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REPRODUCTIVE PERFORMANCE OF THE GUPPY FISH POECILIA RETICULATA [PETERS, 1859] FED WITH LIVE ARTEMIA FRANCISCANA [KELLOG, 1906] CULTURED WITH INERT AND LIVE DIETS
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Reproductive performance of the guppy fish Poecilia reticulata [Peters, 1859] fed with live Artemia franciscana [Kellog, 1906] cultured with inert and live diets

Comportamiento reproductivo del guppy *Poecilia reticulata* [Peters, 1859] alimentado con *Artemia franciscana* [Kellog, 1906] viva cultivada con dietas vivas e inertes

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Abstract

The reproductive performance as fry number, standard length, wet and dry weight and fry sex ratio, was studied in the guppy fish, *Poecilia reticulata*, under the influence of different broodstock diets. Dietary treatments consisted in live *Artemia franciscana* biomass fed with live microalgae (*Tetraselmis suecica* and *Chaetoceros calcitrans*), *Spirulina* dry powder, soy meal, wheat meal, a mixture 50%/50% of each meal and a commecial diet as the control group. After 45 days at laboratory conditions, there were no significant differences ($P > 0.05$) in the number of offspring born/female and individual standard length of fry among the treatments. Newborn fish from the control group were heavier than the rest of the treatments (4.14 and 1.06 mg wet and dry weight, respectively). In average, broodstock fed with brine shrimp cultured with *Spirulina* produced more male brood. The results point out towards the use of the commercial diet to fulfill the reproductive needs of *P. reticulata*.

Key words

Reproduction, *Poecilia reticulata*, *Artemia franciscana*, live diets, inert diets.

Resumen

El comportamiento reproductivo del guppy *Poecilia reticulata* —evaluado como número de crías, longitud estándar, peso húmedo y seco de las crías y su proporción sexual— fue estudiado usando varias dietas para los reproductores. Los tratamientos consistieron en adultos vivos de *Artemia franciscana* cultivada con microalgas vivas (*Tetraselmis suecica* y *Chaetoceros calcitrans*), harina de *Spirulina*, harina de soya, harina de trigo, una mezcla 50%/50% de dichas harinas y una dieta comercial como grupo control. Después de 45 días bajo condiciones experimentales de laboratorio, no se encontraron diferencias significativas ($P > 0.05$) en el número de crías producidas por hembra y en la longitud estándar individual de las crías entre los tratamientos. Las crías producidas por el grupo control fueron más pesadas que las del resto de los tratamientos (4.14 y 1.06 mg de peso húmedo y seco, respectivamente). En promedio, el grupo de reproductores alimentado con *Spirulina* produjo mayor cantidad de machos. Los resultados destacan el uso de la dieta comercial para cubrir con los requerimientos reproductivos de *P. reticulata*.

Palabras clave

Reproducción, *Poecilia reticulata*, *Artemia franciscana*, dietas vivas, dietas inertes.

Introduction

Since cultured fish and shellfish are continuously exposed to stress by stocking, handling, upgrading, feeding and harvesting, reproduction in captivity is commonly hindered and must be induced. There are several methods of management used to achieve the reproductive phase in aquatic organisms. Among them, the manipulation of the broodstock nutritional requirement plays a very important role for seed (gametes and fry) production [Bromage, 1995]. Varying egg and larval quality is one of the main factors interfering with the reliable performance of aquatic organisms. As larval performance during the first period largely depends on the availability of essential nutrients, the endogenous provision of these nutrients through the egg stages, and possibly parental diet, might be an important tool in improving reproductive performance [Lavens *et al*., 1999]. Reproductive responses can vary between species and the nutritional element tested. For example, Dhert *et al*. [1995] demonstrated, that an extra dosage of ascorbic acid in the turbot *Scophthalmus maximus* (Linnaeus) broodstock diet did not show differences in the number of produced eggs compared with a control diet. For the white grouper *Epinephelus aeneus*, [Geoffroy Saint Hilaire, 1809] Hassin *et al*. [1997] evaluated a broodstock diet including dry pellets with 40% protein content, and frozen fish or squid to induce reproduction, but spawning was only observed when a hormonal injection was also administered. Berntsson *et al*. [1997] found that the content of the essential fatty acid 22:6 n-3 (docosahexaenoic acid) from broodstock groups of the European flat oyster, *Ostrea edulis*, fed with different live algae diets, explained 50% of the variation in growth rate among broods.

In the case of freshwater ornamental fish culture, there is little information on the nutritional requirements to cover reproductive needs. Since common food source for ornamental species are live organisms such as *Artemia* and *Daphnia* [Godin and Dugatkin, 1996], its reproductive performance could vary depending on the culture conditions as the type of food. It is demonstrated that the energy and nutritional contents of the zooplankton strongly depend on the biochemical composition of the diet they receive [Sorgeloos and Léger, 1992]. Morimoto [1994] mentions that for most of the aquatic organisms, the nutritional quality of diets given to broodstock significantly affects biochemical composition of the eggs, total number of eggs spawned, percentage of eggs hatched among other factors. Based on the afore-mentioned findings, the reproductive potentials, particularly fecundity and quality of the fry, of the guppy fish are presumible variable, depending on several factors such as the male body coloration and the nutritional content of the diet. Therefore, the present work assesses some reproductive parameteres of the guppy fish *Poecilia reticulata* [Peters, 1859] fed with live biomass of brine shrimp cultured with live and inert diets to study the influence of these diets on its reproduction.

Material and methods

Experimental set-up

The initial stock of guppy fish were obtained from the Centro Acuícola Jala (Colima, México). After acclimation to the laboratory conditions (temperature 27°C, photoperiod 12:12), animals were disinfected [Robertson *et al*., 1993] and stocked in 2 liter plastic containers, where oxygen concentration was kept above 3 mg/l by air diffusers. The experimental stocking density was adjusted to four fish per container at a sexual ratio of one male and three females without no emphasis in the male body coloration pattern. Each experimental

container was filled up to a culture volume of 5 liters with municipal freshwater. Daily, faeces and other particles were extracted out from the bottom of each container by siphoning and 50% of water volume was changed every two days. The experiment lasted for 45 days.

Dietary treatments

Fish were fed with live brine shrimp cultured with inert and live diets, following the culture methodology described by Lavens and Sorgeloos [1996]. *A. fransicana* was cultured at low density (< 500/l) in 100 liters cylindrical tanks with conical bottom, and fed with the tested diets during the experiment. The inert diets were soy meal (SM), wheat meal (WM), a 50% mixture of each meal (50/50), and dried powder of *Spirulina* (SPIR). As live diets, the microalgae *Tetraselmis suecica* [Butch], (TETRA), and *Chaetoceros calcitrans* [Paulsen], (CHAET), were used. Dietary densities for *A. franciscana* were adjusted according to the water turbidity criteria [Bossuyt and Sorgeloos, 1980] The different *A franciscana* dietary groups were biochemically analyzed [A.O.A.C., 1990]. Fish were fed at satiation with the brine shrimp once daily. A control group of fish was fed with a commecial peletized diet containing 30% protein. The 7 dietary treatments consisted each of three replicates and were assigned randomly to the 21 experimental containers.

Evaluation

Once females displayed the morphological characteristics for delivering (big and darkness belly, Fernando and Phang, 1994), they were individually isolated in a 6 liter aquarium provided with a plastic sieve (0.5 cm opening) to allow newborn fish escapement of the maternal attack. After completing delivery, the female was introduced back to its broodstock container and fry were evaluated for fish number, fish size (mm), individual fish dry weight (mg) and wet weight (mg). For the fish dry weight, a sample of 10 organisms from each bearing was taken. Randomly, a sample of 15 animals from each spawning was kept with the original broodstock diet and analyzed for sex ratio after 30 days of delivering.

Statistical analysis

Values for each evaluated parameter represent treatment means (± s.d.) and statistical significance of differences in means was determined using one way analysis of variance [Reyes, 1982]. Tukey's multiple range test was applied to detect significant differences among means ($P < 0.05$). For statistical analysis of the results, the Statgraphics 4.0 computer package was used.

Results and discussion

The use of *Artemia* biomass as sole live food provides energetic and nutritional profits for the predator organism [Sorgeloos *et al*., 1986] and is a very common practice in ornamental aquaculture. However, there is little information about the nutritional requirements for reproduction in freshwater fish species.

Table 1. Proximal content (% dry weight basis) of *A. franciscana* cultured with different diets.

	DIETARY TREATMENTS					
	Tetra*	Chaet	Spir	50/50	SM	WM
Protein	69	58	63	48	52	49
Lipids	1.1	1.3	1.0	3.0	0.6	1.0
Carbohyd.	17.7	31.0	21.0	36.0	35.0	35.0
Ash	11.8	10.0	15.0	12.5	12.5	15.4

* TETRA = Tetraselmis suecica, CHAET = Chaetoceros calcitrans, SPIR = *Spirulina* dry powder, 50/50 = 50% soy meal + 50% wheat meal, SM = soy meal, WM = wheat meal.
Proximal content of the control diet = protein 30%, lipids 3.0%, carbohydrate 6.0, ash 12.0%, humidity 12%, F.N.E. = 37%.

Table 1 shows the proximal content of *A. franciscana* cultured with different diets. The high protein levels (> 48%) in all *Artemia* groups coincide with the brine shrimp protein concentration reported by Correa *et al*. [1994], Millikin *et al*. [1980], Gallagher and Brown [1975] and Good *et al*. [1982], but differs to those obtained by Arriaga and Re Araujo [1997] and Cervantes [1996] who obtained protein values below 30%. In the case of the carbohydrates, Léger *et al*. [1986] reported levels from 9 to 17% in adult brine shrimp, nevertheless, the concentrations obtained in this experiment were higher. For lipids, lower concentrations were found in *Artemia* cultured with the different diets (0.6 to 1.3%) compared with those reported by the aforementioned authors. Differences in *Artemia* biochemical content might be explained by technical factors such as the culture management and animal density, among others. Sorgeloos *et al*. [1986] mentioned that adult brine shrimp protein content varied depending on the provided diet.

Mean results, of the reproductive parameters evaluated for *P. Reticulata*, are summarized in Table 2.

Table 2. Number of bearings, individual and total brood production per treatment, standard length (mm), wet and dry weight (mg), and sexual ratio of *P. reticulata* brood fed with *A. franciscana* biomass produced with different diets.

Reproductive parameters	Dietary Treatments						
	TETRA ^	CHAET	SPIR	50/50	SM	WM	CONTROL
Group of broods	3	4	3	4	2	3	3
Offspring born per female	33 (9)[a*]	34 (14)[a]	51 (12)[a]	36 (9)[a]	57 (13)[a]	51 (11)[a]	32 (10)[a]
Newborn standard length (mm)	6.42 (0.1)[a*]	6.14 (0.1)[a]	6.34 (0.1)[a]	6.39 (0.1)[a]	6.27 (0.1)[a]	6.38 (0.1)[a]	6.49 (0.1)[a]
Newborn wet weight (mg)	3.64 (0.5)[b]	2.09 (0.7)[a]	3.91 (0.5)[b]	3.92 (0.5)[b]	2.73 (0.7)[a]	3.09 (0.9)[ab]	4.14 (0.7)[b]
Newborn dry weight (mg)	0.88 (0.01)[b]	0.62 (0.02)[a]	0.95 (0.07)[b]	0.87 (0.03)[b]	0.69 (0.07)[a]	0.78 (0.03)[ab]	1.06 (0.05)[b]
Brood sexual ratio (female/ male)	3.36	1.57	0.86	1.12	2.05	1.53	0.93

* Values (± standard deviation) with the same superscript in a line are not significantly different ($P < 0.05$).
^ TETRA = Tetraselmis suecica,
CHAET = Chaetoceros calcitrans,
SPIR = *Spirulina* dry powder,
50/50 = 50% soy meal + 50% wheat meal,
SM = soy meal,
WM = wheat meal,
CONTROL = commercial diet.

The fish group fed with *Artemia* cultured with soy meal displayed only two brood delivery events in 45 days. On the other hand, the CHAE and 50/50 treatments presented 4 newborn fish groups. Godin and Dugatkin [1996] pointed out that under optimal conditions, guppy fish broods are produced every 27-30 days per female. Differences with the present work could be due to the different diets used. Especifically for guppy fish, Long and Houde [1989] mentioned that the body pigmentation pattern is a basis for female mate choice. Although male coloration was not the same among the treatments in the present work, this parameter did not apparently affect the reproductive results. No significant differences in number of baby fish per parturition nor mean total length of the newborn fish were detected among the groups, which coincides with the data given by Kavumpurath and Pandian [1993a, b]. For the fish weight, the animals fed with *Artemia* cultured with the control diet were heavier compared to the rest of the dietary treatments. No clear differences in fry sex ratio were detected among the groups (Table 2), which is in analogy with the results obtained by Kavumpurath and Pandian [1993a] for *P. reticulata*.

Finally, Fah and Leng [1986] reported a diatery protein requirement for growth of *P. reticulata* of 30%. Protein inclusion in the diet above that level did not increase the number of fry. In this experiment, bromatological analysis of *Artemia* cultured with the different experimental diets showed protein concentrations higher than 30%, that is, all tested diets for guppy fish (including the control diet) contained at least, the protein concentration recommended by Fah and Leng [1986]. Considering that there were no differences for most of the reproductive parameters evaluated, the biochemical content of the experimental diets covered the reproductive needs of *P. reticulata*. According to Trexler *et al.* [1990] the obtained results might suggest the influence of combined factors (genetic, nutritional, technical) on the reproductive observations, rather than the evaluated *Artemia* diets only. Although, live food is widely used to feed most of the ornamental fish species since it apparently contains most of the nutritional requirements, an artificial diet containing 30% of crude protein seems to be sufficiently energetic to induce reproduction in *P. reticulata*.

Conclusions

Since there were no significant differences for the most of reproductive parameters evaluated for the guppy fish, and due to the fact that reproduction cycle (gonad maturation, sexual behavior, fertilization and presence of baby fish) was completed for all tested diets, it is suggested that all dietary treatments covered the feed needs for reproduction of *P. reticulata*. To know its optimal dietary requirements for reproduction, it is recommended the use of diets with energetic content below to those studied in this work.

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