Environmental and biological factors influence the relationship between a predator fish, *Gambusia holbrooki*, and its main prey in rice fields of the Lower Mondego River Valley (Portugal)

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Abstract

We studied the relationships between a predator fish, *Gambusia holbrooki*, and its main food prey, within the content of a rice field food web. The influence of some environmental and biological factors on these trophic interactions, in combination with existent quantitative information, allowed us to evaluate the ecological viability of using a non-ionic surfactant, *Genapol OXD-080*, to control a plague caused by crayfish (*Procambarus clarkii*) populations in the rice fields. In the Lower Mondego River Valley, Portugal, *G. holbrooki* is abundant in rice fields. It feeds mainly on copepods, cladocerans and rotifers. Surface insects, such as aphids, collembolans, adult (imago) chironomids and other dipterans, are additional food. Large *G. holbrooki* consumed greater amounts of cladocerans and adult chironomids than other smaller size groups, while small fish prefered rotifers. Gravid females ate copepods, cladocerans, and adult chironomids and other dipterans in significantly greater amounts than immatures, males, and non-gravid females. Non-gravid females ate collembolans in significantly greater quantities than any other fish group. The population density of copepods, cladocerans, adult chironomids, and other dipterans, the area covered by aquatic vegetation, and water temperature all had significant effects on the total number of prey caught by *G. holbrooki*. In contrast, a negative correlation was found with rotifers, collembolans, aphids in higher densities, and of increased water volume, dissolved oxygen and pH. *G. holbrooki* holds a key intermediate position in the rice field food chain, feeding in large amounts of aquatic invertebrates and being eaten, in turn, by piscivores. With regard to the toxicity of *Genapol OXD-080* on non-target organisms, LC_{50} values for *G. holbrooki* and some of its main prey were several times lower than the concentration necessary to decrease the activity of crayfish populations in the rice fields. Thus, *Genapol OXD-080* could potentially cause greater damage to the local populations of non-target species and should not be used without taking precautions not to contaminate other important biological reservoirs, such as the rice field irrigation channels.

Introduction

Knowledge of the relationships between different key organisms present in agro-ecosystems such as rice fields, together with laboratory information, is an important requirement to assess environmental impacts resulting from conventional and new types of agricultural practices. Rice culture is the most important agricultural activity in the world (Forés & Comín,

1992). Therefore, rice fields are artificial habitats, but home to complex ecological systems with a large variety of plant and animal species (Linden & Cech, 1990).

A new chemical method for controling plague in rice fields is presently under development in the Lower Mondego River Valley of Central Portugal. Since its introduction to the Iberian Peninsula, *Procambarus clarkii*, the Louisiana red swamp crayfish, caused sig-

nificant losses in rice yield (Anastácio & Marques, 1995; Anastácio et al., 1995). To mitigate damages, a non-ionic biodegradable surfactant, *Genapol OXD-080*, was selected to help control the concentrations of *P. clarkii*. This surfactant is more environment-friendly than pesticides most commonly used (Jørgensen et al., 1997), allowing additionally the use of crayfish as a profitable food source for humans (Fernandes et al., 1994, 1995).

Genapol OXD–080 is a polyglycol esther of fatty alcohol, with the following chemical structure:

$$R - (CH_2)_y - O(CH_2CH_2O)_x - H,$$
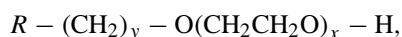
where R represents a synthetic unsaturated fatty alcohol, non-branched, with 12–15 carbon atoms, represented by y (Fonseca et al., 1997), x is defined by 8–10 mols of ethylene oxide. The idea is to control the physiological activity of crayfish by decreasing respiratory exchanges (gill hematosis) without killing them. It prevents damage to the rice plants, specially young plants, caused by crayfish burrowing. An important requirement regarding the ecological viability of this approach is that populations of non-target species are not significantly affected. To assess potential implications for the ecosystem, it is critical to understand the relationships between the organisms that play essential roles in the food web of the rice field. It will contribute, in combination with the existent laboratory information regarding the toxicities of *Genapol OXD-080* on non-target species (Cabral et al., 1997), to determine what changes this surfactant might cause on the local aquatic communities.

The aim of the present study was to characterise the relationships between a key predator fish, *Gambusia holbrooki* (Girard) (Cyprinodontiformes: Poeciliidae), and its main prey, with a primary focus on physico-chemical and biological aspects on the interactions. *Gambusia holbrooki*, also known as mosquitofish, is native from the coastal region of the eastern United States. Like *Gambusia affinis* (Baird & Girard), *G. holbrooki* has been widely introduced through mosquito control programs into warm temperate and tropical regions all over the world (Cech et al., 1992; Haynes & Cashner, 1995; Homski et al., 1994; Hoy, 1985; Lydeard & Belk, 1993; Schaefer et al., 1994; Wurtsbaugh et al., 1980). In the Iberian peninsula the mosquitofish was introduced in 1921 (Albuquerque, 1956). Because this viviparous fish is well known for its consumption of insect larvae, zooplankton and other invertebrates, it plays a crucial role as a predator in agro-ecosystems such as rice fields (Blaustein,

1992; Cech et al., 1992). The following characteristics of mosquitofish make the species suitable for this study: (a) it is abundant, (b) as a small predator, it occupies an intermediate position in the food chain, preying directly on aquatic invertebrates and being eaten by piscivores (Britton & Moser, 1982; Hurlbert et al., 1972), and (c) it is one of the most well studied poeciliids (Haynes & Cashner, 1995).

Materials and methods

Study site

The Lower Mondego River Valley (Figure 1) is located in the central region of Portugal (40° 10′ N, 08° 41′ W). The valley consists of approximately 15 000 ha where the main agricultural crop is rice, which occupies about 60% of the usable area. Non cultivated areas, such as swamps, appear in the periphery of the valley, exhibiting a flourishing wetland fauna and flora. Drainage channels, constituting another biological reservoir, are spread across the whole valley (Anastácio & Marques, 1995).

In the study site mosquitofish and its prey species occur year-around in the irrigation channels, but only seasonally in the rice paddies. During a large part of the year the rice paddies have a very low water level or are completely dry and unable to support mosquitofish. In the main irrigation channels, although levels varied throughout the year, there is always enough water to support a large variety of plant and animal species, which ensures an important pool for faunal recruitment and population renewal to rice fields. The sampling program was therefore focused in the irrigation channels.

Sampling program and laboratory procedures

The sampling program was carried out in a main irrigation channel from April 1996 to May 1997, fortnightly during the most important mosquitofish reproduction period (April–July), and monthly in the remaining period. Samples of mosquitofish, zooplankton, and macroinvertebrates, both benthic and associated with aquatic vegetation, were taken.

During each sampling event, mosquitofish were electrofished in three randomised areas confined by nets laid transverse across the irrigation channel. A semi-portable generator supplied a rectified DC current (350–600V). Sampling always took place between 10 a.m. and 1 p.m., corresponding to the most
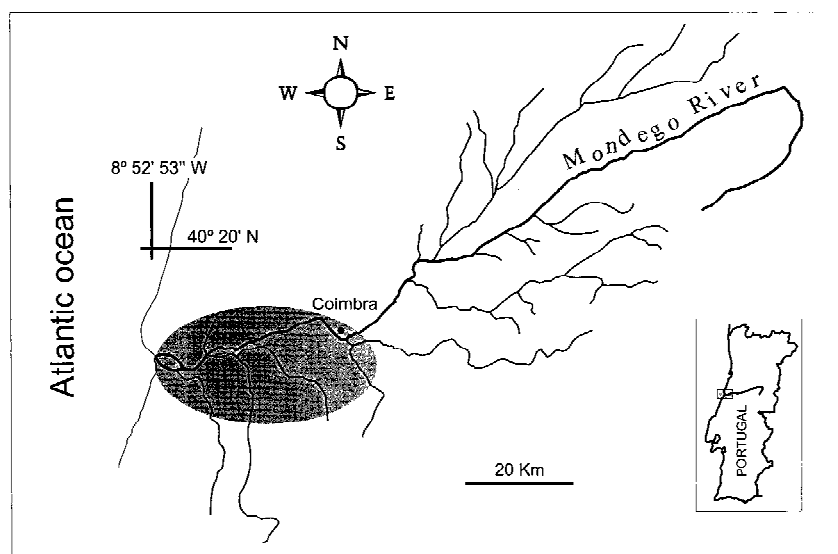
Figure 1. Location of the Lower Mondego River Valley (shaded area).

active mosquitofish feeding period (Crivelli & Boy, 1987). Sampled areas ranged from 3 to 16 m^2 and were shocked during a period of 30–40 min, enough to catch practically all the fish present in each area. All mosquitofish caught were immediately preserved in 4% neutralised formaldehyde, while other fish were returned to the irrigation channel.

In the laboratory fish were washed, counted, and preserved in 70% ethanol until gut dissections could be performed. All individuals were measured and sorted by standard length to the following size groups: 1 (till 10 mm), 2 (11–15 mm), 3 (16–20 mm), 4 (21–25 mm), 5 (26–30 mm), 6 (31–35 mm), 7 (36–40 mm) and 8 (41–45 mm). Moreover, fish were classified as (a) immature (normally with 15 mm or less, if sex could not be determined externally), (b) males (normally no longer than 30-mm and identified by the presence of a gonopodium), (c) non-gravid females and (d) gravid females. We define gravid females as fish with mature ova, i.e. comprised between the stage presenting a clear amber colour without visible embryonic structures and the stage with late embryos, ready for parturition (Meffe, 1987). Five individuals per size group and from each sampled area were examined for gut contents, except for size groups 3, 4, and 5, where females and males had coincident sizes. In this case, five females and five males were examined per group. A total of 484 fish were investigated. For each fish the gut tube was excised from the esophagus up to

the point where it bends ventrally and dissected. Prey items were recorded and identified.

Prey samples were collected from the primary microhabitats in the irrigation channels, including the sediment, water phase, and aquatic vegetation. Three replicates were randomly sampled for each microhabitat. Benthic samples were collected using a small Van Veen grab, capable of collecting up to 5-dm^3 from a 0.0496-m^2 area. Samples were sieved *in situ* using a 0.5-mm mesh size net bag.

Macroinvertebrates attached to aquatic vegetation were collected using simple plastic skimmers, each with a rectangular section of 0.066-m^2 and a 0.5-mm mesh size nylon net. The skimmer was abruptly plunged bellow the *Myriophyllum aquaticum* beds, the most important aquatic plant, and the sample collected after cutting the plants quickly with pruning-shears. To prevent macroinvertebrates from escaping, each skimmer was immediately enclosed in a 0.5-mm mesh nylon net bag and also sieved *in situ*. Both the benthic and *Myriophyllum* samples were maintained alive in plastic containers up to the laboratory and then placed in a cold room (4 °C) for a maximum of 2 days while they were processed.

Samples from zooplankton and macroinvertebrates in the water column were obtained by pulling a 200-μm mesh plankton net horizontally with a digital flow meter to determine the volume of water passing through the net. Samples were immediately preserved

in 4% neutralised formaldehyde and subsequently identified and sorted in the laboratory.

Water temperature, conductivity (μS), dissolved oxygen (mg l^{-1}), and pH, were measured *in situ* at each site. From June 1996 water samples for chlorophyll *a* determination were collected with plastic bottles and analysed according to the technique described in APHA-AWWA-WPCF (1980). Water volume was estimated from depth and width measurements in a 148.7-m long representative section of the irrigation channel, and then extrapolated for the entire channel. The percentage of the water surface area covered by *Myriophyllum aquaticum* was also estimated through three or more independent observations.

Data analysis

To identify the preferential prey items caught by mosquitofish we used the Ivlev's electivity index for fishes (Ivlev, 1961), defined as $E = (r - p)/(r + p)$, where r = proportion of the number of a given prey in the mosquitofish gut content, and p = proportion of the number of the same organism in field samples. Positive values of E (0–1) indicate a preference, negative values (-1 to 0) indicate little or no representation in the gut content. We used the value -0.5 as lower limit to identify 'preferential' prey.

The Kruskal–Wallis one-way analysis of variance by ranks followed by a non-parametric multiple comparisons test with unequal sample sizes (Zar, 1984) was used to assess the significance of differences in the number of preferential prey items between the gut contents of different mosquitofish size and sex groups. Stepwise multiple-regression analysis (Zar, 1984) was used to test any possible correlation between the total number of prey items consumed by mosquitofish and the environmental variables, water temperature, water volume, dissolved oxygen, pH, area covered by aquatic vegetation, and prey availability. A step-down procedure was followed in order to examine the effect of each environmental variable on the others, with the least significant variable being removed at every step. The analysis stopped when all the remaining variables had a significant correlation level ($P<0.05$) (Zar, 1984). Tests for normality, Kolmogorov–Smirnov, and for variance homogeneity, Cochran's C and Bartlett's, were performed for the dependent variable before running the multiple-regression analysis (Zar, 1984). Following the tests results, the total number of prey consumed by mosquitofish was transformed using a logarithmic transformation ($X' = \log[X + 1]$). The non-parametric Spearman rank correlation was used to test relationships between the main zooplankton group densities and phytoplankton abundances, expressed as mg of chlorophyll *a* m^{-3}, taking into account data from the period when both variables were determined. This was an indirect way to assess the potential effects of zooplankton predation by mosquitofish on phytoplankton abundance, considered an indicator of eutrophication in the irrigation channels.

Results

The environmental characteristics (average $\pm$ S.E.) of the irrigation channel during the sampling period were as follows: water temperature: 18.4 ± 1.5°C, with a minimum of 9.4°C and a maximum of 25.2°C; dissolved oxygen, 5.7 ± 0.7 mg l^{-1}, with a minimum of 2.8 mg l^{-1} and a maximum of 10.9 mg l^{-1}; pH, 7.3 ± 0.2, with a minimum of 6.6 and a maximum of 8.8; conductivity, 318.3 ± 36.3 μS, with a minimum of 133.6 μS; and a maximum of 553.0 μS; water volume, $362\,677\pm60\,473$ dm^3, with a minimum of 22 666 dm^3 and a maximum of 586 707 dm^3.

Fourteen invertebrate large groups were collected in the three microhabitats. From these, six groups were divided into 15 subgroups (Table 1). For the present purposes it was considered enough to take into account high taxonomic levels. For each microhabitat, the density of each group and the proportion (%) of the group in the whole set of samples are also given in Table 1. The most abundant invertebrates in the water phase were copepods and cladocerans. Two invertebrate groups normally associated with aquatic and riparian vegetation, arachnids and ants (Formicidae), were also well represented in the water phase. In the macrobenthos, oligochaets and chironomid larvae were the most abundant invertebrate groups. Adult hydrophilids, aphids, chironomids larvae, and crayfish (*Procambarus clarkii*) were very abundant on the aquatic vegetation, being found during the whole study period (Table 1).

The 10 groups and 10 subgroups found as prey items in the mosquitofish gut contents are given in Table 2. The Ivlev's electivity index values were calculated for each mosquitofish size group and for the whole population (Table 2). Since we analysed only a few fish from the peripheral size groups 1 and 8, they were pooled with groups 2 and 7, respectively. Taking the Ivlev's electivity index values into account, for the population considered as a whole (Table 2,

Table 1. Average densities (mean $\pm$ S.E.) for each invertebrate group and respective percentages in relation to the average total number of all invertebrate groups per microhabitat sample (in parenthesis).

Invertebrates	Microhabitats		
	Water phase (ind. dm^{-3} (%))	Sediment (ind. m^{-2} (%))	Aquatic vegetation (ind. m^{-2} (%))
Copepoda	1.03 $\pm$ 0.62 (62.81)	0	0
Cladocera	7.41 $\pm$ 7.41 (16.08)	0	0
Rotifera	0.05 $\pm$ 0.05 (0.24)	0	0
Collembola	5E-4 $\pm$ 5E-4 (0.28)	1.44 $\pm$ 1.44 (3.06)	36.44 $\pm$ 23.61 (6.85)
Ephemeroptera	3E-4 $\pm$ 2E-4 (0.59)	0.96 $\pm$ 0.96 (1.02)	2.52 $\pm$ 1.47 (1.36)
Odonata	2E-4 $\pm$ 2E-4 (0.01)	0.48 $\pm$ 0.48 (0.51)	3.97 $\pm$ 2.91 (1.37)
Hemiptera			
Aphididae	0.01 $\pm$ 9E-3 (2.91)[a]	0	181.82 $\pm$ 121.67 (18)
Others	0	0	1.44 $\pm$ 0.82 (0.22)
Hymenoptera			
Formicidae	2E-3 $\pm$ 2E-3 (10.39)[a]	1.92 $\pm$ 1.48 (0.61)[a]	16.96 $\pm$ 9.42 (3.53)
Others	7E-4 $\pm$ 4E-4 (0.34)[a]	0.96 $\pm$ 0.65 (0.70)[a]	2.89 $\pm$ 1.37 (0.71)
Coleoptera			
Hydrophilidae (AD)	0	0	14.67 $\pm$ 8.45 (15.01)
Dytiscidae (AD)	0	0	8.30 $\pm$ 5.15 (1.21)
Hydraenidae (AD)	0	0	0.36 $\pm$ 0.36 (0.03)
Terrestrial (AD)	0	0	1.35 $\pm$ 0.75 (0.33)
Diptera			
Chironomidae (LV)	0	12.96 $\pm$ 5.43 (21.63)	29.57 $\pm$ 10.98 (17.35)
Others (LV)	0	2.40 $\pm$ 1.51 (4.45)	0.72 $\pm$ 0.49 (0.72)
Chironomidae (AD)	0	0	1.44 $\pm$ 1.11 (0.16)
Others (AD)	0	0	1.80 $\pm$ 1.46 (2.44)
Decapoda			
Procambarus clarkii	0	2.40 $\pm$ 1.67 (2.28)	100.26 $\pm$ 40.79 (20.5)
Arachnida	7E-4 $\pm$ 4E-4 (5.17)[a]	0.96 $\pm$ 0.65 (0.57)[a]	7.94 $\pm$ 3.95 (3.09)
Oligochaeta	0	167.05 $\pm$ 97.55 (65.1)	8.30 $\pm$ 5.15 (2.16)
Gastropoda			
Physidae	0	0	15.87 $\pm$ 12.85 (4.79)
Ancylidae	0	0	2.52 $\pm$ 2.52 (0.11)

The samples (*n*) were collected from the three distinct irrigation channel microhabitats during the study period: water phase (*n*=13), sediment (*n*=14) and aquatic vegetation (*n*=14).
LV, larva; AD, Adult.
[a] Accidental.

right column: Total average), zooplankton (copepods, cladocerans, ostracods and rotifers) constituted the main feeding option for mosquitofish, followed by the insect groups aphids and collembolans, adult chironomids and other dipterans. Ostracods appeared as a preferential prey for all mosquitofish size groups, although they were found in only 7.8% of the fish analysed. However, ostracods were excluded from the statistical analysis because of the lack of information regarding their occurrence in the environment (Table 1). The absence of ostracods in the samples can be explained as a function of our sampling method-

ology for benthic organisms, since ostracods are too small to be retained in a 0.5-mm mesh net. Copepods were the only other group with a positive Ivlev's index for all mosquitofish size groups (Table 2). Young mosquitofish remains were found in 37 gut contents (7.6%), indicating a certain degree of cannibalism.

A Kruskal–Wallis one-way analysis of variance by ranks was used to assess the significance of differences in the numbers of preferential prey items between the gut contents of different mosquitofish size and sex groups. Only cladocerans, rotifers and adult chironomids were caught in significantly different quantities

Table 2. Prey groups Ivlev's electivity index values calculated for the mosquitofish (*Gambusia holbrooki*) size groups considered and for the total population sampled.

Preys	Mosquitofish size classes						
	1 + 2	3	4	5	6	7 + 8	Total average
Copepoda	**0.68** (23)	**0.09** (89)	**0.14** (108)	**0.20** (74)	**0.04** (38)	**0.41** (25)	**0.18** (357)
Cladocera	−**0.07** (31)	−**0.05** (63)	−**0.28** (74)	−**0.32** (54)	−**0.24** (32)	−**0.23** (28)	−**0.20** (282)
Ostracoda	**1** (3)	**1** (7)	**1** (12)	**1** (8)	**1** (5)	**1** (3)	**1** (38)
Rotifera	**0.09** (11)	**0.09** (22)	**0.13** (35)	**0.001** (15)	−1 (5)	−1 (9)	−**0.06** (97)
Collembola	−**0.006** (7)	−**0.33** (55)	−0.50 (62)	−**0.46** (42)	−**0.05** (17)	**0.003** (5)	−**0.37** (188)
Hemiptera							
Aphididae	−**0.02** (32)	−**0.35** (105)	−**0.39** (125)	−**0.31** (88)	−**0.31** (51)	−**0.29** (29)	−**0.32** (430)
Hymenoptera							
Formicidae	−1 (30)	−0.92 (101)	−0.82 (104)	−0.55 (84)	−0.57 (46)	−0.62 (26)	−0.76 (391)
Others	−1 (13)	−0.96 (74)	−0.91 (78)	−0.91 (62)	−0.76 (28)	−1 (7)	−0.92 (262)
Coleoptera							
Hydrophilidae (AD)	−1 (17)	−1 (60)	−1 (82)	−0.84 (61)	−0.94 (31)	−0.88 (16)	−0.95 (267)
Dytiscidae (AD)	−1 (16)−	−1 (45)	−1 (55)	−0.84 (50)	−1 (20)	−1 (9)	−0.96 (195)
Hydraenidae (AD)	−	−0.88 (15)	−0.87 (29)	−**0.38** (22)	−0.67 (6)	−1 (1)	−0.71 (73)
Terrestrial (AD)	−1 (6)	−0.85 (37)	−1 (44)	−0.57 (31)	−**0.21** (10)	−1 (3)	−0.78 (131)
Diptera							
Chironomidae (LV)	−0.82 (33)	−0.78 (54)	−0.88 (67)	−0.76 (57)	−0.94 (32)	−0.98 (28)	−0.85 (270)
Chironomidae (AD)	−0.71 (7)	−0.85 (26)	−0.67 (43)	−**0.38** (33)	−**0.12** (16)	**0.40** (10)	−**0.49** (135)
Others (AD)	−0.83 (12)	−**0.39** (26)	−0.54 (28)	−**0.12** (29)	−0.62 (13)	−0.57 (14)	−**0.45** (122)
Arachnida	−1 (32)	−0.95 (96)	−0.89 (111)	−0.83 (83)	−0.94 (44)	−0.95 (26)	−0.91 (392)

The values in bold indicate a prey preference, taking into account the lower limit selected to identify "preferential" preys (−0.5). The values with parenthesis represent the number of fishes observed.
LV, Larva; AD, Adult.

Table 3. Average number of prey items eaten (mean ± S.E.) by mosquitofish (*Gambusia holbrooki*) taking into account the size groups collected during the study period.

Preys	Mosquitofish size groups						χ^2	p
	1 + 2 (n=39)	3 (n=120)	4 (n=137)	5 (n=101)	6 (n=53)	7 + 8 (n=34)		
Copepoda	16.4 ± 4.0	18.0 ± 3.9	31.6 ± 4.7	40.2 ± 8.5	37.0 ± 10.0	51.1 ± 13.1	9.27	n.s.
Cladocera	6.9 ± 1.9	7.9 ± 2.9	6.4 ± 1.8	7.4 ± 2.7	19.3 ± 7.6	30.4 ± 10.1	15.76	**
	•	•	•	•	••	• • •		
Rotifera	1.5 ± 1.2	7.8 ± 3.4	13.8 ± 4.8	0.6 ± 0.3	0	0	15.36	**
	• • •	• • •	• • ••	••	•	•		
Collembola	0.3 ± 0.2	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.4	3.1 ± 1.2	0.8 ± 0.7	3.41	n.s.
Hemiptera								
Aphididae	1.8 ± 0.4	1.4 ± 0.2	2.6 ± 0.4	3.2 ± 0.6	3.7 ± 0.9	2.1 ± 1.1	4.52	n.s.
Diptera								
Chironomidae (AD)	0.03±0.03	0.02±0.01	0.2 ± 0.1	0.4 ± 0.2	0.6 ± 0.3	1.7 ± 0.9	22.22	***
	•	•	•	•	•	••		
Others (AD)	0.03±0.03	0.07±0.02	0.08±0.04	0.3 ± 0.08	0.08±0.05	0.09±0.05	9.48	n.s.

The number of solid circles bellow the values indicates significant differences between size groups for the medians of a given prey group (Kruskal–Wallis and nonparametric multiple comparison tests). **$p<0.01$; ***$p<0.001$ (n.s., no significant). *n* is the number of individuals.
(AD), adult.

Table 4. Average number of prey items eaten (mean ± S.E.) by mosquitofish (*Gambusia holbrooki*) sex groups considered during the study period.

Preys	Mosquitofish sex groups				χ^2	p
	Immatures (*n*=37)	Males (*n*=138)	Non-gravid females (*n*=193)	Gravid females (*n*=116)		
Copepoda	14.6 ± 4.0	14.2 ± 2.2	24.7 ± 3.8	65.8 ± 8.8	39.56	***
	•	•	•	••		
Cladocera	6.9 ± 2.0	5.8 ± 1.8	5.2 ± 1.8	24.6 ± 5.0	41.10	***
	•	•	•	••		
Rotifera	1.6 ± 1.3	7.3 ± 2.9	5.6 ± 2.1	6.9 ± 4.6	6.52	n.s.
Collembola	0.2 ± 0.1	0.8 ± 0.2	2.3 ± 0.4	0.03 ± 0.02	45.28	***
	•	•	••	•		
Hemiptera						
Aphididae	2.2 ± 0.5	2.4 ± 0.4	2.6 ± 0.4	2.2 ± 0.5	2.15	n.s.
Diptera						
Chironomidae (AD)	0.03 ± 0.03	0.1 ± 0.04	0.02 ± 0.01	1.2 ± 0.3	38.30	***
	•	•	•	••		
Others (AD)	0.03 ± 0.03	0.1 ± 0.04	0.06 ± 0.03	0.3 ± 0.06	16.71	***
	•	• • •	••	• • ••		

The number of solid circles bellow the values indicates significant differences between sex groups for the medians of a given prey group (Kruskal–Wallis and nonparametric multiple comparisons tests). *** $p<0.001$ (n.s., no significant). *n* is the number of individuals.
AD, adult.

by distinct size groups (Table 3). The multiple comparison test showed that large mosquitofish size groups (7+8) clearly consumed greater amounts of cladocerans and adult chironomids than the other size groups, whereas small and medium fish consumed greater amounts of rotifers (Table 3). There were also differences between the sex groups regarding the type of prey caught (Table 4). Copepods, cladocerans, adult chironomids and other dipterans were caught in significantly greater amounts by gravid females than by immature, males, and non-gravid females. Nevertheless, males and non-gravid females fed more on other adult dipterans than immature, showing also significant differences with respect to their diet (Table 4). Non-gravid females ate collembolans in significantly greater quantities than any other sex group.

A stepwise multiple-regression analysis was used to search for significant correlations between the total number of prey items consumed by mosquitofish (TOT) and environmental variables. Twelve independent variables were considered: water temperature (TMP), water volume (VOL), dissolved oxygen (OXI), pH, area covered by aquatic vegetation (VEG), and the densities of copepods (COP), cladocerans (CLA), rotifers (ROT), collembolans (COL), adult chironomids (CHI), other adult dipterans (DIP), and aphids (APH).

Neither of the variables was excluded from the model. The resulting regression equation was:

$$TOT = 3.77 + 0.72(COP) + 0.53(CLA) - 95.85(ROT) - 0.004(COL) + 0.06(CHI) + 0.3(DIP) - 0.003(APH) - 0.00001(VOL) + 0.02(VEG) - 0.06(OXI) + 0.06(TMP) - 0.51(pH); \; (d.f. = 483, R^2 = 0.56, F = 50.03, P < 0.001).$$

The development of dense populations of copepods, cladocerans, and adult chironomids and other dipterans at the study site appeared to have a significant effect in increasing the total number of prey caught by mosquitofish (Figure 2). Other positive correlations regard the influence of changes in the area covered by aquatic vegetation, and water temperature (Figure 2). Nevertheless, it seems likely that these two environmental factors are probably related to the density of prey populations, and therefore with prey availability. When rotifers, collembolans and aphids appeared in higher densities, mosquitofish seemed to capture less prey than when these groups are rare or absent (Figure 2). On the other hand, the increase of water volume, dissolved oxygen and pH values appear negatively correlated with the number of prey caught by mosquitofish (Figure 2).
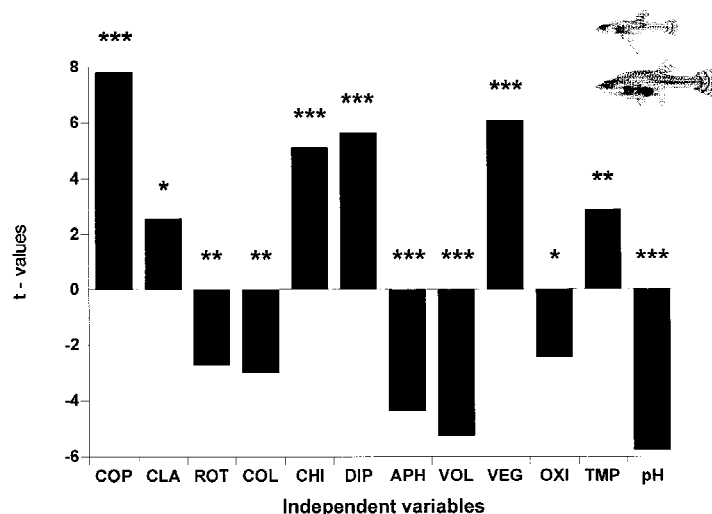
Figure 2. The t-values and significance levels (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) of the correlates, selected by automatic stepwise regression analysis as factors with significative influence on the variation in the total number of preys consumed by mosquitofish (*Gambusia holbrooki*). The codes of the independent variables are respectively: the densities of the seven preferential prey groups: Copepods (COP), Cladocerans (CLA), Rotifers (ROT), Collembolans (COL), adult Chironomids (CHI), other adult Dipterans (DIP), and Aphids (APH), and water volume (VOL), area covered by aquatic vegetation (VEG), dissolved oxygen (OXI), water temperature (TMP) and pH.

The non-parametric Spearman rank correlation was used to test relationships between the densities of the main zooplankton groups and phytoplankton abundance, expressed as mg of chlorophyll *a* m^{-3}. Contrary to other experimental works (e.g. Hurlbert et al., 1972; Hurlbert & Mulla, 1981), that suggested a close association between low densities of zooplankton due to mosquitofish predation and an increase in phytoplankton abundance, we did not find a significant negative correlation between the decrease of copepods and cladocerans densities and an increase in chlorophyll *a* concentration ($n = 11$, $r_s = -0.52$, n.s. and $r_s = -0.18$, n.s., respectively). Of all the measured physicochemical parameters and phytoplankton abundance, only dissolved oxygen showed a close to significant positive correlation with chlorophyll *a* density ($n = 11$, $r_s = 0.61$, $P = 0.054$).

Discussion and conclusions

In general, the results obtained from this study are very similar to those described in the literature. Mosquitofish normally feed primarily near the surface on zooplankton, specially free-living Cyclopoid copepods and cladocerans (Colwell & Schaefer, 1983; Crivelli & Boy, 1987; Daniels & Felley, 1992; Hurlbert & Mulla, 1981). Hurlbert & Mulla (1981) and Crivelli & Boy (1987) found that copepods were much

less affected than cladocerans by mosquitofish predation. However, we observed exactly the contrary, with copepods constituting the most important prey group during the study period. Cladocerans were clearly less important as prey items, and ostracods were found only in a few guts (Table 2). The difference in these findings may be a function of the different availabilities of the prey groups to mosquitofish. In fact, among the water phase invertebrates, copepods composed 62.8% of the samples, whereas the cladocerans accounted for only 16.1% (Table 1). Although mosquitofish prey selectively on larger zooplankters, rotifers seemed to be a relative important prey group for immature fish as well as for young males and females, which constitute the small-medium size classes (Table 3).

Throughout the year, surface insects, like aphids, collembolans, adult chironomids, and other dipterans, were an important additional food source. Nevertheless, mosquito larvae (chironomids and other dipterans), which were relatively abundant in the irrigation channel sediments (Table 1), constituted only a small quantitative fraction of the mosquitofish diet. This observation agrees with the disappointing reports from experiments using mosquitofish for mosquito control around the world (Rupp, 1996). Our estimation of cannibalism (7.6%) in the Lower Mondego River Valley is in accordance with estimates for wild mosquitofish

populations, which ranged from 6% to 13% (Nesbit & Meffe, 1993; Walters & Legner, 1980).

During the non-reproductive season, our results showed a similar diet for immature, males and non-gravid females, which ate very close quantities of the seven preferential prey groups (Table 4). During the reproductive season, from April to July, most of the mature females were gravid (Cabral & Marques, in prep.). These females captured, in general, more prey items than immature, males and non-gravid females. Moreover, during this period of high reproductive investment, gravid females eat more surface insects, especially adult dipterans, than the other population groups (Table 4). This may be explained by the larger size of gravid females that enable them to eat bigger prey items and the need for higher caloric intake (Harrington & Harrington, 1961).

The increased feeding by mosquitofish at higher water temperatures may be a function of increasing metabolic rates (Cech et al., 1985). Based on experimental results, Reddy (1975) observed that mosquitofish ate 150–200% more larvae at 30°C than at 20°C over a 10-h period. The reduction in the number of prey items caught by mosquitofish when dissolved oxygen and pH increased may have been caused by low concentrations of zooplankton. Hurlbert & Mulla (1981) experimentally reported that pH and dissolved oxygen levels were higher in ponds with mosquitofish than in control ponds lacking mosquitofish. They suggested that this difference was primarily caused by a greater abundance of phytoplankton in fish ponds where zooplankton was predated by mosquitofish. In our case, an increase in photosynthesis due to the low concentrations of zooplankton could have indirectly caused the observed increase in dissolved oxygen and pH. Obviously, the low concentrations of zooplankton due to predation (Hurlbert et al., 1972) or to other causes, would also result in a smaller amount of food caught by mosquitofish, which explains the correlations observed (Figure 2).

The correlation of the number of prey items ingested by mosquitofish with the area covered by aquatic vegetation (Figure 2), may be due to the idea that the fish can see their prey easier against the vegetation, or simply because this microhabitat offers better conditions for the growth of the prey species (Linden & Cech, 1990). It is known that mosquitofish are able to penetrate and prey successfully in dense vegetation (Linden & Cech, 1990), such as *Myriophyllum* mats, where the presence of suitable and well

contrasted prey, like adult dipterans, would likely shift predation away from other less accessible food items.

The increase in the number of prey ingested by mosquitofish may be also related to the increased concentration of organisms in the water column that would result from a decrease in the level of the water. Consequently, prey items are more easier captured by mosquitofish at periods when the water volume is low (Figure 2).

The results of this study will be integrated with additional works on risk assessment associated with the application of *Genapol OXD-080* in order to control crayfish populations in the rice fields of the Lower Mondego River Valley. Nevertheless, based on our current knowledge, we are able to make some suggestions concerning the risks with using *Genapol OXD-080*. Because the interaction between the mosquitofish and its prey are a crucial component of the food web in the irrigation channels, we recommend that *Genapol OXD-080* should not be used as an alternative method to control crayfish populations without special precautions. This recommendation is supported by the existent quantitative laboratorial information for these species. Although chemical application is usually restricted to the rice paddies, the risk of contaminating an important biological reservoir, the irrigation channels, must always be considered. For instance, in the case of *Genapol OXD-080*, the usual water discharge from rice paddies may result in surfactant contamination. This question is extremely important, since the LC_{50} value for mosquitofish was 17.2 times lower than the concentration of *Genapol OXD-080* necessary to significantly decrease crayfish physiological activity (Cabral et al., 1997). Therefore, even a small amount of contamination may be harmful to mosquitofish, killing them or affecting some important biological aspects such as metabolism, predatory and reproductive capacity (Cabral et al., submitted).

The possible detrimental effects of *Genapol OXD-080* on microcrustaceans will indirectly affect mosquitofish since they are preferentially zooplankton feeders. The LC_{50} value for the cladoceran *Daphnia magna*, which may be considered to represent freshwater zooplankton, was found to be 13.9 times lower than the concentration of *Genapol OXD-080* necessary to decrease crayfish gill hematosis (Cabral et al., 1997). Some works concerning the effects of surfactants on invertebrates focused on the surface insects often caught by mosquitofish. For adult chironomids substances residing at the surface of the water phase, namely surfactants and oils, may act as

selective control agents because these insects depend on the air/water interface in all stages of their life cycle (Corbet et al., 1995). For aphids, Imai et al. (1994) observed that several different surfactants revealed strong aphicidity. Only collembolan species seem to be more tolerant of surfactants. For instance, Holmstrup & Krogh (1996) detected differential degrees of damage, according to age, of an anionic surfactant on collembolan populations from agricultural and grassland soils in temperate regions.

Despite the fact that *Genapol OXD-080* appears to be potentially harmful to the biological structure and function of the irrigation channel communities, its application in the rice paddies will have a comparatively much lower impact. In fact, at the time of the application, rice paddies present an extremely impoverished faunal community (Frias et al., in prep.), while the irrigation channels contain a large variety of plant and animal species that will re-colonise the paddies following the early spring flood. Since *Genapol OXD-080* is rapidly biodegradable, provided that it does not contaminate the irrigation channels in high concentrations, it would be possible to ensure this important ecological pool for faunal recruitment and population renewal. This may be feasible if the water discharge from rice paddies was not performed during or immediately after the application of *Genapol OXD-080*. These aspects must be taken into account when establishing a 'best possible strategy' for crayfish management in rice fields, including the use of surfactants.

Acknowledgements

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