The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB), University of Hawaii at Manoa Library
and
University of Hawaii Aquaculture Program in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

EFFECT OF FEEDING FREQUENCY ON GROWTH AND FECUNDITY IN AN ORNAMENTAL FISH, *BETTA SPLENDENS* (REGAN)

Raja James* and Kunchitham Sampath

Department of Zoology, V.O. Chidambaram College, Tuticorin 628 008, Tamil Nadu, India

(Received 14.10.03, Accepted 11.2.04)

Key words: *Betta splendens*, feed intake, feeding frequency, growth, reproduction

Abstract

Experiments were performed to investigate the influence of feeding frequency on growth, gonad weight and fecundity in an ornamental fish, *Betta splendens.* Five groups of 30-day-old juveniles (0.045±0.01 g; 14.43±2.2 mm) were fed either one meal in three days, one meal in two days, one meal per day, two meals per day or three meals per day for 77 days. Mean body length and weight increased linearly in all groups. However, the feeding, growth and fecundity rates increased with feeding frequency up to two meals a day, thereafter they declined. Fish fed once in three days consumed only 266 mg feed (dry weight) that was converted into 61 mg flesh (dry weight). Fish fed once in two days consumed 384 mg and gained 116 mg flesh. For fish fed once a day, 543 mg were consumed and converted to 175 mg flesh. For those fed twice a day, 1000 mg feed produced 279 mg flesh and for those fed three times a day, 886 mg food produced 242 mg flesh. The growth rate was significantly and drastically lower in all groups after spawning began, perhaps due to diversion of assimilated food energy for egg production. The gonad developed significantly ($p<0.05$) earlier in fish given at least one meal per day and weighed 85.5, 158 and 246.5 mg (wet weight) in fish fed once, twice and three times per day, respectively, on day 35 while it weighed only 56 and 93.5 mg on day 63 in fish fed once in three or two days, respectively. A similar trend was noted in the gonadosomatic index. Female *B. splendens* laid 274 and 454 eggs in groups fed once in three or two days, and 1833, 2202 and 632 eggs in groups fed once, twice or three times a day, respectively. Feeding three times a day resulted in total mortality of the spawners during the third spawn. Two meals a day elicited the maximum growth and reproductive performance and hence is considered optimal for *B. splendens*.

* Corresponding author. E-mail: piojames@yahoo.com

Introduction

Growth and reproduction are influenced by numerous factors. Feed quality, quantity, composition and ration size and feeding frequency 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). Frequent feeding improves fish growth (Andrews and Pages, 1975; Chua and Teng, 1978). However, increasing the frequency of feeding beyond a particular level may lead to feed wastage and increase production costs (Sampath, 1984). Identification of the optimal feeding frequency helps reduce feed wastage and maximize growth and reproduction.

Food quantity is an important factor affecting reproduction in fish. Hester (1964) reported that a scarcity of food reduced fertility in the guppy, *Poecilia reticulata.* Female fish need adequate protein, fat, vitamins and minerals for egg development and spawning. Yolk is composed of phospholipo proteins, an amalgam of minerals (phosphorous), protein and lipid. Protein is also required to form follicles in the embryo. The absence of any one of these nutrients can reduce the survival of larvae that hatch from these eggs (James and Sampath, 2002).

Many authors studied the effect of feeding frequency on food intake and growth in edible fishes (Marian et al., 1982; Jobling, 1983; Sampath, 1984; Sampath and Ravindran, 1988) but little attention has been paid to the impact of feeding frequency on growth and reproductive performance in ornamental fishes (James and Sampath, 2003a). To our knowledge, the influence of nutrition on growth or reproduction was studied in ornamental fishes (Degani, 1993; Degani and Gur, 1992; Degani and Yehuda, 1996) but no report has yet been published on the influence of feeding frequency on growth and reproductive performance in tropical fishes. Hence, the present study was undertaken to study the effects of feeding frequency on growth, gonad development and fecundity in the Siamese fighting fish, *Betta splendens*.

Materials and Methods

Experimental fish. The Siamese fighting fish, *B. splendens* (Regan), is available in a great variety of colors and is one of the most popular of all aquarium fish species. The male has large fins and intensive multi-coloration while the female has smaller fins and somber coloration.

The male builds a bubble nest on the water surface, usually beneath floating leaves of aquatic plants. When both sexes are ready for spawning, a few trial pairings take place. The male is aggressive, expands his tail fin, opens his operculum and drives off his partner. The male changes his attitude when the female shows readiness for spawning. The female waggles her body in front of the male, the male curves his body and takes the female in a bodily embrace. The male turns the female over so that her underside faces upwards. The male begins to loosen his grip as soon as the female starts to lay the strings of eggs. The male collects the fallen eggs (sometimes the female also collects them) and squeezes them into the bubble nest. Sometimes eggs stay on the female's fins and the male does not collect these until they fall off (Richter, 1988).

The male betta incubates the eggs intensively and fry hatch within 36 h at 25-27°C. Fry are small with a whitish yolk sac and begin to sink. The male continually collects them, warms them in his mouth and returns them to the nest until they are free swimming. The fry prefer *Infusoria* as their initial diet, followed by *Artemia* nauplii and other live diets as development proceeds. The betta grows and attains sexual maturity very quickly, depending on the nature of its diet (James and Sampath, 2003b). It grows to a maximum length of 60 mm.

Fish and maintenance. Three hundred and seventy five uniform-sized juvenile *B. splendens* of 30 days (0.045±0.01 g; 14.43±2.2 mm) were collected from two laboratory brooders obtained from the same parents. They were sorted into 15 groups of 25 each. Triplicates of five feeding schedules were tested: one meal in three days, two days or one day (at 6:00), two meals a day (6:00,

18:00), or three meals a day (6:00, 12:00, 18:00). Each group was reared in a 110-l circular cement cistern (53.34 x 45.72 cm) containing 50 l fresh water in a static condition.

The water was clean, unchlorinated well water and its quality was monitored biweekly. Temperature averaged 28±1ºC, pH 7.8±0.05, water hardness 316±15 mg CaCO3/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 at the bottom.

Feeding. Fish were fed fresh minced beef liver *ad libitum* in a feeding tray for 2 h, after which unconsumed feed was removed and dried in a hot air oven at 80ºC. The protein, fat, ash and nitrogen free extract contents of the beef liver were 35.44, 3.86, 13.91 and 46.79% respectively (James and Sampath, 2002). Feed consumption was estimated by subtracting the amount of unconsumed dry feed from the total dry weight of feed offered. The feeding rate (mg/g live fish/day) was computed as the amount of feed consumed/the initial wet weight of the fish/number of days. Feed samples and unconsumed feed were weighed in an electrical monopan balance to 0.1 mg accuracy.

Growth calculations. Before beginning the experiment, the total wet weight of the fish in each aquarium was weighed in an electrical monopan balance. Five fish from the stock were sacrificed to estimate their water content (Maynard and Loosli, 1962) and determine the initial dry weight of the fish. All animals in each aquarium were weighed every 14 days and dry weight was calculated by using the percent water content of the fish sacrificed at the beginning the experiment. Growth was calculated by subtracting the dry weight on the day of calculation from the initial dry weight. The growth rate (mg/g live fish/day) was calculated as growth/initial wet weight of fish/number of days. Gross conversion efficiency (%) was calculated as growth/feed consumption x 100. 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 graph sheet.

Gonad estimations. Every 14 days, the body weight of three fish of every treatment (one from each of the three replicates) was measured and recorded. Once the gonad began to develop and until spawning began, three females from each treatment were sacrificed every 14 days to estimate the gonad weight. The ovaries were removed and weighed in an electrical monopan balance and the gonadosomatic index (GSI) was computed according to the formula of Dahlgren (1979): GSI (in %) = wet weight of gonad/wet weight of the fish x 100.

Spawning. After attaining sexual maturity, two males and two females were randomly selected from each replicate and used for the spawning studies. One male *B. splendens* was paired with one female in a plastic trough containing fresh water at a depth of 15 cm. The female found shelter against the highly aggressive male on the water surface or under the plant tufts. The male built a foam nest, made of bubbles covered with saliva.

Both partners were ready to spawn within 4-7 h. The females laid eggs within 24-30 h. Both the males and females collected the falling eggs and squeezed them into the nest. After completion of spawning, the female was removed from the trough to protect her from aggressive attacks by the male. The male displayed a strong instinct to incubate the eggs.

The number of eggs laid by the female was counted using a sterilized needle, without causing much disturbance. Fecundity is the number of eggs laid in a single spawn by an oviparous female (Mc Fadden et al., 1965). The male incubated the eggs intensively for 36 h, after which the fry hatched. The number of fry hatched and the number of unhatched eggs were counted. The hatching rate was calculated as: number of eggs hatched/number of eggs laid x 100. The experiment lasted 77 days during which some pairs spawned three times. The remaining animals in the experimental tanks were removed and sacrificed for growth estimation.

Statistical analysis. Students *t* test was applied to determine the significance of mean values between experimental groups. One-way ANOVA was applied to test the signifi-

cance of the feeding frequency on egg production. Regression analysis was carried out based on the method of least square (Zar, 1974).

Results and Discussion

The feeding frequency significantly influenced growth, gonad weight and fecundity in the fighting fish, *B. splendens*. The mean body length and weight generally increased with time and the increase in feeding frequency (Fig.1). The slopes of the length gain of fish fed twice (5.05) and three (5.31) times a day did not differ significantly (t = -0.255; p>0.05) but the slope of the weight gain of fish fed twice a day was significantly higher than that of fish fed three times a day. James and Sampath (2003a) observed that mean body length and weight of red swordtail *Xiphophorus helleri* were higher in fish receiving more frequent meals than in those receiving fewer meals. Hislop et al. (1978) reported that haddock, *Melanogrammus aeglefinus,* fed a low ration exhibited poor growth in terms of body length and weight, supporting the present observation. An increase in mean body length and weight was observed in juvenile dwarf gourami, *Colisa lalia,* as the protein level and rearing period increased (Shim et al., 1989).

The feeding rate increased with increased feeding frequency. Test animals receiving two meals a day consumed more food and grew better than the other groups. Fish that received food once in three days, twice in three days, once a day, twice a day and three times a day consumed 266, 384, 543, 1000 and 886 mg (dry weight) of food, which was converted into 61, 116, 175, 279 and 242 mg (dry weight) of flesh, respectively. Fish receiving three meals a day consumed more feed than fish receiving two meals a day, but the amount did not statistically differ (t = 2.16; p>0.05) before spawning commenced. The feeding rate significantly declined after spawning began in all groups (Table 1).

The results obtained for growth rate and gross conversion efficiency were similar to those for feeding rate. The reproductive cycle of *B. splendens* is very short (14 days). More food energy is required prior to spawning for gonad development and production of eggs than once spawning begins. James and Sampath (2003a) observed a higher food intake in *X. helleri* before commencement of breeding and a drastic decline afterwards. Townshend and Wooton (1984) found that females of the convict cichlid, *Cichlasoma nigrofasciatum*, channel a higher proportion of ingested food energy to gonad development and fecundity than males, so that the female reproductive output is more sensitive to variations in food supply. In our study, food energy obtained from less frequent feedings (once in two or three days) was apparently insufficient for the fish to fully satisfy their somatic growth and reproduction requirements. This was evident by a lower spawning frequency, number of eggs and percent hatched (Table 2).

The gonad weight and gonadosomatic index increased with time and the increase in feeding frequency (Table 3). Animals that received one meal in two or three days required 63 days to attain sexual maturity, while those that received two or three meals a day reached sexual maturity on day 35. This difference may be due to variations in final body weight as fish that received 1-3 meals per day reached 660 mg wet weight on day 35, whereas those offered less frequent feeding weighed only 480 mg wet weight on day 63. Shim et al. (1989) reported that female gourami (*C. lalia*) fed high protein diets (35%) had a greater ovary weight and gonadosomatic index than those fed low protein diets (5%). James and Sampath (2003a) found that females of *X. helleri* fed frequent meals had a higher gonad weight and gonadosomatic index than those fed less frequent meals. Gonad weight in *C. nigrofasciatum* relatively increased with the increase in ration and females on a low ration had a negligible proportion of oocytes whereas females on a high or medium ration had a distinct proportion of oocytes (Townshend and Wooton, 1984). In the current study, it is likely that more frequent feeding resulted in a higher proportion of oocytes and gonad weight than low feeding frequencies, evidenced by the gonad weight and gonadosomatic index.

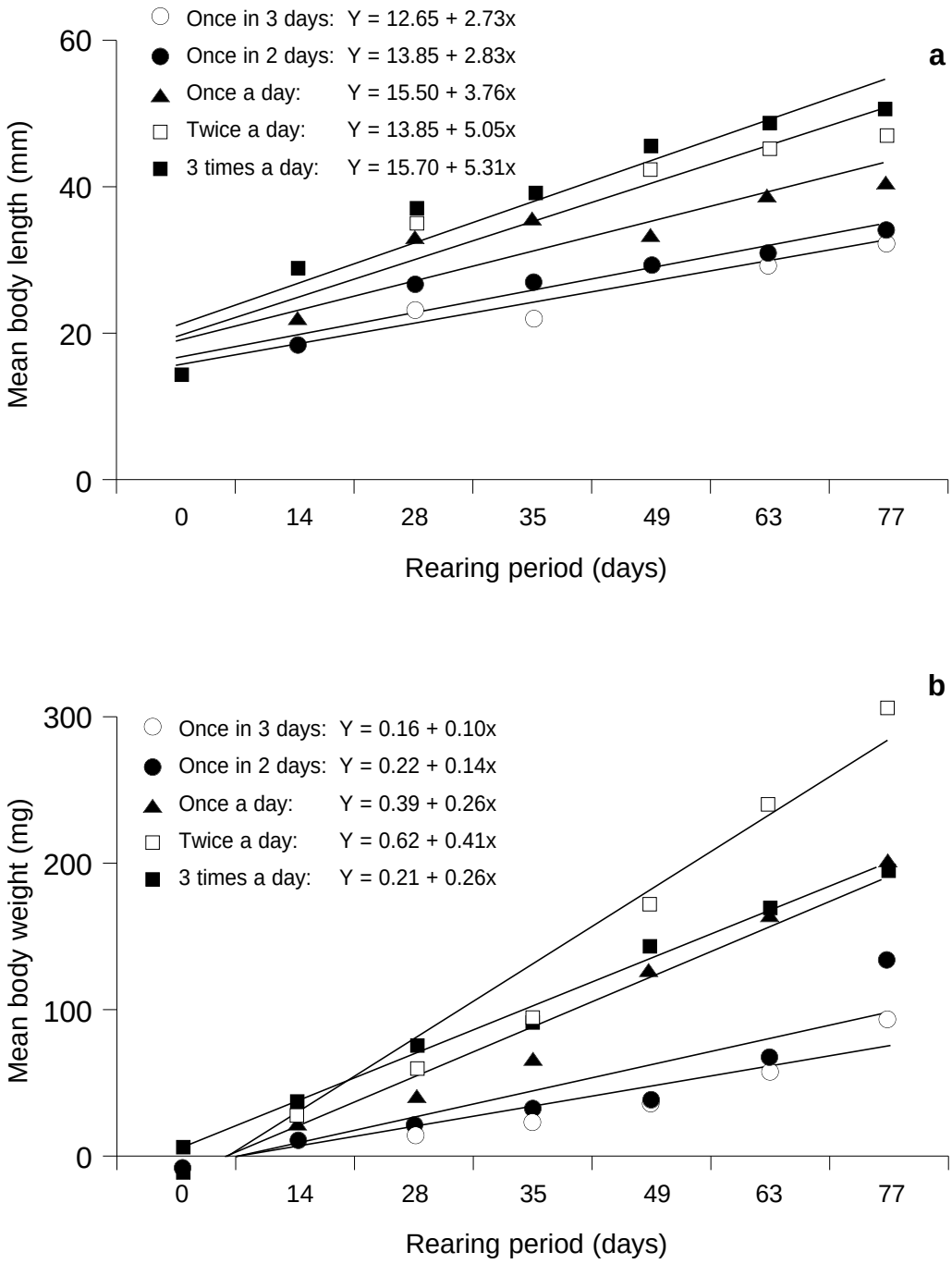

Fig. 1. Mean body length (a) and weight (b) in relation to meal frequency in *Betta splendens* as a function of rearing period.

Table 1. Effect of feeding frequency on feeding and growth rates and gross conversion efficiency in *Betta splendens* before and after commencement of spawning (mean±SD, n = 3).

	Feeding frequency				
	Once in three days	*Once in two days*	*Once a day*	*Twice a day*	*Three times a day*
	Feeding rate (mg/g live fish/day)				
Before spawning	39.36±3.3	58.18±2.8	69.83±5.2	138.26±6.5	154.82±8.7
After spawning	22.77±1.6	25.92±1.8	24.71±1.4	30.28±1.2	14.69±0.9
	Growth rate (mg/g live fish/day)				
Before spawning	9.83±0.7	14.28±1.1	29.14±1.5	39.98±2.6	45.61±2.8
After spawning	4.48±0.6	6.10±0.5	5.41±0.3	7.46±0.6	3.44±0.4
	Gross conversion efficiency (%)				
Before spawning	26.18±1.7	30.65±1.8	45.02±1.9	31.98±1.7	32.63±1.4
After spawning	19.67±1.5	23.61±0.8	21.85±1.8	24.17±1.1	23.23±1.6

Significance of differences between twice a day and three times a day before spawning, by Student's *t* test:
for feeding rate: $t = 2.16$, $p>0.05$
for gross conversion efficiency: $t = 2.09$, $p>0.05$

Table 2. Effect of feeding frequency on fecundity in *Betta splendens* (mean±SD, n = 6).

	Feeding frequency														
	Once in three days			*Once in two days*			*Once a day*			*Twice a day*			*Three times a day*		
	No. eggs spawned	*No. eggs hatched*	*Hatching rate (%)*	*No. eggs spawned*	*No. eggs hatched*	*Hatching rate (%)*	*No. eggs spawned*	*No. eggs hatched*	*Hatching rate (%)*	*No. eggs spawned*	*No. eggs hatched*	*Hatching rate (%)*	*No. eggs spawned*	*No. eggs hatched*	*Hatching rate (%)*
First spawn		-			-		429±15	408±20	95.2±3.1	559±35	523±23	94±5.4	230±16	154±12	67±5
Second spawn		-			-		594±22	558±31	94±2.8	752±28	705±30	94±4.3	302±20	179±11	59.2±4
Third spawn	274±18	198±20	72.4±3.5	454±44	385±32	84.7±4.5	810±34	772±22	95.4±3.5	891±39	827±21	93±5.2	Animals died		
Total no. eggs laid	274±18			454±44			1833±25.0			2202±38.0			532±18.0		

Significance of differences between feeding frequency and total no. eggs laid, by *t* test:
for twice a day vs three times a day: $t = 82.63$, $p<0.01$
for twice a day vs once a day: $t = 10.13$, $p<0.01$
for twice a day vs once in two days: $t = 67.23$, $p<0.01$

Significance of difference between feeding frequency and total no. eggs laid, by one-way ANOVA: F-value = 24.72, $p<0.01$

Table 3. Gonad weight (wet weight) and gonadosomatic index (GSI) in *Betta splendens* fed at different frequencies (mean±SD, n = 6).

Day	*Feeding frequency*									
	Once in three days		*Once in two days*		*Once a day*		*Twice a day*		*Three times a day*	
	Gonad wt (mg)	*GSI (%)*	*Gonad wt (mg)*	*GSI (%)*	*Gonad wt (mg)*	*GSI (%)*	*Gonad wt (mg)*	*GSI (%)*	*Gonad wt (mg)*	*GSI (%)*
28	Not developed		Not developed		23±0.8	6.33±0.2	34±2.3	8.50±0.5	112±9.3	15.83±7.2
35[1]	Not developed		3.5±0.02	2.23±0.08	85.5±4.7	15.89±0.7	158±10.3	30.76±0.8	246.5±15.4	37.16±3.1
49	Not developed		28.5±1.3	6.39±0.08						
63[2]	56±3.7	12.90±0.6	93.5±5.7	19.83±0.7						

[1] Fish fed once, twice or three times a day began spawning.
[2] Fish fed once in two or three days began spawning.

Significance of differences in gonad weight on day 35, by *t* test
between twice a day and three times a day: $t = 10.68$, $p<0.01$
between twice a day and once a day: $t = 14.33$, $p<0.01$
between twice a day and once in two days: $t = 33.51$, $p<0.01$

Significance of differences in GSI, by *t* test
between twice a day and three times a day: $t = 4.48$, $p<0.05$
between twice a day and once a day: $t = 30.98$, $p<0.01$
between twice a day and once in two days: $t = 79.25$, $p<0.01$

The feeding frequency significantly (one-way ANOVA: df 4, 14; F = 24.72; $p<0.01$) affected fecundity. The number of eggs laid per spawn and the frequency of spawning increased with the increase in feeding frequency up to twice a day and thereafter declined, indicating that females fed less frequently might have had fewer oocytes undergoing vitellogenesis than females fed more frequently, resulting in poor fecundity. Total fecundity declined in female haddock *M. aeglefinus* (Hislop *et al.*, 1978) and stickleback *Gasterosteus aculeatus* (Wooton, 1977) fed low rations. Fish fed once or twice a day spawned three times during the experiment but those fed less frequently spawned only once. The reduction in number of spawnings was similar to that of *G. aculeatus* (Wooton, 1977), *C. nigrofasciatum* (Townshend and Wooton, 1984) and *X. helleri* (James and Sampath 2003a). The response of *B. splendens* to feeding frequency suggests that food supply could be an important factor in determining the fecundity and frequency of spawning. However, feeding more frequently than the optimum (twice a day) not only led to food wastage and reduction of feed intake and spawning but also to loss of the female brood during the third spawning. There was total mortality amongst the females fed three times a day. Anatomical observations of the fish revealed an accumulation of fat in the gut that, perhaps, obstructed the digestive process. Fat deposition throughout the ovary may have prevented the release of eggs, resulting in mortality. Consumption of high protein diets beyond the optimum requirement led to deposition of fat and food wastage (Cowey, 1979). Based on the present study, two meals a day is the optimum feed schedule for maximizing growth and reproduction in *B. splendens*.

References

Andrews J.W. and J.W. Pages, 1975. The effects of frequency of feeding on culture of catfish. *Trans. Am. Fish. Soc.*, 104:317-321.

Bulkley R.V., 1972. Diethylstilbestrol in catfish feed. *Trans. Am. Fish. Soc.*, 101:537-539.

Chua T.W. and S. Teng, 1978. Effects of feeding frequency on the growth of young estuary grouper *Epinephelus tauvina* (Forskal) cultured in floating net cages. *Aquaculture*, 14:31-47.

Cowey C.B., 1979. Protein and amino acid requirements of finfish. pp.4-5. In: J.E. Halver, K. Tiews (eds.). *Fin Fish Nutrition and Fish Feed Technology.* Proc. World EIFAC, IUNS Symp., June 20-23, 1978, Hamburg. Heenemann, Berlin.

Dahlgren B.T., 1979. The effects of population density on fecundity and fertility in the guppy *Poecilia reticulata* (Peters). *J. Fish Biol.*, 15:71-91.

Degani G., 1993. Growth and body composition of juvenile *Pterophyllum scalare* (Lichtonstein) (Pisces:Cichlidae) at different densities and diets. *Aquacult. Fish. Manag.*, 24:725-730.

Degani G. and N. Gur, 1992. Growth of juvenile *Tricogaster leerii* (Bleeker, 1952) on diets with various protein levels. *Aquacult. Fish. Manag.*, 23:161-166.

Degani G. and V. Yehuda, 1996. Effects of diets on reproduction of angelfish, *Pterophyllum scalare* (Cichlidae). *Indian J. Fish.,* 43:121-126.

Hester F.J., 1964. Effects of food supply on fecundity in the female guppy (*Lebistes reticulatus*). *J. Fish. Res. Bd. Can.*, 21:1-4.

Hislop J.R.C., Robb A.P. and J.A. Gauld, 1978. Observation on effects of feeding level on growth and reproduction in haddock (*Melanogrammus anglefinus* L.) in captivity. *J. Fish Biol.*, 13:85-98.

James R. and K. Sampath, 2002. Effect of feed types on growth and fecundity in ornamental fish, *Betta splendens* (Regan). *Indian J. Fish.*, 49:279-285.

James R. and K. Sampath, 2003a. Effects of meal frequency on growth and reproduction in the ornamental red swordtail, *Xiphophorus helleri. Israeli J. Aquacult. - Bamidgeh*, 55:197-207.

James R. and K. Sampath, 2003b. Effect of animal and plant protein diets on growth and fecundity in ornamental fish, *Betta splendens* (Regan). *Israeli J. Aquacult. - Bamidgeh*, 55:39-52.

James R., Muthukrishnan J. and K. Sampath, 1993. Effect of food quality on temporal and energetics cost of feeding in *Cyprinus carpio* (Cyprinidae) *J. Aqua. Trop*., 8:47-53.

Jobling M., 1983. Effect of feeding frequency on food intake and growth of Arctic charr, *Salvelinus alpinus* (L). *J. Fish Biol.*, 23:177-185.

Jobling M., 1998. Feeding and nutrition in intensive fish farming. pp. 68-113. In: K. Black, A.D. Pickering (eds.). *Biology of Farmed Fish.* Sheffield Acad. Press, Sheffield.

Marian M.P., Ponniah A.G., Pitchairaj R. and M. Narayanan, 1982. Effect of feeding frequency on surfacing activity and growth in air breathing fish, *Heteropneustes fossilis*. *Aquaculture*, 26:237-244.

Maynard A.L. and K.S. Loosli, 1962. *Animal Nutrition*. McGraw Hill, New York.. 533 pp.

Mc Fadden J.T., Cooper E.L. and J.K. Anderson, 1965. Some effect of environment on egg production in brown trout (*Salmo trutta*). *Limnol. Oceanogr.*, 10:88-95.

Richter H.J., 1988. pp. 105-112. In: *Gouramis and Other Anabantoids*. TSH Plaza, Neptune City, New Jersey.

Sampath K., 1984. Preliminary report on the effects of feeding frequency in *Channa striatus*. *Aquaculture*, 40:301-306.

Sampath K. and T.J. Pandian, 1984. Interactions of feeding frequency and density on feed utilization in air-breathing murrel, *Channa striatus. Proc. Indian Acad. Sci.*, 93:445-453.

Sampath K. and J. Ravindran, 1988. Optimum meal frequency in the fry of *Cirrhinus mrigala* (Hamilton). pp.65-67. In: M. Mohan Joseph (ed.). *Proc. 1st Indian Fish. Forum.* Asian Fish. Soc., Indian Branch, Mangalore.

Shim K.F., Landesman L. and T.J. Lam, 1989. Effect of dietary protein on growth, ovarian development and fecundity in the dwarf gourami *Colisa lalia* (Hamilton). *J. Aqua. Trop.*, 4:111-123.

Townshend T.J. and R.J. Wooton, 1984. Effects of food supply on the reproduction of the convict cichlid, *Cichlasoma nigrofasciatum*. *J. Fish Biol.*, 24:91-104.

Wooton R.J., 1977. Effect of food limitation during the breeding season on the size, body components and egg production of female sticklebacks (*Gasterosteus aculeatus*). *J. Anim. Ecol.*, 46:823-834.

Zar J.M., 1974. *Biostatistical Analysis*. Prentice Hall, New Jersey. 260 pp.