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EFFECTS OF MEAL FREQUENCY ON GROWTH AND REPRODUCTION IN THE ORNAMENTAL RED SWORDTAIL, *XIPHOPHORUS HELLERI*

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Key words: fertility, feeding frequency, growth, red swordtail

Abstract

Experiments were performed to investigate the influence of meal frequency on growth, gonad weight and fertility in an ornamental fish, the red swordtail *Xiphophorus helleri.* Five groups of uniform-sized 30-day juveniles (0.04±0.01 g, 13.10±0.61 mm) were fed either one meal in 3 days (1/3), one meal in 2 days (1/2), one meal a day (1/1), two meals a day (2/1) or three meals a day (3/1) for 210 days. Group 3/1 had the highest mean body weight throughout the experiment, followed successively by groups 2/1, 1/1, 1/2 and 1/3. The growth rate was higher in all groups before breeding began and drastically declined afterwards; the significant reduction might have been due to the diversion of assimilated food energy to fry production. The gonad weight and gonadosomatic index (GSI) increased with the increase in meal frequency and as the rearing period progressed. Fish in groups 1/1, 2/1 and 3/1 began to develop gonads on day 42 and reached the breeding stage on day 70; fish in 1/2 and 1/3 began to develop gonads on days 70 and 98 and reached the breeding stage on days 98 and 112, respectively. The increase in meal frequency significantly reduced the interbrood interval and fertility. Fish in group 3/1 produced the highest number of fry (704). Those in groups 2/1, 1/1, 1/2 and 1/3 released 450, 372, 70 and 8 fry, respectively. Groups 3/1, 2/1, 1/1, 1/2 and 1/3 bred four, three, three, two and one times, respectively. Based on the present study, three meals a day is optimum for *X. helleri* growth and reproduction in a culture system.

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Introduction

Xiphophorus is one of the important ornamental fish. *Xiphophorus* means 'sword-bearer'. The swordtail, *X. helleri* (Adams, 1962), was named in honor of the collector, Carl Heller. In 1962, in Florida, O. Adams developed the first red lyre form of *X. helleri*. Nowadays, lyre swordtails are bred in a range of colors. *X. helleri*, one of the successful live-bearers (ovo-viviparous), was brought to Europe from southern Mexico and Guatemala where it occurs on the Atlantic slopes. The wild form is green but there are many different shapes and colors. Swordtail prefers neutral or slightly alkaline fresh water at a temperature of 20-24°C. It is omnivorous in nature and feeds on live foods and artificial diets as well as leaves and fruits that fall into the water. The male grows to a maximum length of 10 cm and the female to a maximum of 12 cm.

The swordtail has a short maturation period of 3-4 months. The male develops a long spike at the bottom of the caudal fin. The anal fin also changes and develops into the gonopodium which is the intromittent organ by which the male fertilizes the eggs within the female. When a reproductive female does not have a fertile male during maturation, the ripe ovary degenerates and the female grows a sword, becoming a fully functional male within 1-2 months. No reverse transformation has been known to occur. Before parturition, the mature female can be recognized by the increased size of its belly and a conspicuous, dark pregnancy spot on its ventral side. A healthy female can release as many as 150 young in a single breeding (James, 1998). The young feed on planktonic organisms and accept artificial feeds. The interval between each breeding cycle is 4-5 weeks (Sweeney, 1992; Stratton, 1993; James, 1998).

Feed is the most potent exogenous factor that affects growth and other physiological mechanisms in organisms. Feed quantity considerably affects fish reproduction (Tyler and Dunn, 1976; Townshend and Wooton, 1984). Supplementary feeding is typically practiced in aquaculture to enhance growth of organisms to marketable size within a short period. Whereas limited feeding negatively affects survival, food intake and growth, excess feeding not only pollutes the environment by leaching nutrients but also increases production costs (Sampath, 1984). Identification of the optimal meal frequency for ornamental fish species would help reduce feed wastage and increase growth, gonad development and reproductive performance.

Many authors have studied the effect of meal frequency on food intake, growth and conversion efficiency in edible fishes (Marian et al., 1982; Jobling, 1983; Sampath, 1984; Sampath and Ravindran, 1988) but little attention has been paid to the growth and nutrition of tropical oviparous fishes (Shim et al., 1989; Degani and Gur, 1992; Degani, 1993; Degani and Yehuda, 1996). To our knowledge, no study has yet been published on the effects of nutrition on reproductive performance or fertility in ovo-viviparous tropical fishes. Studies combining growth, gonad development and reproduction are particularly lacking in ornamental fish. Hence, the present work was undertaken to study the effects of meal frequency on growth, gonad development and fertility in the ornamental red swordtail, *X. helleri*.

Materials and Methods

Fish and maintenance. Three hundred and seventy-five 30-day-old juvenile *X. helleri* (0.04±0.01 g; 13.10±0.61 mm) were collected from two laboratory brooders obtained from the same parents. They were sorted into fifteen groups of 25. Three groups received three meals a day (3/1), three received two meals a day (2/1) and three, each, received one meal a day (1/1), one meal in two days (1/2) or one meal in three days (1/3). The treatments and feeding schedules are given in Table 1. Each group was reared in a 110-l circular cement cistern (1.75' x 1.50') containing 90 l fresh water.

Fish were fed minced pieces of fresh beef liver *ad libitum* in a feeding tray for two hours, after which unconsumed feed was removed and dried in a hot air oven at 80°C. The contents of protein, fat, ash and nitrogen free

Table 1. Frequency of meals and feeding schedules.

Meal frequency (no. meals/no. days)	*Feeding schedule (h)*
One meal in three days (1/3)	08:00
One meal in two days (1/2)	08:00
One meal in one day (1/1)	08:00
Two meals in one day (2/1)	06:00, 18:00
Three meals in one day (3/1)	06:00, 12:00, 18:00

extracts of the beef liver were 35.44, 3.86, 13.91 and 46.79%, respectively (James and Sampath, 2002). Feed consumption (mg) was estimated by subtracting the amount of unconsumed dry feed from the total dry weight of the feed offered. The feeding rate (mg/g live fish/day) was computed as: the amount of feed consumed (in mg)/(the initial wet weight of the fish in g x the number of days).

The water was clean, unchlorinated well water and its quality was monitored biweekly. Temperature was 28±1°C, pH 7.8±0.05, salinity 0.13±0.01‰ and DO 4.04 ppm. The tanks were drained twice a week and replenished with fresh water to remove accumulated feces at the bottom.

Before beginning the experiment, the total wet weight of the fish in each aquarium was weighed. Five fish from the stock were sacrificed to estimate their water content (Maynard and Loosli, 1962); the initial dry weight of the fish was determined using this estimation.

Growth and gonad estimations. All animals in each aquarium were collected every 14 days and the wet weight was taken. The wet weight was converted to dry weight using the percent water content of the fish sacrificed before beginning the experiment. Growth was calculated as the difference between the dry weight at the beginning of the experiment and that on the day of calculation. The growth rate (mg/g live fish/day) was calculated as: growth (in mg)/(the initial wet weight of the fish in g x the number of days since the last measurement). The mean body weight (g) was calculated by dividing the total wet fish weight in the aquarium by the number of fish in the aquarium.

Every 14 days, three fish were chosen (one from each of the three replicates receiving the same treatment) and their mean body weight was measured and recorded on a graph. Once the gonad began to develop and until breeding began, two females from each treatment were sacrificed at 14 day intervals. Their ovaries were removed and weighed and the gonadosomatic index (GSI) was computed according to the formula of Dahlgren (1979): GSI (in %) = wet weight of gonad (in mg)/wet weight of fish (mg) x 100. The maturity of the gonad was determined according to the development of the ova inside. Meffe (1985) identified six stages that indicate the readiness of the animal for breeding.

Fish, feed samples, unconsumed feed and the ovary were weighed in an electric monopan balance to an accuracy of 0.1 mg.

Breeding. Two females were randomly chosen from each replicate and reared with a male in a separate tank containing a sufficient quantity of macrophytes of the *Hydrilla* species, until the completion of the experiment. The remaining test animals were removed from the experimental tanks and sacrificed. When the breeding females released their young, the young were isolated from their parents and counted. Fertility was measured as the number of intrafollicular embryos produced by a female (Dahlgren, 1979). The time between two breeding cycles is known as the interbrood interval (IBI; Snelson et al., 1986). The experiment continued until 210 days to allow sufficient time to elapse for a second breeding to occur.

Statistical analysis. The Students *t* test was applied to determine the significance of differences between mean values in different groups. Two-way ANOVA was applied to find the significant effects of meal frequency and rearing period on the feeding and growth rates. One-way ANOVA was applied to test the significance of meal frequency on the production of young (Zar, 1974).

Results

Meal frequency influenced the growth, food intake, ovarian weight and fertility in *X. helleri*. The mean body weight increased as the feeding frequency increased (Fig.1). Animals subjected to three meals per day had the highest mean body weight throughout the experimental period, followed by groups 2/1, 1/1, 1/2 and 1/3, in that order.

The feeding (Table 2) and growth (Table 3) rates increased significantly (two-way ANOVA) as the frequency of meals increased ($p<0.01$) but decreased with time ($p<0.05$). The feeding and growth rates were five times higher in the 3/1 group than in the 1/3 group up to 28 days, however, the difference gradually declined as the experiment progressed. Growth was higher in all the groups before commencement of breeding than after, however, it dramatically rose before the release of young and dropped thereafter. For instance, in group 2/1, the growth rate before each parturition was 4.07, 4.96 and 2.01 mg/g live fish/day and it dropped to 1.26, 0.94 and -0.85 mg/g live fish/day after parturition. Similar trends were observed in the other treatments.

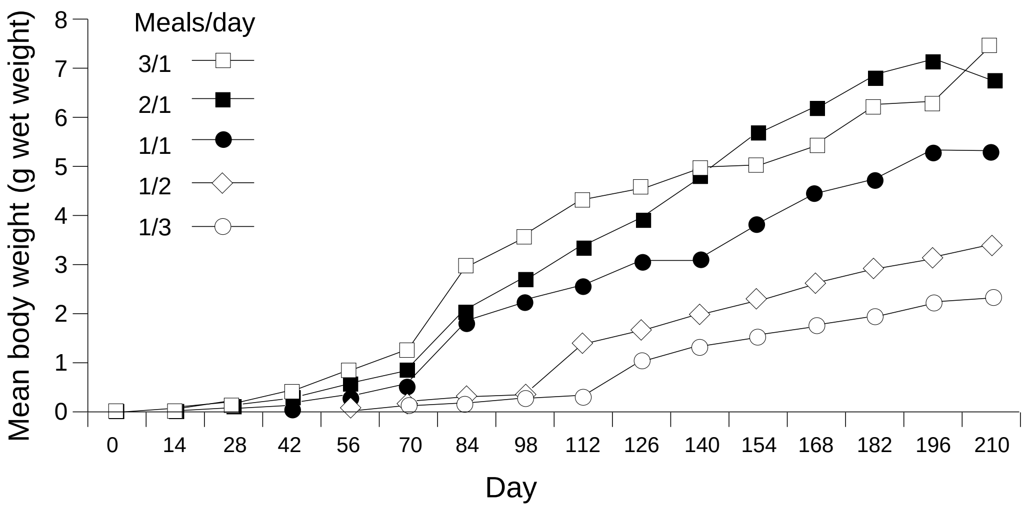

Fig. 1. Effect of meal frequency on mean body weight in red swordtail *Xiphophorus helleri*.

Table 2. Feeding rate (mg/g live fish/day) in red swordtail, *Xiphophorus helleri.* Each value is the mean (±SD) of three replicates.

Day	*Meal frequency (no. meals/no. days)*				
	1/3	*1/2*	*1/1*	*2/1*	*3/1*
14	30.38±3.2	39.95±2.3	88.28±4.8	133.05±9.4	145.48±7.8
28	20.29±2.3	37.29±2.5	86.33±5.1	126.83±7.6	109.47±6.5
42	34.08±2.8	42.15±3.1	85.44±4.8	78.18±4.5	77.87±5.6
56	40.79±3.6	47.91±3.3	59.60±4.3	58.45±3.3	49.01±2.8
70	31.44±3.0	30.79±2.0	37.70±2.8*	38.43±2.1*	35.67±2.4*
84	18.52±1.6	24.37±1.5	26.56±1.7	36.12±1.5	26.24±1.8
98	20.08±1.7	26.00±1.5*	15.23±0.9	26.72±1.5	21.71±1.4
112	25.66±1.9*	29.54±1.8	23.54±1.3	32.88±2.2	25.22±2.0
126	18.36±1.3	20.84±2.2	18.29±1.1	28.67±2.4	21.80±1.6
140	23.45±2.2	16.62±1.9	18.22±1.4	24.55±1.5	29.22±1.9
154	19.83±2.0	19.12±2.0	19.44±1.0	28.61±1.7	24.49±1.3
168	25.20±1.8	20.64±2.2	16.95±1.2	25.86±1.5	28.71±1.7
182	18.02±1.1	18.93±1.5	19.45±1.4	29.48±1.8	29.59±2.2
196	21.41±1.0	18.09±1.7	21.60±1.8	24.32±2.1	22.91±2.3
210	20.17±2.1	15.94±1.6	19.35±1.3	26.32±2.4	21.84±1.8

* Breeding commenced
Two-way ANOVA:
Meal frequency vs feeding rate: F.0.01 - Df 4,24 = 5.91 ($p<0.01$)
Rearing period vs feeding rate: F.0.05 - Df 4,24 = 3.85 ($p<0.05$)

The gonad weight and gonadosomatic index increased with the increase in meal frequency and progression of time (Table 4). Fish in groups 1/1, 2/1 and 3/1 began to develop gonads on day 42 and reached the breeding stage on day 70 while fish in groups 1/2 and 1/3 began to develop gonad on days 70 and 98 and attained the breeding stage on days 98 and 112, respectively.

With the increase in meal frequency, the reproductive performance of *X. helleri* increased significantly (one-way ANOVA, $p<0.01$) and animals released more young and earlier (Table 5). In the first breeding, fish in groups 2/1 and 3/1 did not significantly differ (t value = 1.85; $p>0.05$), however, they differed in subsequent breedings. Animals in group 1/2 had a longer interbrood interval (45

Table 3. Growth rate (mg/g live fish/day) in red swordtail, *Xiphophorus helleri*. Each value is the mean (±SD) of three replicates.

Day	*Meal frequency (no. meals/no. days)*				
	1/3	*1/2*	*1/1*	*2/1*	*3/1*
14	9.62±1.2	12.10±0.7	19.02±1.2	25.10±1.8	36.67±2.3
28	8.75±1.0	10.42±0.5	33.40±2.7	59.87±4.2	56.07±3.5
42	9.52±0.8	10.57±1.1	20.79±1.2	32.35±2.5	29.11±1.8
56	10.43±0.8	16.94±1.4	24.93±1.4	22.41±1.8	19.42±0.7
70	11.19±1.2	7.91±0.6	14.98±1.1*	9.28±0.5*	11.76±0.5*
84	5.54±0.4	12.90±1.2	7.43±0.6	5.41±0.3	7.64±0.6
98	6.13±0.7	5.07±0.4*	4.89±0.2	6.89±0.3	4.56±0.4
112	7.86±0.5*	6.26±0.7	3.77±0.3	5.78±0.5	5.04±0.6
126	4.18±0.3	4.28±0.6	4.19±0.3	4.07±0.2	1.28±0.3
140	6.29±0.7	4.57±0.3	0.07±0.0	1.26±0.2	2.21±0.1
154	3.10±0.2	3.53±0.2	5.79±0.4	4.96±0.3	0.26±0.0
168	3.97±0.4	3.15±0.1	4.06±0.3	0.94±0.0	1.83±0.0
182	2.23±0.3	2.46±0.1	1.41±0.0	3.00±0.2	2.33±0.1
196	3.26±0.4	2.32±0.2	2.93±0.1	2.01±0.0	1.31±0.0
210	1.16±0.0	1.98±0.0	0.03±0.0	- 0.85±0.0	-0.92 ±0.0

* Breeding commenced
Two-way ANOVA:
Meal frequency vs growth rate: F.0.01 - Df 4,24 = 5.15 (p<0.01)
Rearing period vs growth rate: F.0.05 - Df 4,24 = 3.57 (p<0.05)

days) than animals in groups 3/1 and 2/1 (average 31.5 days). Fish reared in group 3/1 bred more often (four times) while fish reared in groups 2/1, 1/1, 1/2 and 1/3 bred three, three, two and one times, respectively, during the 210-day experiment.

Discussion

The present study showed that meal frequency significantly influenced growth, ovarian weight and reproductive performance in *X. helleri*. The mean body weight increased with the increase in meal frequency and the rearing period. Hislop et al. (1978) reported that *M. anglefinus* fed a low ration exhibited poor growth in terms of body length and weight. An increase in mean body length and weight was observed in juveniles of the dwarf gourami *Colisa lalia* as protein levels and the rearing period increased (Shim et al., 1989). Food

Table 4. Wet gonad weight and gonadosomatic index (GSI) in red swordtail, *Xiphophorus helleri*. Each value is the mean (±SD) of six observations.

	Meal frequency (no. meals/no. days)									
	1/3		*1/2*		*1/1*		*2/1*		*3/1*	
Day	*Gonad weight (mg)*	*GSI (%)*	*Gonad weight (mg)*	*GSI (%)*	*Gonad weight (mg)*	*GSI (%)*	*Gonad weight (mg)*	*GSI (%)*	*Gonad weight (mg)*	*GSI (%)*
42	-	-	-	-	1.00±0.01	0.31±0.01	2.50±0.18	0.63±0.00	3.50±0.13	0.84±0.01
56	-	-	-	-	3.30±0.14	0.50±0.01	14.00±0.34	1.46±0.13	22.50±0.52	2.40±0.11
70	-	-	1.00±0.00	0.20±0.00	16.00±0.78*	1.56±0.07*	69.00±4.43*	4.30±0.28*	196±9.52*	9.69±0.47*
84	-	-	8.50±0.45	1.07±0.01						
98	4.00±0.24	0.68±0.03	50.00±5.02*	4.53±0.26*						
112	43.00±3.34*	5.76±0.56*								

* Breeding began

Table 5. Reproductive performance in red swordtail *Xiphophorus helleri*. Each value is the mean (±SD) of six observations.

	Meal frequency (no. meals/no. days)									
	1/3		*1/2*		*1/1*		*2/1*		*3/1*	
	Day young released	*No. young*	*Day young released*	*No. young*	*Day young released*	*No. young*	*Day young released*	*No. young*	*Day young released*	*No. young*
First breeding	167±15	8±2	151±14	28±4	136±5	72±6	133±6	107±8	124±5	131±5
Interval (days)	-	-	45	-	32	-	34	-	29	-
Second breeding	-	-	196±17	42±5	168±8	123±8	167±4	150±10	153±10	183±11
Interval (days)	-	-	-	-	29	-	31	-	30	-
Third breeding	-	-	-	-	197±10	177±12	198±5	193±14	183±5	189±15
Interval (days)	-	-	-	-	-	-	-	-	25	-
Fourth breeding	-	-	-	-	-	-	-	-	208±4	204±13
Total no. breedings/ young	1	8±0	2	70±4.6	3	372±9.0	3	450±10.7	4	707±14.8

intake and growth were higher in the early rearing period and gradually decreased as the juveniles grew. An increased metabolic rate was perhaps responsible for the higher food intake during the early period. Faster gastric evacuation can help fish digest more food per unit time and greater stomach volume can help ingest relatively larger quantities of food (Elliott, 1975). Haines (1973) showed that the RNA:DNA ratio in fish decreased with the increase in fish size, suggesting a decline in the rate of protein synthesis.

The slower growth rate in *X. helleri* prior to and during breeding could be due to diversion of more assimilated energy to ovary development and fry production. Cessation of growth, in association with gonad development, has been observed in salmonids (Hardy, 1983; Washburn et al., 1990) and it has been suggested that this could be the result of hormonal changes occurring during the late phase of gametogenesis. Rathinam (1993) reports a loss of body weight during parturition in the live-bearers, *M. latipinna* and *X. helleri*. *X. helleri* is ovo-viviparous. It bears young developed from eggs retained in the mother's body, but without any close tissue connections between the two for the supply of extra nourishment to the embryo. The embryo is nourished from food stored in the eggs. During development, the uptake of organic substances and intake of water molecules from the maternal body (Balinsky, 1970) could result in considerable loss of energy in the female *X. helleri*. Townshend and Wooton (1984) found that females channel a higher proportion of ingested food energy to gonad development than males, so that the female reproductive output is more sensitive to variations in food supply. Food energy obtained from less frequent feedings (groups 1/3 and 1/2) was apparently insufficient for fish to fully satisfy their requirements for both somatic growth and reproduction. This was evidenced by fewer breedings and a lower number of fry released per breeding.

The present investigation reveals that fish fed more frequently had a higher ovary weight and GSI than fish fed less frequently. Shim et al. (1989) reported that female gourami fed high protein diets (35%) had a greater ovary weight and GSI than females fed low protein diets (5%). Townshend and Wooton (1984) reported that an increase in ration relatively increased the gonad weight in *Cichlasoma nigrofasciatum* and that females on a low ration had a negligible proportion of oocytes whereas females on high and medium rations had a distinctive proportion of oocytes. It is likely that the high (groups 2/1 and 3/1) and medium (group 1/1) rations positively influenced oocyte production and gonad weight, confirmed by the ovarian weight and GSI.

Food availability is an important factor affecting reproduction in fish (Wooton, 1982). Our results show that reducing the food supply increased the interbrood interval and reduced the frequency of breeding and fry production in *X. helleri*. The interbrood interval of live-bearers is determined by the age and condition of the female, availability of food, lipid stores and degree of energetic stress suffered during previous reproductive cycles (Wetherington, 1982). Snelson et al. (1986) found that the females of the sailfin molly, *Poecilia latipinna*, fed low food rations had longer interbrood intervals, supporting the present investigation. They suggested that a shortage of food made the female brooder sacrifice a substantial amount of somatic tissue to load the yolks, causing the longer interbrood interval. It is likely that *X. helleri* fed low rations had poor lipid stores and required more days to load the yolks, leading to a longer interbrood interval (45 days) and reduced frequency of breeding and fry production, since the opposite trend was obtained in fish fed more frequently. The average interbrood interval and fertility of wild swordtail fish is 30-35 days and 60 fry per breeding (Zaidi et al., 1997). The present study reveals that the number of fry produced per breeding increased with the increase in meal frequency, suggesting that *X. helleri* prefers frequent feedings (three meals a day) and responds well in terms of growth, gonad development and reproductive performance.

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