3
0
0
50
100
150
200
Number of spraints



Low stretch
Number of prey items
16
12
8
4
0
0
50
100
150
200
250
Number of spraints

Figure 2. Spatial and seasonal variation of otter trophic diversity (Shannon index, *H'*) in Bullaque River. Vertical bars indicate SE. Means marked with different letters are significantly different from one to another (Tukey test, $p > 0.05$)