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EFFECT OF FEED TYPE ON GROWTH AND FERTILITY IN ORNAMENTAL FISH, *XIPHOPHORUS HELLERI*

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Abstract

The effects of feed type on feed intake and conversion, ovary weight, and fertility were studied in red swordtail, *Xiphophorus helleri*, for 235 days. Five groups of 30-day-old juveniles (0.05 ± 0.001 g; 16.84 ± 1.59 mm) were fed *Artemia*, earthworms, liver, pelleted feed, or a mixed diet. Mean body length and weight were higher in fish that consumed the mixed diet or *Artemia* than in those that consumed the other diets. Pelleted feed produced the highest feeding rate but the poorest conversion rate. Fish fed pelleted feed consumed 143 mg/g/day, which was converted into 13 mg/g/day flesh. Those fed the *Artemia*, earthworm, liver, or mixed diets consumed 46, 56, 89, and 84 mg/g/day, respectively, which were converted into 16, 18, 21, and 24 mg/g/day, respectively. The conversion rate dropped after parturition, possibly due to allocation of a major portion of the feed energy to development of gonads and young. Irrespective of feed type, gonads appeared on day 42. On day 84, the gonad weight of fish fed earthworms was 16 mg (wet weight), significantly ($p < 0.01$) increasing to 25 mg in fish fed *Artemia*, 31 mg in fish fed liver, 86 mg for pellets, and 145 mg for the mixed diet. A similar trend was noted in the gonadosomatic index. Females fed the mixed diet, *Artemia*, and liver released 753, 612, and 509 fry in four breeding cycles while those fed earthworms and pellets bred only three times, releasing 315 and 155 fry, respectively.

Introduction

Growth, gonad development, and reproduction in fish are influenced by many factors. Feed composition, quality and quantity, and ration size are among the most important (Sampath and Pandian, 1984; James et al., 1993; Jobling, 1998). In aquaculture, production of

fish to market size within a short period is of utmost importance (Bulkley, 1972). Sometimes, there is a shortage of the live foods used in large-scale commercial culture and spawning of ornamental fishes. Dry feed formulations have been tried as substitutes for

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live food for edible and ornamental fishes (Khan and Jafri, 1994; Lochmann and Phillips, 1994; James and Sampath, 2002).

Female fish need adequate protein, fat, vitamins, and minerals for egg development and spawning/breeding. Yolk is composed of phospholipids, proteins, an amalgam of minerals (phosphorous), protein, and lipid. Protein is required for formation of follicles in the embryo. The absence of any of these nutrients can reduce larvae survival (James and Sampath, 2002).

Many authors have studied the effect of nutrition on growth of edible fishes (Degani et al., 1985; Wah Lam and Shephard, 1988; James et al., 1993; Khan and Jafri, 1994; Kim et al., 1996). There are reports on the influence of nutrition on growth or reproduction in ornamental fishes (Degani, 1991; Degani and Gur, 1992; Degani and Yehuda, 1996; James and Sampath, 2004) but, to the best of our knowledge, no reports have yet been published on the impact of live food, pelleted feeds, or a mixed diet on growth, gonad development, and reproductive performance in tropical fishes. The present study was undertaken to study the effects of feed quality on growth, gonad development, and fertility in the red swordtail fish, *Xiphophorus helleri*.

Materials and Methods

Fish and maintenance. Three hundred and seventy five 30-day-old juvenile *X. helleri* (0.05 ± 0.001 g; 16.84 ± 1.59 mm) were collected from laboratory bred brooders obtained from the same parents. They were sorted into five groups, corresponding to five types of feed. Each group comprised 25 individuals, reared in a circular cement tank (0.525 x 0.45 m) containing 90 l static water. Triplicates of each group were maintained.

The clean unchlorinated well water was monitored biweekly. Temperature averaged $28 \pm 1^\circ\text{C}$, pH 7.8 ± 0.05 , salinity $0.13 \pm 0.01\%$, hardness 316 ± 15 mg CaCO_3/l , ammonia 1.01 ± 0.12 mg/l, and DO 4.04 ppm. The tanks were drained twice a week and replenished with fresh water to remove accumulated feces from the bottom.

Feeding. *Artemia*, earthworm, beef liver, pelleted feed (45% protein), and mixed diet (each of the above feeds in equal proportions) were chosen as the experimental feeds. *Artemia* were collected every two days from the local salt pan and maintained in a cement tank containing sea water. Before feeding to fish, the *Artemia* were washed 3-5 times in fresh water to remove salt. Earthworms were collected from nearby agricultural land and left in tap water for 12-15 h to allow evacuation of the gut. Beef livers were procured from a beef stall in the local market and kept in the refrigerator. Earthworms and beef livers were minced and traces of water were removed by pressing the minced pieces between folds of filter paper. Pelleted feed was prepared following the square method (Hardy, 1980). Pelleted feed contained 45% animal protein since *X. helleri* fed this level had higher growth and fertility than those fed 35% protein (James, 1998).

Feed was weighed and given *ad libitum* in a feeding tray daily at 08:00 for two hours. Unconsumed feed was collected and dried in a hot air oven at 80°C . Food consumption was estimated by subtracting the weight of the unconsumed dry feed from the weight of the offered feed. The feeding rate was computed as the amount of feed consumed (mg)/initial wet weight of fish (g)/number of days. Fish, feed samples, and unconsumed feed were weighed in an electric monopan balance to an accuracy of 0.1 mg.

Growth calculations. Before beginning the experiment, the wet weight of each fish was weighed in an electric monopan balance. Five fish from the stock were sacrificed to calculate the water content (Maynard and Loosli, 1962) and determine the initial dry weight of the fish. The fish in each aquarium were collected and weighed every 14 days and the dry weight was calculated by using the percent water content of the fish sacrificed at the beginning of the experiment. Growth was calculated by subtracting the dry weight on the day of calculation from the initial dry weight. The growth rate was calculated as growth (in mg)/initial wet weight of fish (g)/number of days. Mean body weight was calculated by dividing the

total wet fish weight in the aquarium by the number of fish in the aquarium. The mean body length was measured using a clean graph sheet.

Gonad estimations. Gonad weight was measured from day 42 until the commencement of breeding on day 84. Two females from each treatment were sacrificed every 14 days (totally four times). The ovaries were removed and weighed in an electric monopan balance and the gonadosomatic index (GSI) was computed according to the formula of Dahlgren (1979): $GSI \text{ (in \%)} = \text{wet weight of gonad (mg)}/\text{wet weight of the fish (mg)} \times 100$. Gonad maturity was based on the development of the ova inside. Meffe (1985) identified six stages that indicate the readiness of the animal for breeding.

Breeding. Two female breeders were randomly chosen from each triplicate and reared with a male in a tank containing a sufficient quantity of the macrophyte, *Hydrilla* sp. The remaining test animals were removed from the experimental tanks. When the breeding females released their young, the young were isolated from the parents and counted. Fertility was measured as the number of intrafollicular embryos produced by a female (Dahlgren, 1979). The experiment was conducted for 235 days, until completion of at least three breeding in each feed group.

Feed analyses. The protein and lipid contents of the experimental diets were determined in a spectrophotometer following Lowry et al. (1951) and Bragdon (1951), respectively. Moisture content was analyzed by drying in an electric hot air oven at 100°C. Mineral content was estimated following the method of Paine (1964). Nitrogen free extract (NFE) was calculated by subtracting the protein, lipid, and mineral contents from the dry weight of the feed samples.

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

Results

Protein content was highest in *Artemia* while nitrogen free extract was highest in liver (Table 1). Fish fed the mixed and *Artemia* diets had the greatest growth (Fig 1). Slopes of the length and weight gains of fish fed the mixture (0.040 and 0.271) and *Artemia* (0.043 and 0.311) did not differ statistically (for length $t = 0.79$, $p > 0.05$; for weight $t = 1.65$, $p > 0.05$) but there were significant differences between fish fed the mixture and liver (for length $t = 14.73$, $p < 0.05$; for weight $t = 2.74$, $p < 0.05$)

Table 1. Proximate composition (%) of experimental diets.

Component	Feed type				
	<i>Artemia</i>	Earthworm	Liver	Pellet feed	Mixture
Crude protein	56.5	38.7	35.4	45.5	41.8
Crude fat	7.9	5.9	3.9	7.5	5.4
Ash	8.6	16.4	13.9	11.7	10.9
Nitrogen free extract (NFE)	27.0	39.0	46.8	35.3	41.9
Moisture	86.4	69.8	74.9	7.9	62.6

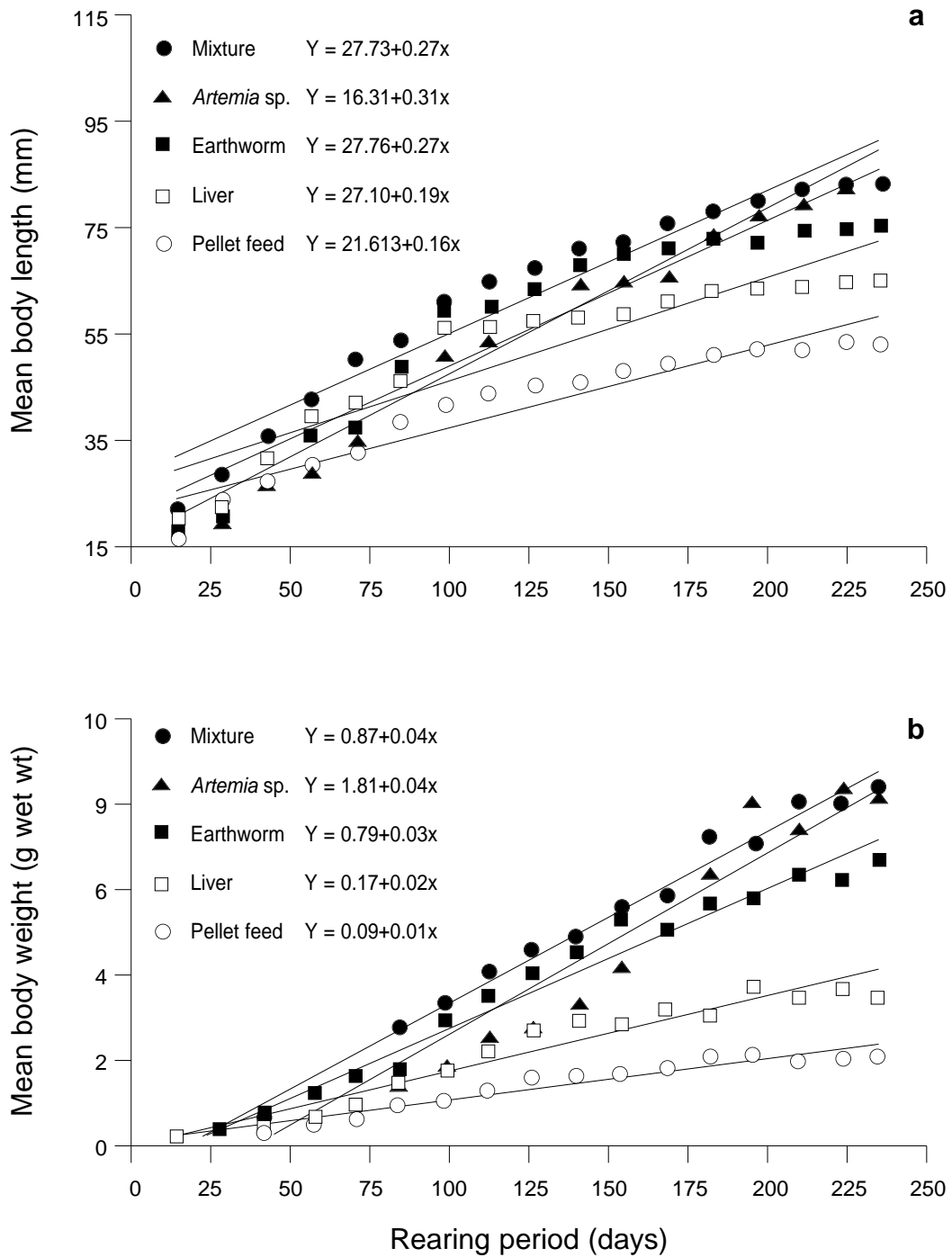


Fig. 1. Mean body length (a) and weight (b) in red swordtail, *Xiphophorus helleri*, fed different diets.

and between fish fed *Artemia* and liver (for length $t = 2.82$; $p < 0.05$; for weight $t = 4.68$; $p < 0.05$).

Fish fed the pelleted feed had the highest feeding rate but the lowest conversion rate (Table 2). Differences during the early rearing period (days 0–84) were highly significant (ANOVA $p < 0.01$), rates dropped in all feed groups after commencement of breeding on day 84, and dropped further after parturition on day 210.

Feed type significantly ($p < 0.05$) influenced gonad weight and gonadosomatic index (Table 3). Irrespective of feed type, the gonad appeared on day 42 and the weight gradually increased. There was a positive correlation between gonad weight and rearing period ($p < 0.05$).

Fish fed the mixture, *Artemia*, and liver diets completed four breeding cycles and produced more fry than the fish fed the earthworm and pellet diets, which bred only three times (Table 4). The number of fry increased with each breeding until the third breeding and declined in the fourth.

Discussion

The mixed diet promoted high food consumption and growth. Perhaps the variety in the diet stimulated appetite and the protein (42%) and nitrogen free extract (42%) components promoted growth. Nandeesh et al. (1994) reported that mixed schedules were superior to single high protein schedules because nitrogen retention was higher in the fish fed the mixed schedules. *Artemia* also produced high food consumption and growth, perhaps because its large size, movement, and high protein content (56.5%) stimulated the predatory responses of the fish. Adult *Artemia* provide more protein than *Artemia* nauplii and all the amino acids essential to fish (nauplii are deficient in histidine, methionine, phenylalanine, and threonine; Claus et al., 1979). Also, live *Artemia* contain enzymes (Bengtson et al., 1991) that help in initial digestion of juvenile fishes and apparently enhanced their appetite, feed intake, and growth. Likewise, coho salmon (*Oncorhynchus kisutch*) fry fed *Artemia* grew faster than those fed supplementary diets (Kim et al., 1996).

The poor growth rate observed in fish fed the pelleted feed suggests that pellets are not suitable to juvenile *X. helleri*. Degani (1991) found that juvenile *Trichogaster trichopterus* fed live feed grew faster than those fed formulated feed because of the palatability, high consumption, and chemical composition of the live feed. Although liver has a low protein content, feed consumption and growth were better than in fish fed earthworms and pellets. This may be due to the softness, palatability, and higher NFE content of liver, which provided energy for fish growth.

The growth rate dropped just before and during the breeding periods. The reproductive cycle of swordtail fish was short. Female *X. helleri* allocate a major portion of assimilated energy to gonad development and fry production and, therefore, only a small fraction of energy could be spared for growth during and just prior to breeding. *X. helleri* is an ovo-viviparous fish. The young developed from eggs retained in the mother's body, but with no close tissue connections to supply extra nourishment to the embryo. The embryo is nourished by nutrients stored in the eggs. During development, the absorption of organic substances and water molecules from the maternal body (Balinsky, 1970) can result in considerable energy loss in the mother. Rathinam (1993) observed a loss of body weight during parturition in the live-bearers *Mollinesia latipinna* and *X. helleri*. Townshend and Wootton (1984) found that females allocate a higher proportion of feed energy to gonad development than males. 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 is the result of hormonal changes during the later phase of gametogenesis.

The present study reveals that the composition and palatability of a diet significantly affect reproduction in *X. helleri*. Female *X. helleri* fed the mixed diet produced 753 fry and completed four breeding cycles within 207 days while those fed *Artemia* or liver completed four breeding cycles in 220 and 223 days, respectively, producing 612 and

Table 2. Effect of feeding different types of feed on feeding and conversion rates (mg/g live fish/day) in *Xiphophorus helleri*. Values are means±SD of three observations.

Day	Feeding rate				Conversion rate					
	Artemia	Earthworm	Liver	Pellet feed	Mixture	Artemia	Earthworm	Liver	Pellet feed	Mixture
84*	46.04±2.3	55.93±2.8	89.32±5.8	143.18±8.5	84.20±4.2	16.05±1.1	17.73±1.4	20.64±1.3	13.24±0.8	23.60±1.7
98	28.65±2.2	12.07±0.7	42.29±3.7	57.19±1.8	37.93±1.6	7.81±0.5	3.22±0.4	6.53±0.8	6.10±0.3	4.40±0.2
112	27.77±1.7	14.75±1.1	38.39±2.8	63.69±3.4	36.59±2.8	9.56±1.0	4.54±0.5	7.83±0.5	3.36±0.2	5.58±0.4
126	21.50±1.4	12.74±0.8	35.33±1.5	47.08±1.5	26.56±2.1	2.49±0.1	4.07±0.3	4.77±0.3	6.37±0.4	3.00±6.2
140	11.48±0.7	9.07±0.4	22.06±1.6	28.35±1.1	23.49±1.4	-3.05±0.0	2.91±0.3	2.33±0.2	0.25±0.0	1.77±0.1
154	12.40±0.9	5.02±0.6	14.96±0.8	25.89±2.1	21.93±1.3	-2.04±0.0	4.39±0.4	-0.86±0.0	1.25±0.1	3.62±0.4
168	19.74±1.4	6.33±0.3	27.02±2.1	24.51±1.2	23.95±1.5	8.85±0.4	-1.01±0.0	3.16±0.4	1.84±0.2	1.34±0.0
182	14.46±0.8	7.66±0.5	18.91±1.1	35.69±2.3	22.66±2.2	1.95±0.1	2.62±0.3	-0.93±0.0	3.64±0.2	5.12±0.6
196	9.45±0.3	7.77±0.4	19.15±1.6	27.81±1.9	26.55±1.8	6.54±0.3	0.13±0.0	5.91±0.6	0.14±0.0	-0.73±0.0
210	8.66±0.4	7.22±0.2	14.73±0.9	28.46±1.7	21.02±1.6	-2.06±0.0	2.53±0.1	-1.96±0.0	-0.97±0.0	3.46±0.4
224	11.37±1.0	6.29±0.7	14.02±1.0	19.96±0.9	18.98±1.5	5.88±0.4	-0.31±0.0	1.85±0.1	0.81±0.0	-0.33±0.0
235	9.12±0.3	8.06±0.8	17.24±1.4	27.62±2.4	19.55±1.4	-3.36±0.0	2.19±0.2	-2.33±0.0	-0.05±0.0	1.63±0.2

* Average feeding and conversion rates days 0–84, before commencement of breeding.

Two-way ANOVA:

Feed type vs feeding rate: F. 0.01 – Df 4, 29 = 10.68 ($p < 0.01$)Feed type vs growth rate: F. 0.05 – Df 4, 29 = 2.72 ($p > 0.05$)Rearing period vs growth rate: F. 0.01 – Df 4, 29 = 7.35 ($p < 0.01$)Rearing period vs feeding rate: F. 0.01 – Df 4, 29 = 11.50 ($p < 0.01$)

Table 3. Effect of feeding different types of feed on gonad weight (mg wet weight) and gonadosomatic index (%) in *Xiphophorus heli-*
leri. Values are means±SD of six observations.

Day	Artemia		Earthworm		Liver		Pellet feed		Mixture	
	Gonad wt	GSI	Gonad wt	GSI	Gonad wt	GSI	Gonad wt	GSI	Gonad wt	GSI
42	1.00±0.01	0.58±0.00	0.75±0.00	0.46±0.00	0.67±0.00	0.23±0.00	1.00±0.01	0.61±0.00	1.00±0.00	0.24±0.00
56	3.33±0.04	0.85±0.01	2.67±0.12	0.78±0.00	5.33±0.34	0.90±0.02	3.00±0.14	0.93±0.02	11.33±0.74	1.02±0.08
70	6.00±0.45	1.27±0.03	5.00±0.34	1.20±0.02	9.33±0.64	1.43±0.06	7.00±0.53	1.83±0.04	36.00±2.10	2.92±0.24
84	25.00±1.30	1.97±0.05	15.50±1.01	1.29±0.02	31.00±2.10	2.34±0.04	86.00±5.30	15.90±1.30	145.0±8.30	6.53±0.38

Significance of differences in gonad weight on day 84 by *t* test

between mixture and *Artemia*: $t = 32.00$, $p < 0.01$

between mixture and earthworm: $t = 34.85$, $p < 0.01$

between mixture and liver: $t = 29.83$, $p < 0.01$

between mixture and pellet feed: $t = 11.14$, $p < 0.01$

Significance of differences in GSI on day 84 by *t* test

between mixture and *Artemia*: $t = 26.54$, $p < 0.01$

between mixture and earthworm: $t = 30.73$, $p < 0.01$

between mixture and liver: $t = 24.47$, $p < 0.01$

between mixture and pellet feed: $t = 15.49$, $p > 0.01$

Table 4. Effect of feeding different types of feed on breeding and number of young released. Values are means±SD of six observations.

Breeding	Artemia		Earthworm		Liver		Pellet feed		Mixture	
	Day	No.	Day	No.	Day	No.	Day	No.	Day	No.
I	136±5	105±8	150±7	81±6	139±9	68±5	139±7	30±4	122±6	130±7
II	167±4	144±10	180±10	105±10	169±7	128±8	166±5	51±5	151±8	179±10
III	194±5	198±12	208±7	129±8	195±15	188±10	192±6	74±6	178±7	242±13
IV	220±5	165±15	-	-	223±4	125±7	-	-	207±5	202±13
Total		612±15.1		315±12.0		509±10.2		155±5.0		753±5.8

Significance of differences between feed type and no. of young released, by *t* test

between mixture and *Artemia*: $t = 19.53, p < 0.01$

between mixture and earthworm: $t = 73.61, p < 0.01$

between mixture and liver: $t = 46.56, p < 0.01$

between mixture and pellet feed: $t = 174.85, p < 0.01$

One-way ANOVA

Feed type vs no. young: $F = 0.01 - Df 4, 14 = 1125 (p < 0.01)$

509 fry. While adult *Artemia* contain a high level of protein and all the essential amino acids, the mixed diet contains a balanced composition of protein and nitrogen free extract which might have stimulated gonad development and enhanced fertility. Although liver has a low protein content, its high nitrogen free extract and palatability enhanced feed intake which, in turn, enhanced growth and fertility. Fish fed pelleted feed had poorer fertility than the other groups, possibly due to a deficiency of nutrients essential to ovary development although the feeding rate was high. Based on the present study, the mixed diet, followed by the *Artemia* and liver diets, was most suitable to increasing fertility in *X. helleri*.

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