Growth responses of juvenile red swamp crayfish, *Procambarus clarkii* Girard, to several diets under controlled conditions

J Oliveira & A Fabião

Departamento de Engenharia Florestal, Instituto Superior de Agronomia, Lisboa Codex, Portugal

Correspondence: Dr António Fabião, Instituto Superior de Agronomia, Departamento de Engenharia Florestal, Lisboa Codex, Portugal

Abstract

The growth responses of juvenile crayfish, *Procambarus clarkii* Girard, to diets based on common fresh feedstuffs available from agriculture and freshwater ecosystems were studied under controlled laboratory conditions. The dietary treatments were: fresh leaves and shoots of watercress, fresh potato tubers, fresh fish meat from mosquitofish, and all the possible combinations of those ingredients on alternate feeding days. After 22 days, the results showed a significantly higher growth rate ($P = 0.05$) in crayfish fed with mosquitofish meat. The differences were not significant between the other treatments, but those which included fish meat induced growth rates consistently higher than those based only on plant ingredients. This trend was maintained until the end of the experiment. These results suggest that a dietary supplement based on animal ingredients might significantly improve growth rate and yield, provided the economic feasibility of such a production process be confirmed in field trials. However, it was not clear whether this was because of protein, or lipid concentration and composition of mosquitofish meat. Further research must also clarify the role of vegetable ingredients in dietary composition, as well as the effect of microbial populations occurring during the decomposition of plant material on its nutritional properties for crayfish.

Introduction

Red swamp crayfish, *Procambarus clarkii* Girard, was first reported in southern Portugal by Ramos &

Pereira (1981), in the Guadiana river basin, where it probably appeared as a result of the natural expansion of populations of the species introduced in Spain, which escaped to the wild. From that river basin, it spread through the adjacent major lotic systems and small reservoirs to central Portugal, occurring nowadays with a wide distribution in the centre and south of the country, where it seems to be especially well adapted to reservoirs and slow-flowing waters (Correia 1993; Ilhéu & Bernardo 1993a,b).

The occurrence of red swamp crayfish in irrigation reservoirs and channels, and in rice fields poses some problems to rice culture, because of the excavating behaviour and trophic ecology of this species. In some circumstances, the galleries excavated by red swamp crayfish cause water loss from the reservoirs and rice ponds, and the rice yields may be significantly decreased by such water loss and by the damage to rice plants, both through excavation and feeding (Correia 1993; Ilhéu & Bernardo 1993a,b). This leads to the widespread use of very toxic pesticides (mainly organophosphorous insecticides) by most farmers, in an attempt to control the population growth of crayfish, and this has undesirable environmental consequences (Ganhão, Germano & Grilo 1991).

Although red swamp crayfish is the main freshwater crayfish species commercially farmed and captured globally (Holdich 1993), the commercial exploitation of natural and farmed populations is scarce and not organized in Portugal. The few farmers and freshwater fishermen who export crayfish to Spain and occasionally sell small amounts in the Portuguese market are exceptions, and are of

little significance within a national context. The management and commercial exploitation of Portuguese natural crayfish stocks could be economically viable and possibly decrease the environmental costs related to the control of crayfish populations in the rice fields.

The prices that have been paid for imported red swamp crayfish in western Europe during the last few years, both fresh and frozen (Holdich 1993; Laurent 1993; Ackefors & Lindqvist 1994), seem to be attractive enough to encourage a more well-organized commercial exploitation in Portugal, mainly in rice fields and small irrigation reservoirs in the centre and south of the country where red swamp crayfish are already present and constitute a pest with costs to agricultural production.

Such a management of natural stocks of red swamp crayfish could include practices to optimize the production levels through higher standing biomass and improved growth rates. Within this context, supplemental feeding may constitute an adequate measure to increase the yield of crayfish in small ponds and irrigation reservoirs (Avault & Brunson 1990; Ackefors & Lindqvist 1994). However, the trophic ecology of red crayfish is complex (Lorman & Magnuson 1978; Avault & Brunson 1990; Ilhéu & Bernardo 1993a), apparently with a dietary preference for plant debris, but still with a significant carnivorous component. Ilhéu & Bernardo (1993b) concluded that the feeding preferences of adult red swamp crayfish could be carnivorous, but because of an opportunistic feeding strategy, such a preference is limited by the mobility and access to shelter of the potential preys, causing a shift in feeding behaviour to plant biomass and detritus, which is easier to catch.

It is largely unknown what potential dietary supplements, among those easily available from Portuguese farms and water bodies, would be most efficient in promoting higher growth rates (especially in the early growth stage). The aim of this study was to assess, under controlled conditions, the growth responses of juvenile crayfish to diets based on common feedstuffs available as agricultural by-products and from freshwater ecosystems, used alone and in combination.

Materials and methods

The experimental trial consisted of 14 polyethylene reservoirs (55 × 45 × 38 cm), filled with 36 l of tap

water each and placed in a laboratory with controlled temperature ($22 \pm 2^\circ\text{C}$) in the Forestry Department of the Instituto Superior de Agronomia in Lisbon. The water was stored for approximately 12 h in a large separate reservoir in order to eliminate chlorine residues. The calcium concentration was adjusted with CaCl_2 to about 150 p.p.m. Ca, to maintain chemical conditions that were adequate for the survival of crayfish during the moult (Bardach, Ryther & McLarney 1972; Goyert & Avault 1977; Landau 1992). A piece of brick was placed in each reservoir to provide shelter for the crayfish. Complete water replacements were performed after 11 and 22 days, after water storage and adjustment of calcium concentration as described above. The dissolved oxygen was kept at saturation levels by using diffusion aerators connected to a compressed air source.

Juvenile crayfish were hatched from females collected from the natural environment and kept in aquaria until hatching, following a technique similar to that described by Trimble & Gaudé (1988). Three weeks after hatching, 126 juveniles with an average length of 20 mm (ranging from 15 to 25 mm) and an average weight of 0.18 g (ranging from 0.08 to 0.46 g) were selected for the experiment and placed in the polyethylene reservoirs, nine crayfish in each reservoir. The experimental design consisted of seven treatments (diets) with two replicates. The diets were: (1) fresh leaves and shoots of watercress, *Nasturtium officinale*, commercially available; (2) commercially available fresh potato tubercles, *Solanum tuberosum*; (3) fresh fish meat from mosquitofish, *Gambusia holbrooki* Girard, a very common and prolific introduced freshwater fish in central and southern Portugal, obtained from water bodies and ponds of the Institute campus; (4) a combination of 1 and 2 on alternate feeding days; (5) a combination of 2 and 3 on alternate feeding days; (6) a combination of 1 and 3 on alternate feeding days; and (7) a combination of 1, 2 and 3 on alternate feeding days.

The main nutritional characteristics of watercress and potato were obtained from published tables (Staron 1984) and those of mosquitofish were evaluated in the laboratory. The dry matter content was assessed after oven drying at 104°C until the weight was constant. Crude protein was determined by the Kjeldhall method. Lipids (total sap) were extracted with petroleum ether after hydrolysis with 3N hydrochloric acid using a SOXTEC System 1047 Hydrolysing Unit and SOXTEC System HT 1043

Extraction Unit (TECATOR AB, Höganäs, Sweden). The ash content was determined by overnight combustion at 550 °C. An analysis of variance was performed with the statistical package GENSTAT 5.0, Release 3.1 (Numeric Algorithm Group, Oxford Science Publications, Clarendon Press, Oxford, UK) to determine whether there were any significant differences (0.05 probability level) between treatments in average length or weight of juvenile crayfish at the beginning of the experiment.

The crayfish were fed *ad libitum* every second day, alternating regularly the feedstuffs in treatments 4 to 7, for 57 days, when 50% of the crayfish in the fastest-growing treatment attained a length of 7.0 cm. Such a length may be considered a minimum for acceptance by the international red swamp crayfish market (Cange, Miltner & Avault 1982; De La Bretonne & Romaine 1989; Huner 1989). The watercress was simply placed in the water, providing access to the leaves and shoots for the crayfish. Potato and fish meat were divided into small pieces and spread on the water surface. The uneaten food was removed regularly from the reservoirs. Every eleventh day, and when the experiment finished (day 57), all the individuals were collected, counted, measured (total length from rostrum to telson) to the nearest millimetre and weighed to the nearest 0.01 g on an electronic balance. The Fulton's condition factor (Bagenal & Tesch 1978)— $K = 100w/l^3$, where w is body weight and l is total length—was calculated for each individual. The water pH was monitored weekly during the experimental period. It ranged between 6.2 and 8.5, which was within the tolerance range of red swamp crayfish (Morales 1987).

The data obtained were treated by analysis of variance applied to the replicate means on each measuring date, using the statistical package GENSTAT 5.0. Whenever significant differences between treatments occurred, the Duncan's new multiple range test (Steel & Torrie 1980) was used for mean separation analysis. The 0.05 probability level was used for all comparisons.

Results

The nutritional composition of the feeds used in the experiment is shown in Table 1. The mosquitofish meat seems to be the best balanced diet, because of its high protein content and reasonable amounts of lipids and minerals. Watercress has a reasonable

Table 1 Main nutritional composition of the feedstuffs used in the experiment (dry matter: % of fresh weight; nutritional ingredients: % of dry weight)

Composition	Watercress	Potatoes*	Mosquitofish meat
Dry matter	9.5	24.0	20.2
Proteins	33.0*	9.2	67.4
Lipids	—	0.4	14.4
Carbohydrates†	7.9*	82.0	3.9
Fibre	37.0*	2.9	—
Ashes	22.0*	5.4	16.4

*Mean concentrations, from Staron (1984).

†Non-parietal carbohydrates.

protein content for a vegetable, but with a high amount of fibre and a very high water content. Potato tubers are probably the worst diet for crayfish, since the concentrations of proteins, lipids and minerals are poor and the carbohydrates (mainly starch) are not easily digestible.

The initial length, weight and condition factor were not significantly different between treatments. The results obtained throughout the experiment are shown in Table 2. Treatment 3, feeding with fresh mosquitofish meat, started to induce a significantly higher growth rate ($P < 0.05$), both in length and weight, after day 22 of the experiment. This difference was maintained until the end of the experiment, at day 57. The differences between other treatments were not statistically significant ($P > 0.05$), but the treatments including fish meat gave results consistently higher than those based only on vegetable products, as shown in Fig. 1. The average length and weight at the end of the experiment were, respectively, higher than 3.0 cm and 1.0 g only in the treatments including fish meat, alone or in combination with other feedstuffs. No significant difference between the results obtained after feeding with watercress and potatoes, either used alone or in combination with fish meat, was observed. The condition factor did not show any clear trend or relation to the type of diet during the experiment.

At the end of the experiment, the highest survival rate (72%) was obtained in treatment 5, consisting of feeding with a combination of fish meat and potatoes, but feeding with fish meat alone (treatment 3) and with a combination of watercress and potatoes (treatment 4) gave a close second best,

Table 2 Mean initial values for individual body weight, total length and Fulton's condition factor in each treatment and results obtained throughout the experiment for those parameters. Values followed by different superscript characters in the same column were significantly different (0.05 probability level)

Treatment*	Initial size	Growth				
		After 11 days	After 22 days	After 33 days	After 44 days	After 57 days
Length (cm)						
1	2.0	2.3	2.3 ^b	2.6 ^b	2.6 ^b	2.8 ^b
2	2.0	2.1	2.2 ^b	2.2 ^b	2.4 ^b	2.5 ^b
3	2.0	3.0	3.6 ^a	4.3 ^a	5.0 ^a	5.9 ^a
4	2.0	2.5	2.4 ^b	2.3 ^b	2.5 ^b	2.7 ^b
5	2.0	2.6	2.5 ^b	2.6 ^b	3.0 ^b	3.7 ^b
6	2.0	2.3	2.8 ^b	3.0 ^b	3.5 ^b	3.9 ^b
7	2.0	2.4	2.8 ^b	2.9 ^b	3.3 ^b	3.7 ^b
Weight (g)						
1	0.18	0.24	0.28 ^b	0.29 ^b	0.48 ^b	0.50 ^b
2	0.18	0.21	0.22 ^b	0.21 ^b	0.25 ^b	0.40 ^b
3	0.18	0.58	1.32 ^a	2.10 ^a	3.45 ^a	6.18 ^a
4	0.17	0.23	0.27 ^b	0.27 ^b	0.31 ^b	0.54 ^b
5	0.18	0.35	0.51 ^b	0.71 ^b	1.04 ^b	1.53 ^b
6	0.18	0.32	0.37 ^b	0.40 ^b	0.66 ^b	1.31 ^b
7	0.19	0.28	0.55 ^b	0.61 ^b	0.90 ^b	1.30 ^b
Condition factor						
1	2.1	1.9	2.2	2.1	2.5	2.1
2	2.1	2.2	2.0	2.3	2.0	2.4
3	2.1	2.0	2.4	2.3	2.5	2.5
4	2.0	1.8	2.1	2.0	1.9	2.5
5	2.1	1.8	2.0	2.0	2.1	2.2
6	2.1	1.9	2.0	2.1	2.2	2.2
7	2.1	1.8	2.1	2.0	2.2	2.3

* (1) fresh leaves and shoots of watercress; (2) fresh potato tubercles; (3) fresh fish meat from mosquitofish; (4) a combination of 1 and 2 on alternate feeding days; (5) a combination of 2 and 3; (6) a combination of 1 and 3; (7) a combination of 1, 2 and 3.

both with approximately 67% survival. The lowest survival rate, 39%, occurred after feeding with potatoes alone (treatment 2), whereas feeding with fish meat plus watercress (treatment 6), or plus watercress and potatoes (treatment 7), both had a survival rate of 61%, and with watercress alone (treatment 1) of approximately 56%. It was not clear if the low survival rate obtained with treatment 2 was related to a low nutritional status, since it occurred mainly in one of the two replicates between day 22 and day 33 of the experiment. In fact, the survivors of this treatment had a good condition factor at the end of the experiment (2.4, see Table 2), almost as high as in treatments 3 and 4, where the highest value of 2.5 was measured. However, the differences between treatments based on the

condition factor were not found to be significant at the 0.05 probability level.

Discussion and conclusions

The occurrence of microbial populations associated with decomposing vegetable crayfish feeds is believed to play an important role in their nutritional value, mainly because of the decrease of the carbon:nitrogen ratio throughout the decomposition period (Goyert & Avault 1977; Landau 1992). Red swamp crayfish has a clear tendency for detritivorous feeding behaviour (Avault & Brunson 1990; Ilhéu & Bernardo 1993a), probably taking advantage of a higher protein content in the

Figure 1 Increases in length (top) and weight (bottom), during the experimental period, of red swamp crayfish fed with the treatment diets. Coarse black line: fed with mosquitofish meat only; dotted lines: fed with diets including mosquitofish meat; fine black lines: fed with vegetable ingredients only.

decomposing plant materials used as food, when compared with fresh vegetable tissues, and a better quality of such protein, related to the occurrence

of microbial populations and benthic organisms associated with the detritus-based ecosystem (D'Abramo & Robinson 1989). As a matter of fact, fresh vegetables are not frequently used as crayfish feeds, and references on their nutritional relevance for this species are very scarce. The present results obtained in the treatments using just fresh vegetable feedstuffs suggest that they must represent a very poor dietary supplement, at least in such a fresh condition.

Huner & Meyers (1979) found that protein of animal origin might have a significant positive effect on the growth of juvenile red swamp crayfish. They concluded that a concentration of 15% of gross animal protein in the diet was the lowest acceptable value for feeding red swamp crayfish with near-optimal growth responses in a controlled experimental system. However, they did not find any significant differences in growth rate within the range of 15–44% of gross animal protein concentration and suggested, based on their own data and on data obtained by other authors, that gross animal protein concentrations within the range of 15–62% would not result in any differences.

In the present experiments, where a diet with 65% protein of animal origin was used in treatment 3, and protein concentrations within the range of 15–65% were accessible to the crayfish in treatments 5, 6 and 7, significant differences in growth response were found between the diet with the highest protein concentration and the others. In addition, the differences between diets with and without animal protein, excluding treatment 3, were not significant (see Table 2), although a clear trend for higher growth rates was observed in treatments where the animal component was available, compared to those where it was not. Although such results suggest a conclusion different from that obtained by Huner & Meyers (1979), and somewhat different from those stated by Brown, Robinson, Clark & Lawrence (1989), Brown, Williams, Robinson, Akiyama & Lawrence (1986) and Reigh, Braden & Craig (1990), it must be stressed that those authors did not use fresh animal protein, and that the experimental conditions in the present experiment did not include any decomposing feedstuffs, meaning that the microbial populations occurring during the decomposition processes were not contributing significantly as an extra, uncontrolled, source of protein to the diets.

The nutritional requirements of crustaceans and, in particular, of red swamp crayfish are not yet

completely studied (Reigh, Braden & Craig 1990; Van-Wormhoudt & Bellon-Humbert 1994). The digestibility of proteins in the diet of crustaceans depends mainly on the proportion of amino acids, and especially, of limiting amino acids. However, the proteolytic characteristics are variable from one species to another and it is not clear what is the optimum protein composition for red swamp crayfish. Lipids also play an important role in the nutrition of aquatic animals, especially in decapod crustaceans, which are incapable of producing linoleic and linolenic acids (D'Abramo & Robinson 1989; Van-Wormhoudt & Bellon-Humbert 1994). The high lipid concentration in the mosquitofish-based diet may have played an important role in the observed differences between treatments in this experiment. This suggests that, in future experiments, the nutritional characteristics of mosquitofish meat must be studied and described in greater detail, allowing a better understanding of its potential as a dietary component for red swamp crayfish.

The main conclusion of this experiment is that a feeding supplement of mosquitofish meat, with a high concentration of gross animal protein and lipids, may contribute to faster growth of juvenile red swamp crayfish in populations confined in a spatially limited environment. The mosquitofish is an abundant and fast-growing fish species in the natural conditions of central and southern Portugal, allowing easy availability of the protein and lipid source, but conclusions on the economic feasibility of such a production process would be premature without confirmation from field trials. On the other hand, a well-balanced diet, including vegetable and animal feedstuffs, may contribute significantly to the quality of the crayfish production (Morales 1987), providing an adequate amount of carotene through the vegetable components. This experiment was not conclusive, since the treatments corresponding to a balanced diet were not significantly different from those without an animal component.

In addition to a more detailed study of the nutritional characteristics of the mosquitofish meat, and to the feasibility of using this species as a source of protein and lipids in the diets of red swamp crayfish, any future research must clarify what the proportions of vegetable components are that still allow a near-optimal growth rate in red swamp crayfish populations, and supply the diet with adequate amounts of carotene and other essential

growth factors, which may be or may not be present in natural and/or astacicultural environments. The effect of a previous decomposition of vegetable ingredients, including agricultural by-products, on their nutritional characteristics and potential as a supplement for red swamp crayfish diets must also be clarified.

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