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Modeling mosquitofish (*Gambusia holbrooki*) responses to *Genapol OXD-080*, a non-ionic surfactant, in rice fields

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Abstract

An ecotechnological approach to control crayfish (*Procambarus clarkii*) infestation in rice fields of the Lower Mondego River Valley (Central Portugal) has recently been investigated. The application of the biodegradable non-ionic surfactant *Genapol OXD-080*, a fatty alcohol polyglycol ether, in rice paddies at a given concentration (50 mg/l) has been considered as a non-harmful chemical method to mitigate damage caused by crayfish digging activities to rice crops. Therefore, an important requirement regarding the ecological viability of this approach is that populations of non-target species are not significantly affected. A simple mosquitofish (*Gambusia holbrooki*) population model, in which the relationships with its main food prey were considered, was developed to assess the potential risk to a non-target key species of contaminating the irrigation channels following surfactant application. The model is based on data concerning mosquitofish life cycle and population dynamics, as well as mosquitofish diet and interactions with its main prey species. Quantitative information regarding the acute and sublethal effects of *Genapol OXD-080* on mosquitofish and other non-target organisms was obtained from laboratory experiments. Three concentrations of *Genapol OXD-080* (0.75, 1 and 2.5 mg/l) were used to simulate a small amount of contamination in irrigation channels. If contamination occurred, the mosquitofish population would tend to decline dramatically, even when submitted to a very small concentration of *Genapol OXD-080* (e.g. 0.75 mg/l, 66.7 times lower than the concentration planned to be used in rice paddies). Thus, *Genapol OXD-080* could potentially cause vast damage to local mosquitofish populations, and therefore should not be used without taking all precautions to avoid contaminating important biological reservoirs, such as the rice field irrigation channels.

Keywords: Mosquitofish; *Gambusia holbrooki*; Surfactant; Genapol; Crayfish; Risk assessment model; Rice field

1. Introduction

The Louisiana red swamp crayfish, *Procambarus clarkii*, was introduced to the Iberian Penin-

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sula mainly as food value, aiming for a double production of rice and crayfish. Crayfish populations have rapidly increased without control, invading most of the wetland areas and rice fields. In the Lower Mondego River Valley (Central Portugal), crayfish infestations have caused serious damage to drainage systems and rice crops, as a consequence of digging activities. Consequently, farmers now consider this species a pest, continually working to eradicate crayfish populations with toxic xenobiotic chemicals. These methods were ineffective, owing to high resistance of crayfish to toxic compounds, and causing on the other hand a severe environmental impact (Anastácio and Marques, 1995; Anastácio et al., 1995).

An alternative chemical procedure to control the physiological activity of crayfish populations without killing them was investigated. This method aimed to assist rice farming and simultaneously to allow crayfish production in rice fields, taking profit from both activities. In previous studies, a non-ionic surfactant, *Genapol OXD-080*, formulated by Hoechst, has been shown, at a given concentration, to reduce crayfish gill hematosis without killing them, reducing their physiological activity, but allowing a further recovery (Fonseca et al., 1997). *Genapol OXD-080*, a polyglycol ether of fatty alcohol, is biodegradable and presents low toxicity in comparison to pesticides commonly used, although illegally, by farmers (Jørgensen et al., 1997). Nevertheless, it was important to assess the environmental risk due to the application of *Genapol OXD-080*, namely with regard to non-target aquatic organisms usually present in rice paddies, where the surfactant is directly applied, and in irrigation channels that might become contaminated. The irrigation channels are important biological reservoirs containing a large variety of plant and animal species able to re-colonise the paddies following the early spring flood. This question has a crucial importance, since the concentration of *Genapol OXD-080* necessary to significantly decrease crayfish physiological activity appears to be drastically harmful to non-target organisms in rice paddies (Cabral et al., 1997).

For the present purposes, the eastern mosquitofish, *Gambusia holbrooki* (Girard) (Cyprinodontiformes: Poeciliidae), originally from the eastern coastal region of the United States, was considered the most suitable non-target key species in the rice paddies and irrigation channels (Cabral et al., 1998). Several decades after its introduction in Portugal, this species plays an important role as a predator in rice fields (Cabral et al., 1998; Cabral and Marques, 1999). Although it is not native to the Iberian Peninsula, mosquitofish presents the following characteristics that justified its choice: (1) is a well established locally abundant species, (2) is a species that is particularly likely to be exposed to the surfactant, (3) is a predator with an intermediate position in the food chain, preying on aquatic invertebrates and being controlled by piscivores (Hurlbert et al., 1972; Britton and Moser, 1982), (4) is one of the most intensively studied poeciliids (Haynes and Cashner, 1995) whose ecology and behavior provide easy opportunities to measure effects, and (5) is a species which closely represents the typical characteristics of a large number of others in the rice field community.

The aim of the present paper was to develop a simple model able to provide some basis for the assessment of the ecological viability of using *Genapol OXD-080* as an alternative chemical method to control crayfish in rice fields. Concretely, the model should predict, simultaneously, (1) the direct effect of a hypothetic surfactant contamination, at relatively low concentrations, over the mosquitofish population in irrigation channels, and (2) the indirect effect of the same event on the relationships between this key predator fish and its main prey species.

2. Study site

The Lower Mondego River Valley is located in the central region of Portugal (40°10′N, 08°41′W). The valley consists of about 15 000 ha where the main agricultural crop is rice, which occupies about 60% of the usable area. Non-cultivated areas, such as wetlands, appear in the periphery of the valley, exhibiting flourishing fauna and flora.

In the study site, mosquitofish and its prey occur throughout the irrigation channels, but only seasonally in the rice paddies. During a large part of the year the rice paddies present a very low water level or are completely dry and unable to support mosquitofish. In the main irrigation channels, although levels vary around the year, enough water always exists to support a large variety of plant and animal species. Therefore, the present study, carried out during 1996–1997, was focused in the irrigation channels.

3. Conceptualization of the model and equations

The model is based on the mosquitofish life cycle and population dynamics (Cabral and Marques, 1999), mosquitofish diet and interactions with its main prey (Cabral et al., 1998). Quantitative information regarding the acute and sublethal effects of *Genapol OXD-080* on mosquitofish and other non-target organisms was obtained from laboratory studies (Cabral et al., 1997, 1999). To develop the model we used STELLA 5.0.

The model includes only three state variables, all related to mosquitofish population structure taking into account sex and age, respectively, immature, adult females and adult males (Fig. 1). Difference equations describing the processes affecting state variables, expressed in densities (individuals/m^2), are presented in Table 1. As initial densities (Table 1, Process equations), we considered data on population dynamics from April 1996 (Cabral and Marques, 1999), the starting point of the model.

3.1. Mosquitofish mortalities

Daily mortality rates of females (femalemort) and males (malemort) (Fig. 1 and Table 1, Process equations) were based on mosquitofish life cycle data (Cabral and Marques, 1999). The daily mortality rate of immature (immamort) (Fig. 1 and Table 1, Process equations) was obtained from the literature and assumed to be mainly a consequence of cannibalism by adults, always present in high densities, on juveniles. Hubbs (1991) experimentally reported that most of the cannibalism is practised by females, and that about 50% of immature individuals confined in an aquarium together with an adult will survive 30 days (about 1.67%/day or 0.0167/day). In the mortality equations (Table 1, Process equations), the factors underlined represent the concentration (in mg/l) of surfactant (surfapplication) at which the mortality is 0.5 (50%) for each mosquitofish group considered, and were based on LC50 data (Cabral et al., 1997, 1999). In the same equations, factors in bold were rated at one order of magnitude greater than the normal daily mortality rates of adults, representing the fraction of fish that may die by starvation. In fact, if food is not available, high mosquitofish metabolic rates will cause fish to starve to death quickly (Wurtsbaugh and Cech, 1983).

Because mosquitofish is a dimorphic species and mature males are much smaller than mature females, the daily mortality rate by starvation for immature individuals was considered similar to that of males. These rates only affect total mortality when the prey fraction available each day for a given mosquitofish group is smaller than the daily consumption of the same group (see more details about this balance in Section 3.3).

3.2. Mosquitofish fecundity and growth

The equations for the daily adult input from immatures (femaleinput and maleinput) (Fig. 1 and Table 1, Process equations) are rated by the average sex-ratio recorded from the field (Cabral and Marques, 1999). The daily recruitment rate (recruitment) and the daily transference rate from immatures to adults (immattransf) were assumed as adjustment factors (Table 1, Process equations). In the first case, recruitment only starts after the 70th day, which coincides approximately with the appearance of newly recruits in June and depends on the mean number of embryos per female (embryos) and the mean proportion of gravid females (gravid female) (Cabral and Marques, 1999). On the other hand, gravid females depend on the photoperiod, the major factor determining the seasonal fecundity of the population studied (Cabral and Marques, 1999), and on the presence of males (Fig. 1 and Table 1, Regulating equations and parameters). The photoperiod value of 14.15 represents the period of time at which the first late embryos, ready for parturition, were recorded (Cabral and Marques, 1999).

3.3. Competition and food consumption

The intra-specific competition pressure for food resources (comp press) is a variable created to express the balance between the prey fraction available for each mosquitofish group and the respective daily prey consumptions (Fig. 1 and Table 1, Regulating equations and parameters). The consumption rates (cons rate) and daily consumptions (cons) (Fig. 1 and Table 1, Regulating equations and parameters) were based on

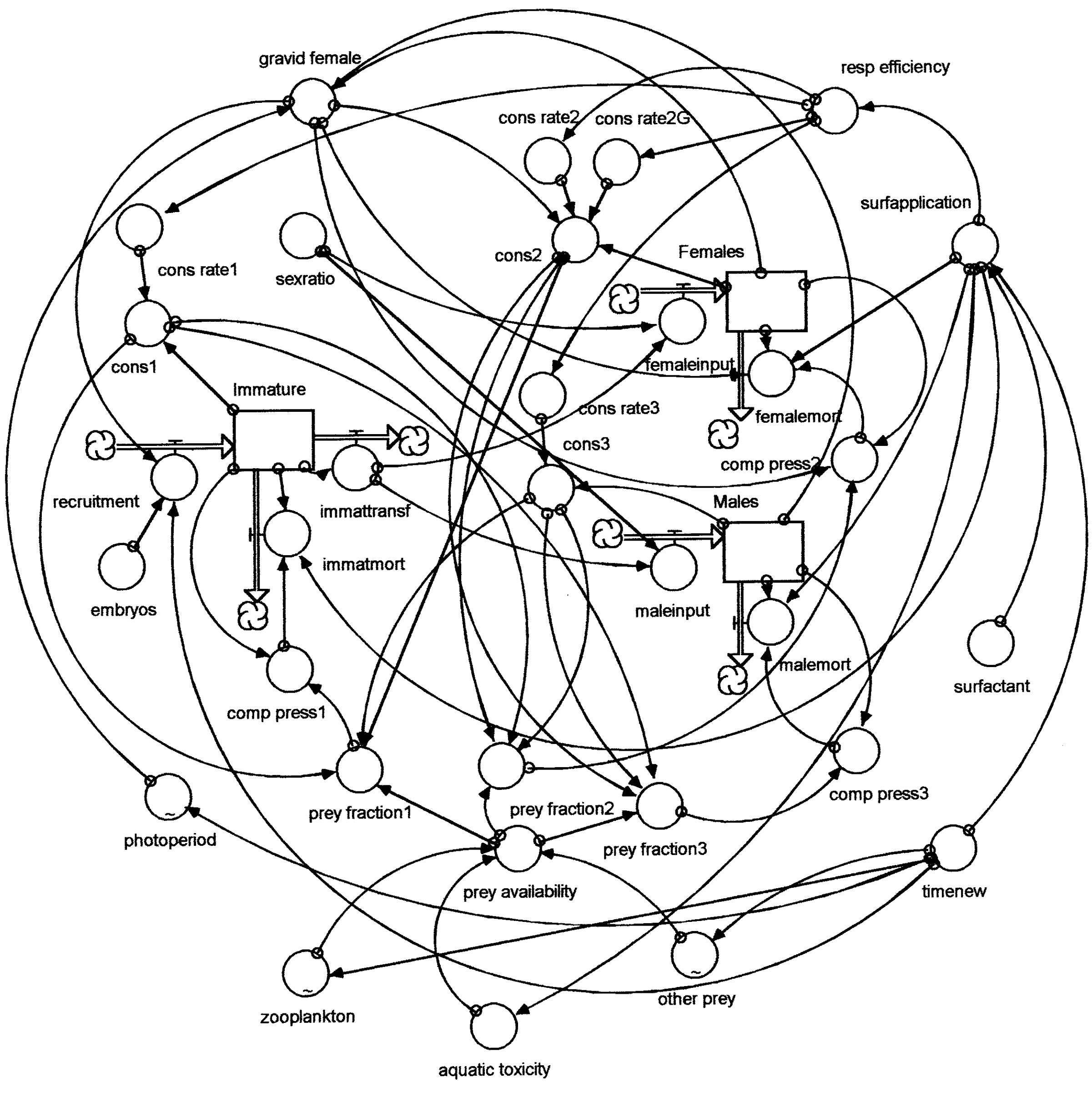

Fig. 1. Conceptual diagram of *Gambusia holbrooki* and its main prey relationships in rice field irrigation channels of the Lower Mondego River Valley.

Table 1
Equations used in STELLA for mosquitofish (*G. holbrooki*) population[a]

Difference equations
Immature(t) = Immature(t − dt) + (recruitment − immatmort − immattransf)*dt
Females(t) = Females(t − dt) + (femaleinput − femalemort)*dt
Males(t) = Males(t − dt) + (maleinput − malemort)*dt

Process equations
(a) Immature
Initial density of Immature = 0
recruitment = if timenew > 70 then 0.00875*embryos*gravid female else 0
immatmort = if surfapplication < 2.9 then 0.0167*Immature*(1 + 3.1510418*surfapplication) + **0.0836*(26*Immature − comp press1)/26** else Immature
immattransf = 0.3*Immature
(b) Females
Initial density of Females = 28
femaleinput = sexratio*immattransf
femalemort = if surfapplication < 3.5 then 0.00746*Females*(1 + 5.5020108*surfapplication) + **0.0746*((41*(Females − gravid female) + 102*gravid female) − comp press2)/41** else Females
(c) Males
Initial density of Males = 5.8
maleinput = (1 − sexratio)*immattransf
malemort = if surfapplication < 2.9 then 0.00836*Males*(1 + 6.125897*surfapplication) + **0.0836*(31*Males − comp press3)/31** else Males

Regulating equations and parameters
(a) Fecundity
embryos = 28
gravid female = IF photoperiod > 14.15 and Males > 0 then 0.46*Females else 0
sexratio = 0.8
(b) Intra-specific competition[a]
comp press1 = if prey fraction1 > 26*Immature then 26*Immature else prey fraction1
comp press2 = if prey fraction2 > 41*(Females − gravid female) + 102*gravid female then 41*(Females-gravid female) + 102*gravid female else prey fraction2
comp press3 = if prey fraction3 > 31*Males then 31*Males else prey fraction3
(c) Food consumption[a]
cons1 = cons rate1*Immature
cons2 = cons rate2*(Females − gravid female) + cons rate2G*gravid female
cons3 = cons rate3*Males
cons rate1 = 26*resp efficiency
cons rate2 = 41*resp efficiency
cons rate2G = 102*resp efficiency
cons rate3 = 31*resp efficiency
prey fraction1 = if prey availability > cons1 + cons2 + cons3 then prey availability else prey availability-(cons2 + cons3)
prey fraction2 = if prey availability > cons1 + cons2 + cons3 then prey availability else prey availability − (cons1 + cons3)
prey fraction3 = if prey availability > cons1 + cons2 + cons3 then prey availability else prey availability − (cons1 + cons2)
(d) Respiratory efficiency
resp efficiency = IF surfapplication < 3 then *1/(1 + 0.62667*surfapplication)* else 0

Environmental variables
aquatic toxicity = if surfapplication < 4 then 1/(1 + 0.27778*surfapplication) else 0
prey availability = 3200*(zooplankton*aquatic toxicity + other prey*aquatic toxicity)
surfactant = given values in mg/l
surfapplication = if timenew > 12 and timenew < 43 then surfactant else 0

Table functions
other prey = GRAPH(timenew)
(0.00, 70.7), (30.4, 30.3), (60.8, 0.00), (91.3, 5.05), (122, 0.00), (152, 55.6), (183, 1687),
(213, 379), (243, 379), (274, 0.00), (304, 273), (335, 591), (365, 70.7)
photoperiod = GRAPH(timenew)
(0.00, 13.3), (30.4, 14.5), (60.8, 15.0), (91.3, 14.8), (122, 13.8), (152, 12.5), (183, 11.3),
(213, 10.3), (243, 9.25), (274, 9.50), (304, 10.5), (335, 11.8), (365, 13.3)
timenew = TIME-365*INT(TIME/365)
zooplankton = GRAPH(timenew)
(0.00, 245), (30.4, 7.94), (60.8, 65385), (91.3, 0.00), (122, 2.93), (152, 1.97), (183, 0.00),
(213, 99.6), (243, 99.6), (274, 1798), (304, 1527), (335, 80.0), (365, 20.0)

[a] The terms 1, 2, 2G, and 3 represent, respectively, immature, females, gravid females and males.

mosquitofish diet data (Cabral et al., 1998). Consumption rates were affected by the respiratory efficiency (resp efficiency) of mosquitofish, which depends on the surfactant concentration in the environment (surfapplication) (Fig. 1 and Table 1, Regulating equations and parameters). In the equation describing respiratory efficiency (Table 1, Regulating equations and parameters), the factor in italic represents the effect of the surfactant on respiratory processes, considering the lowest concentration at which a significant decrease of the average oxygen consumption was observed in mosquitofish submitted to *Genapol OXD-080* (Cabral et al., 1999).

3.4. Environmental factors

The prey component is composed of two table functions, zooplankton and other prey. The tables (Fig. 1 and Table 1, Table functions) were based on temporal data on abundances recorded expressed in densities (ind./m^2) (Cabral et al., 1998). Prey availability was estimated from the sum of the abundances of these two main prey groups (Fig. 1 and Table 1, Environmental variables). In this equation, the value 3200 was the lowest multiple of daily prey availability (in density) necessary to support the mosquitofish population in a normal situation without surfactant contamination. Both prey groups are affected by the surfactant concentration (surfapplication) present in the water (aquatic toxicity) (Fig. 1 and Table 1, Environmental variables). In the equation of 'aquatic toxicity', the factor underlined represents the concentration (in mg/l) of surfactant for which the main prey mortality becomes 0.5 (50%) (Cabral et al., 1997).

The surfactant application (surfapplication), expressed in mg/l, is carried out only from the 13th to the 42nd day (from the end of April), which coincides with the time of the year when the application of *Genapol OXD-080* in the rice fields should take place and because it is assumed that the surfactant effect lasts about 1 month (Fig. 1 and Table 1, Environmental variables). This period was necessary to prevent damage to the young rice plants, caused by crayfish burrowing (Anastácio and Marques, 1997).

4. Simulation results and verification

The simulated densities fit well with average densities observed for adult mosquitofish in irrigation channels (Cabral and Marques, 1999), where the surfactant was not applied. With regard to immature mosquitofish, in the simulations, the peak of density is slightly earlier and the individuals disappearing too soon in comparison with the real population, observed from field sampling

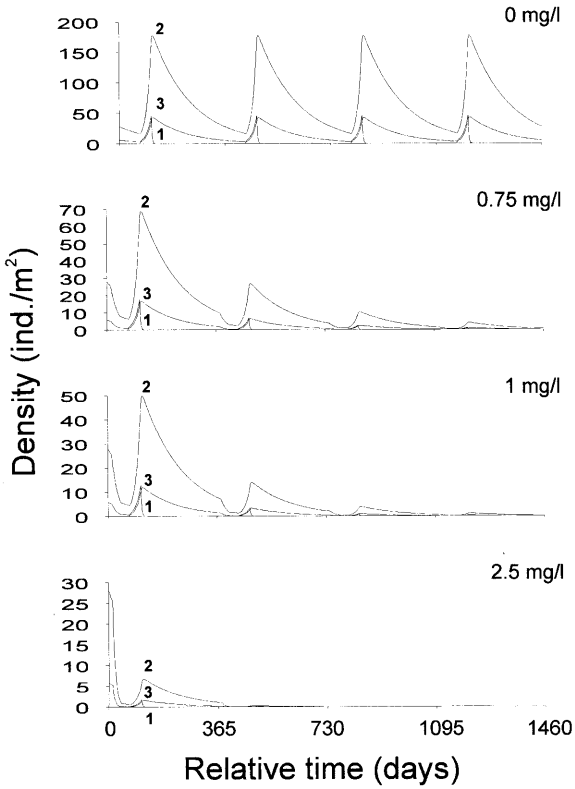

Fig. 2. Computer simulation, for *Gambusia holbrooki* population as a whole (but discriminated in group densities: immature [1], females [2] and males [3]), of a normal situation where no surfactant was used (0 mg/l) and when a gradient of surfactant contaminations (0.75, 1 and 2.5 mg/l) occurred in a rice field irrigation channel for 4 years.

(Cabral and Marques, 1999). Taking into account the available field data, particularly regarding adult mosquitofish, the model behaves as we would expect in a scenario where no surfactant was used.

Three concentrations of *Genapol OXD-080* (0.75, 1 and 2.5 mg/l) were used to simulate a small amount of contamination in irrigation channels. If contamination occurred, the mosquitofish groups considered should tend to decline dramatically even for the smallest concentrations (0.75 mg/l) of *Genapol OXD-080* (Fig. 2). This concentration is 66.7 times lower than the concentration that would be necessary to use in rice paddies to control crayfish activity (Cabral et al., 1999). Thinking of the population as a whole, if irrigation channels become contaminated by *Genapol*

OXD-080, mosquitofish should tend to decline through time, for all the concentrations considered, which contrasts with a normal steady-state scenario (Fig. 2).

5. Discussion

The model developed in this study is a useful contribution for the assessment of the environmental risk associated with the application of *Genapol OXD-080* to control crayfish population activity in rice paddies. Because the interactions between mosquitofish and its prey are a crucial component in the irrigation channels food web, we recommend that *Genapol OXD-080* not be used as an alternative method to control crayfish populations without special precautions. In fact, even a small amount of contamination in irrigation channels may be harmful to mosquitofish, killing them or affecting important biological aspects such as metabolism, predatory, and reproductive capacity (Cabral et al., 1999).

In the respiratory metabolism tests and for short periods (a few hours), we considered that the range of concentrations used in the model was a gradient of more or less sublethal doses (Cabral et al., 1997, 1999). Nevertheless, a significant decrease in mosquitofish metabolism was observed even when submitted to a very small concentration of *Genapol OXD-080* (0.75 mg/l), demonstrating that oxygen consumption rates change dramatically in the presence of surfactant (Cabral et al., 1999). The reversible disruption of the respiratory processes in the gill membrane caused by the surfactant (Abel, 1974; Lewis, 1991) constitutes a credible explanation for this. However, for longer periods, such as the 96 h of the LC50 tests or the 40 days of the mosquitofish clutch survival tests, different and drastic responses were obtained (Cabral et al., 1999). The probable period of surfactant contamination in irrigation channels, if it takes place, falls within this interval of time. The model behaves as expected when a gradient of surfactant contaminations was simulated. The decline of mosquitofish population was caused by the persistence of negative factors on mosquitofish mortalities and/or the indirect effect of the surfactant on prey populations. In the first case, the mortality factors were elaborated to affect directly and proportionally the mosquitofish mortality during the contamination period (1 month), even at concentrations smaller than the LC50 value. In fact, for long periods of surfactant exposure, the decline of fish populations may occur at 'sublethal' concentrations, probably by some form of increasing internal poisoning to which gill damage was only a contributory or complicating factor (Abel, 1974).

The possible detrimental effects of *Genapol OXD-080* on microcrustaceans will indirectly affect mosquitofish since they are preferentially zooplankton feeders (Wurtsbaugh et al., 1980; Hoy, 1985; Lydeard and Belk, 1993; Homski et al., 1994; Schaefer et al., 1994; Cabral et al., 1998). The LC50 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 studies concerning the effects of surfactants on other non-target organisms often caught by mosquitofish (Cabral et al., 1998) also consider a potential negative impact on chironomids (Corbet et al., 1995), aphids (Imai et al., 1994) and collembolan (Holmstrup and Krogh, 1996).

Despite the fact that *Genapol OXD-080* appears to be potentially harmful to the biological structure and function of 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.). Since *Genapol OXD-080* is rapidly biodegradable, provided that it does not contaminate the irrigation channels, it would be possible to ensure this important ecological pool for faunal recruitment and population renewal. This may be feasible if there is no water discharge from rice paddies into irrigation channels during or in the next days 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.

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