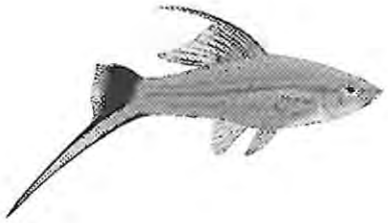

ASPECTS OF NUTRITION OF THE SWORDTAIL FISH, *XIPHOPHORUS HELLERI*, (FAMILY: POECILIIDAE) UNDER INTENSIVE CULTURE CONDITIONS.

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CONTENTS

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ABSTRACT

The aim of this work was to investigate the nutrition of a popular ornamental fish, the swordtail (*Xiphophorus helleri*), under intensive aquaculture conditions. The study focused on the formulation and manufacture of artificial dry diets, as well as the influence of livefeed supplementation on growth and nutritional indices of both juvenile and broodstock fish.

A combination of 65% fishmeal and 35% soya oil cake meal was found to closely approximate the essential amino acid profile of *X. helleri* (r^2 = 94,3%) and lysine was identified as the first limiting amino acid. The influence of dietary protein and energy on the growth of *X. helleri* was investigated by means of a growth trial comparing a range of nine test diets combining 3 crude protein (45, 38 and 30%) and 3 digestible energy (16,5, 14,5 and 12,5 kJ/gram) levels. A high crude protein content (45% dry matter) and a protein to energy ratio of ≥ 27 mg protein per kJ digestible energy was shown to promote optimal growth rate and feed conversion ratio in juvenile *X. helleri*. The final body protein and lipid content of the fish were significantly correlated with the dietary protein to energy ratio.

The nutritional value of identical dietary formulations prepared by drum-drying or cold extrusion techniques and presented either as flake or crumble particles was evaluated in a growth trial and through analysis of vitamin C levels in the diets. Up to 27 % of vitamin C activity was destroyed during the drum-drying of flake diets, while 80 % of the remaining vitamin C was lost through leaching within 10 minutes of immersion in water. A crumble-type diet was shown to be acceptable to *X. helleri*, and yielded significantly better growth rate and feed conversion than the equivalent flake feed. Almost 19% of fish fed the flake diets developed vitamin C deficiency symptoms including scoliosis and lordosis, while this was completely prevented by feeding crumbles, indicating a significant restriction of leaching losses from this type of particle.

The use of livefeed (*Daphnia* spp.) as a daily supplement to dry feeds was shown to result in a significant improvement in growth rate and survival of juvenile swordtail fish. Furthermore, the synergistic action of nutrients contained in the livefeed resulted in an improvement in the feed

conversion efficiency of the artificial formulation. While mortalities of 13,4% and 15% were recorded in fish fed exclusively on flake feed or *Daphnia*, no mortality occurred in the group fed the supplemented diet. Furthermore, the incidence of vitamin C deficiency symptoms was prevented by daily supplementation with *Daphnia*. Fish fed exclusively on *Daphnia* exhibited significantly more intense pigmentation due to carotenoids contained in the livefeed.

In broodstock fish maintained primarily on flake diets, growth rate, feed conversion ratio as well as reproductive performance, as measured by fecundity, were significantly improved by daily livefeed supplementation. Weekly supplementation showed no measurable advantage over flake feed only. A direct correlation was demonstrated between fecundity and size of female fish with a mean fecundity of 190 embryos per female in those fed a daily *Daphnia* supplement. This was significantly higher than the yield from the non-supplemented or weekly supplemented treatments (133 and 140 embryos per female respectively). Despite these differences, the number of young harvested during the experiment did not differ between treatments and this discrepancy was ascribed to parental cannibalism of newborn juveniles. This phenomenon constitutes a major potential bottleneck in production with estimates of up to seventy percent of young lost in some experimental tanks.

In conclusion, the present study demonstrated that established aquaculture nutrition principles and techniques are applicable to the tropical ornamental fish species, *X. helleri*. It was demonstrated that by using balanced crumble feed formulations, and regular livefeed supplementation, production yields under intensive conditions may be significantly improved. Suggestions for future work on poeciliid production in South Africa include investigation of the economic implications of the recommendations stemming from this project, and further research into effective restriction of parental cannibalism of newborn fish.

CHAPTER 1

INTRODUCTION

HISTORICAL PERSPECTIVE AND MOTIVATION FOR RESEARCH

On a global scale the aquarium fish trade has been relatively static in recent years, as evidenced by the stable fish prices realized on international markets (Bleher, 1993). Cheong (1993) estimated the world trade in ornamental fish to be approximately 1,5 billion fish per annum. In the United States, the world's largest importer of aquarium fish, the market has experienced gradual growth with imports of over $500 million worth of fish per year (Shariff and Subasinghe, 1992). Production of ornamental fish in South Africa constitutes an important sector of the overall aquaculture industry in the country. On the basis of generated income, it is third overall, after the trout and mussel industries (Hecht *et al.*, 1992), with a product gate value of R5 million (Britz and de Kock, 1994). As a result of the stable nature of the market for ornamental fish, expansion in the production sector would require new marketing channels to be established, and on an international basis, market share captured from other producers. To achieve this goal, South African producers need to offer a larger variety and volume of quality fish in order to compete with foreign producers, in particular those in Asian countries such as Singapore. Asian producers are increasingly experiencing a number of potentially restricting environmental factors, including deteriorating water quality and emergence of fish diseases, which may adversely affect their competitiveness. This may in turn increase the demand for South African produced fish, provided a quality product is offered at a competitive price.

The present project was undertaken in collaboration with Amatikulu Hatchery (Pty.) Ltd., the largest producer of ornamental fish in South Africa. The project formed part of an ongoing research and development effort aimed at increasing fish production and quality. Amatikulu Hatchery currently produces in excess of eighty different fish species and strains (Hecht *et al.*, 1992). The bulk of South African ornamental fish producers, including Amatikulu Hatchery, are situated in the subtropical areas of northern Kwazulu-Natal. Despite the cooler water temperatures elsewhere in the country, entrepreneurs are increasingly establishing ornamental fish

farms, utilising heated and insulated recirculating production systems.

Of the aquarium fish species produced in South Africa, approximately one quarter belong to the family Poeciliidae (Cyprinodontiformes), the livebearing toothcarps. In Singapore, the largest ornamental fish exporter in the world, twenty percent of the fish produced belong to this family (Fernando *et al.*, 1991). These fish, which include the swordtail (*Xiphophorus helleri*), guppy (*Poecilia reticulata*), mollies (*P. velifera* and *P. sphenops*) and platies (*X. variatus* and *X. maculatus*) are traditionally among the most popular species in the aquarium trade.

At Amatikulu Hatchery, the livebearers are farmed by independent "satellite" farmers, each producing two or more strains under contract. Each farmer owns a unit within an "aquaculture park" of 15 farmers. Each unit comprises four breeding tanks (4000 l), nine grow-out tanks (12500 l), and 5 smaller holding tanks (2000 l). The tanks function on a flow-through system, with ground water supplied directly from a borehole. A simple removable downpipe acts as a drainage port. Aeration is provided in the smaller tanks. The entire complex is enclosed in a cover of nylon shadecloth, primarily aimed at excluding predatory birds.

The motivation for research into livebearer production was based on a realisation that potential yields were not being achieved. Examination of production figures for the year prior to initiation of this project, revealed that of the poeciliid fish produced at Amatikulu Hatchery, the swordtail exhibited markedly lower production levels than the other species, despite the stocking of equivalent numbers of broodstock. This could be attributed either to lowered fecundity, or post-partal loss of fry. The swordtail was thus selected for use as a research model during this project. Interviews with the farmers and Amatikulu Hatchery management, and examination of the culture system revealed that low winter temperatures (minimum water temperature: 11°C), nutrition and cannibalism appeared to be the major factors adversely affecting production. A decision was made to shift production into insulated plastic tunnels to overcome the temperature problem, and nutrition was chosen as the focus of the present study. In addition, an attempt was made to quantify the rate of cannibalism during nutritional trials with broodstock fish.

X. HELLERI AS A RESEARCH MODEL: NATURAL HISTORY AND BIOLOGY

X. helleri is classified as a generalized poeciliid and is typical of most of the livebearer species common in the aquarium trade (Thibault an Schultz, 1978). It should thus be possible to apply the recommendations stemming from this research to other species, in particular the closely related *X. maculatus* and *X. variatus*, the platy fish. Historically, most studies involving poeciliid fish have been performed under laboratory conditions, investigating aspects of genetics or physiology (Meffe and Snelson, 1989). While the genetics of *X. helleri* is well understood, it's ecology and natural history remains largely undocumented (Meffe and Snelson, 1989).

X. helleri (Heckel 1848) originates in northern South America, with a range extending from Rio Nautla, Veracruz in the north, southward to northern Honduras (Rosen and Bailey, 1963). Numerous naturally occurring genetic strains occur as a result of isolation of small populations of fish in a wide variety of biotypes. Typical habitats range from the margins of inland lakes, swamps, and rivers to the riparian zones of fast-flowing high altitude streams, either overgrown or lacking in vegetation. As a result of the variety of forms, three separate subspecies were initially distinguished, *X. helleri helleri*, *X. helleri strigatus* and *X. helleri guentheri*. These are all now synonomised as *X. helleri*, the green swordtail.

Swordtails have been known in the aquarium trade since 1909, and were first popularized in Germany. Over the years, many forms, varying in colour and fin shapes have been developed both through selective breeding and hybridization, especially with *X. maculatus*. These two species freely interbreed in captivity, producing fertile young. Interestingly, although these species coexist commonly in the wild, hybridization has not been recorded (Stevens, 1983).

In nature swordtails are omnivorous, their diet including aquatic and terrestrial invertebrates, as well as plant (both macrophyte and algal) material (Arthrington, 1989).

Poeciliid fishes, although all viviparous, display a wide variety of reproductive adaptations within the family. In mature males the third, fourth and fifth rays of the anal fin are modified to form an intromittent organ, the gonopodium, used for internal fertilization of the female fish. Sperm is

transferred to the female genital tract in sperm bundles (spermatozeugmata) where it may be stored and possibly nourished for up to six months, effectively allowing numerous broods to be fertilized from a single mating (Meffe and Snelson 1989). In females, the ovaries are fused into a single large organ, which when filled with ova or embryos may occupy most of the peritoneal cavity. Fertilization occurs within the egg follicles, and the developing embryos remain in the follicle until shortly before birth.

Like most other fish species, *X. helleri* are classified as lecithotrophic, and the nutrients used by the embryo during gestation are stored within the ova as yolk prior to fertilization. A simple placental barrier exists, consisting of a chorion formed by the expanded pericardial sac and an amnion, the pericardial sac's inner wall (Meffe and Snelson, 1989). Maternal contribution during gestation is restricted largely to gaseous exchange and supply of minerals. By contrast, in matrotrophes, such as *Heterandria formosa* the placental barrier is far more complex, forming a so-called pseudo- or follicular placenta, allowing extensive maternal nutrient contribution during gestation.

In generalised species, including *X. helleri*, all embryos within a brood are at a similar stage of development at the end of the gestation period, and ovulation occurs shortly before parturition. The embryo then moves into the ovarian cavity, down the gonoduct and into the environment. The entire clutch are born within a matter of hours. In certain of the specialized species, superfetation occurs, and groups of embryos of various developmental stages occur simultaneously within the ovary. In these species smaller broods of young are born more frequently.

Brood size in *X. helleri* may range from twenty to over two hundred, depending on the size of the female, and environmental conditions (Meffe and Snelson, 1989). The gestation period has been determined as thirty five days (Siciliano, 1972), but the interbrood interval may vary between four and six weeks, depending on environmental circumstances, particularly water temperature. The variation in interbrood interval in generalised poeciliids is determined by the length of the period between parturition and subsequent fertilization, the yolk loading period (Snelson *et al.*, 1986). Female fish attain sexual maturity at four to six months of age.

RESEARCH APPROACH

Correct nutrition is essential for optimal reproductive success, growth and health of fish. The specific nutritional requirements will depend on the fish species under consideration. Published information regarding diets fed to ornamental fish in commercial aquaculture is scant. In Singapore, swordtails are fed on diets formulated by farmers according to individual preferences, and are generally based on fish meal, wheat flour and skim milk. These diets contain a protein content of between 18,3 and 26,3 % crude protein (dry weight). Much emphasis is placed upon the regular supplementation of diets with livefood, including cladocerans such as *Daphnia* sp. and tubificiid worms (Fernando *et al.*, 1991). This is considered particularly important in broodstock fish.

All fish produced at Amatikulu Hatchery were fed a staple drum-dried flake diet produced by a feed factory on the farm. A few of the farmers fertilised their ponds prior to stocking in order to stimulate primary production, thus supplementing the available food supply with naturally occurring phyto- and zooplankton. Three areas were investigated in order to produce an optimal diet for poeciliids: dietary formulation with respect to protein and energy content, the processing technique employed, and the possible benefits of livefeed supplementation. To ensure that the results of this study were of relevance to the farmers, research was designed around the resources available on the farm. In as far as possible, diets were made from practical feed ingredients, using the available commercial feed manufacturing processes. In work on livefeeds, *Daphnia* sp., a livefeed already produced on the farm was evaluated as a supplement to the fishes staple flake feed diet.

Since a balanced supply of protein and amino acids is essential for optimal growth, health and reproductive success in fish (Wilson, 1989), and protein is the most cost sensitive dietary ingredient, the essential amino acid and protein requirements of *X. helleri* were initially focused upon. Fish require a protein source which will provide a well-balanced mixture of essential and non-essential amino acids. Using the essential amino acid profile of *X. helleri* and various practical protein sources, a guide to its requirement pattern was determined. These results were used to formulate an "ideal" protein source for inclusion in test diets. In order to determine the

optimal dietary protein content, and protein to energy ratio for swordtails, an experiment was designed comparing diets containing a range of protein and energy levels. As fish eat to satisfy their energy demands (Lee and Putnam, 1973), it is important to establish an optimum ratio between dietary protein and energy, in order to avoid wastage of protein (metabolized via gluconeogenesis for energy production), depressed feed consumption, or suboptimal growth.

A need had been identified within the ornamental aquaculture industry for the development of a pellet or crumble type feed which would be more stable in water than the flake formulation employed at Amatikulu Hatchery (Hecht and Britz, 1990). As concern existed regarding the possible loss of nutrients through heat degradation during the drum-drying process, or via leaching from the flake diet, research was planned comparing the nutritional value of feeds produced via different processing methods. A growth trial was thus designed to evaluate two aspects of the artificial feed: the effect of heat versus cold processing, and the hypothesis that a crumble diet may be more effective in retaining its nutritional value than a flake feed. Vitamin C was selected as an indicator of nutrient loss for this work, as it is a heat-labile, water soluble essential nutrient producing macroscopically visible deficiency symptoms.

As livefeeds are commonly considered by aquaculturalists to effectively stimulate improved production and growth when used as a supplement to formulated feeds, the influence of *Daphnia* sp. supplementation on the growth and survival of juvenile swordtails was investigated. Besides these parameters, this trial was designed to evaluate the efficacy of *Daphnia* sp. supplementation in preventing flake-induced vitamin C deficiency symptoms, as well as its effect on the pigmentation of the fish.

In view of the perceived low fecundity of *X. helleri* broodstock at Amatikulu Hatchery, and in recognition of the fact that the level and quality of nutrition are critical in achieving optimal reproductive success in fish (Wootton, 1982), a trial was planned evaluating the growth and fecundity of broodstock fish. Diets of drum-dried flake feed combined with various levels of livefeed supplementation were evaluated. This experiment was also used to evaluate the rate and importance of parental cannibalism of new born fish.

Based on the results and observations stemming from the experimental work carried out during the course of this project, recommendations are made for the nutritional management of *X. helleri,* maintained under intensive farming conditions. Observations concerning broodstock husbandry techniques are also included.

CHAPTER 2

AMINO ACID REQUIREMENTS OF SWORDTAILS AND FORMULATION OF AN "IDEAL" PROTEIN FROM PRACTICAL FEED INGREDIENTS.

INTRODUCTION

Fish, in common with other vertebrates, require ten essential amino acids (EAA) in their diets. These include arginine (arg), histidine (his), isoleucine (ile), leucine (leu), lysine (lys), methionine (met), threonine (thr), tryptophan (trp), phenylalanine (phe) and valine (val) (Steffens, 1989). It is generally accepted that cystine spares part of the methionine requirement in some fish (Ketola, 1982), and although not conclusively proved, tyrosine probably has a sparing effect on phenylalanine in fish diets. Very little work has been published regarding the amino acid or protein requirements of ornamental fish species, and no reference to the precise amino acid needs of *X. helleri* or other poeciliid fish species was available. As an understanding of the balance of essential amino acids required by a species is fundamental to the formulation of an optimal diet, an investigation was undertaken to determine the ideal proportions of EAA required for inclusion in a swordtail diet. Based on this requirement, an "ideal" protein was formulated from practical ingredients, for use in the subsequent investigation into the protein and energy requirements of *X. helleri*.

The quantitative requirement for each individual amino acid varies considerably between fish species, as may the ratio between the amino acids. Requirements have been calculated for a number of aquaculture species, including various salmonids, *Ictalurus punctatus*, *Anguilla* sp., *Sparus aurata* and *Cyprinus carpio* (Wilson, 1985). These results have been obtained by various methods, including the use of test diets containing crystalline amino acids (Mertz, 1972), or in some cases by using radioactive-labelled amino acids (Walton *et al.*, 1984). Since the determination of quantitative EAA requirements of fish by means of growth trials is very exacting, and has only been established for a limited number of fish, an alternative approach has become established. It is now widely accepted (Wilson, 1985; Benitez, 1989) that the amino acid profile of a species, which is readily analysed using ion-exchange chromatography and spectrophotometry

(Benitez, 1989), gives a reasonable indication of the balance of dietary amino acids required. Thus, for "new" aquaculture species such as *X. helleri*, about which very little is known nutritionally, this assumption can be used to provide an approximation of the ideal dietary EAA balance. By combining protein rich feed ingredients to match the essential amino acid profile of the organism under investigation, an "ideal" protein can be formulated for inclusion in artificial diets.

The amino acid profile of whole body tissue of *X. helleri*, the staple Amatikulu Hatchery farm flake, and selected practical feed ingredients were determined in order to formulate a protein source for this species. Two protein sources, fishmeal (Danish low-temperature treated) and soya oil cake meal, were selected, after preliminary investigation into their essential amino acid profiles. This was due to their common use in practical fish feeds, availability, cost and the relative balance between the EAA present in each.

MATERIALS AND METHODS

Quantitative amino acid determination was performed on a Beckman 6300 High Performance Analyzer according to the methods of Simpson *et al.* (1976). Sixteen swordtail fish (twelve females and four males - Initial mass: 25,16g ; Dry mass: 5,82g) were starved for thirty six hours, sacrificed and freeze-dried for 24 hours. Simultaneously, a sample of the standard flake diet formulated for general use at Amatikulu hatchery was analysed. Tryptophan levels could not be determined with the techniques available to the author. The amino acid profiles of Danish low-temperature treated fish meal and soya oil cake meal were provided by their suppliers. Representative samples of the feed components were analysed for proximate composition according to the standard of the Association of Official Analytical Chemists (AOAC, 1984). Moisture was determined after drying samples at 100°C for 48 hours in a convection oven. Ash content was determined after samples were burnt at 550°C for 7 hours in a muffle furnace (Crucibles used were first treated for 8 hours at this temperature). Protein (Kjeldahl method) and lipid content (Soxhlet apparatus) were determined on samples sent to the University of Natal. An optimal combination of these two proteins approximating the essential amino acid profile of *X. helleri* was determined using a modified form of the essential amino acid ratio (A/E ratio)

developed by Ogata *et al.* (1983). The A/E ratio is defined as: $A/E Ratio = \frac{EAA}{Total EAA} * 1000$, where EAA = Essential amino acid value in g/100g dry matter and total EAA includes tyrosine and cystine (combined with phenylalanine and methionine respectively). Because the levels of tryptophan and cystine could not be obtained for *X. helleri,* the above formula was used with the exclusion of these two amino acids as well as tyrosine.

As the level of tryptophan in *X. helleri* tissue was not available, an indication of whether the proposed protein combination would provide sufficient tryptophan was obtained by comparing the essential amino acid profile of the protein source to that of the channel catfish, *Ictalurus punctatus* (Wilson and Poe, 1985), a warmwater omnivorous fish. The A/E ratio (including tryptophan, but excluding tyrosine and phenylalanine) of channel catfish was then compared to that of the protein source. On the basis of the similarities in overall EAA profiles of *I. punctatus* and *X. helleri*, it was assumed that if sufficient tryptophan was provided by the protein source to meet the requirements of *I. punctatus*, then this would apply to *X. helleri* as well.

In addition, a further index of comparison of the EAA profiles of *X. helleri* and the proposed protein combination was used to confirm that a close match existed. The ratio between the first limiting amino acid (in this case lysine) and each individual essential amino acid was calculated for *X. helleri* and the protein source according to the formula: $\frac{E.A.A.(g/100\,g)}{Lysine\ (g/100\,g)}$.

In order to quantify how closely the A/E ratio and lysine ratio of *X. helleri* and the protein sources matched each other, simple regression analyses were performed, and the coefficients of determination (r^2) calculated.

RESULTS

Amino acid levels of *X. helleri*, Amatikulu standard farm flake feed, fish and soya oil cake meal, expressed as percentage protein (g/100g protein) are presented in Table 2.1. The crude protein content of the samples submitted for analysis, as well as results of the proximate analyses of the fish meal and soy oil cake meal are included.

TABLE 2.1. Amino acid content (g/100g protein), crude protein content of swordtail fish and various protein sources and proximate analyses of fish meal and soya oil cake meal (% dry matter). (Bold font indicates essential amino acids). Soya O.C.M. = Soya oil cake meal.

Amino Acid	*X. helleri*	Standard Flake	L.T. Fishmeal	Soya O.C.M.
Arginine	5,67	5,39	6,59	7,06
Histidine	2,31	2,35	2,84	2,61
Isoleucine	3,78	4,11	5,33	6,05
Leucine	6,83	7,20	8,58	8,32
Lysine	7,73	7,07	9,07	6,42
Phenylalanine	3,89	4,05	4,73	5,07
Methionine	2,60	2,32	3,58	1,49
Threonine	3,78	4,11	4,93	3,97
Valine	4,40	4,85	6,43	5,24
Tryptophan			1,28	1,41
Aspartic Acid	9,27	9,63	10,55	
Serine	4,05	4,13	4,88	4,61
Glutamic Acid	12,58	15,65	14,59	
Proline	4,66	4,91	4,88	
Glycine	7,17	5,90	5,99	5,47
Alanine	5,71	5,79	6,65	
Tyrosine	2,77	2,32	3,98	3,56
Cystine			1,12	1,30
Crude Protein	54,5%	37,5%	72,8%	48,3%
Carbohydrate			0	25,7
Fat			10,2	0,9
Ash			11,3	5,0
Crude Fibre			0,2	5,0
Moisture			7,3	10,5

Iteration of various ratios of fishmeal and soya oil cake meal revealed that a combination of 65 percent fish meal and 35 percent oil cake meal best approximated the A/E ratio of *X. helleri* (Table 2.2). Furthermore, comparison of these ratios indicated that lysine was the main limiting amino acid. The crude protein content of this fishmeal/soya combination was 64% (dry matter basis).

TABLE 2.2. Modified essential amino acid ratios (Ogata *et al.*, 1983) for *X. helleri* and various protein sources. FM = Fishmeal, SM = Soya oil cake meal. r^2 = Coefficient of determination with respect to profile of *X. helleri*. (First limiting amino acid underlined)

Amino Acid	*X. helleri*	Standard Diet	L.T. Fishmeal	Soya Oil Cake Meal	65:35 FM:SM
Arginine	137	130	127	153	133
Histidine	57	57	55	56	55
Isoleucine	92	99	102	130	109
Leucine	167	174	165	180	168
Lysine	189	<u>170</u>	<u>174</u>	<u>139</u>	<u>166</u>
Phenylalanine	95	98	90	110	95
Methionine	63	56	69	32	60
Threonine	92	99	95	86	92
Valine	108	117	123	113	120
r^2 (%)		95,8	96,2	69,6	94,3

Comparison of the relationship between the A/E ratios of channel catfish and those of the fishmeal / soya combination revealed that in this species tryptophan is not a limiting amino acid (Table 2.3). On the basis of the similarity of the *X. helleri* and *I. punctatus* amino acid profiles it was assumed that this would be true for *X. helleri* as well.

TABLE 2.3. Modified A/E ratios (Ogata *et al.*, 1983) for *X. helleri, I. punctatus* and protein source. r^2 = Coefficient of determination with respect to profile of *X. helleri.*

Amino Acid	*X. helleri*	*I. Punctatus*	65:35 FM:SM
Arginine	137	144	129
Histidine	57	47	54
Isoleucine	92	93	106
Leucine	167	160	164
Lysine	189	183	162
Methionine	63	63	59
Phenylalanine	95	89	93
Threonine	92	89	90
Tryptophan	?	**16**	**25**
Valine	108	111	118
r^2 (%)		98,5	94,3

Comparison of the EAA profiles of *X. helleri* and the fishmeal / soya combination using the first limiting amino acid (in this case lysine) as a reference also revealed a high degree of similarity (Table 2.4).

TABLE 2.4. Ratios between essential amino acids and lysine content of *X. helleri* and protein source. r^2 = Coefficient of determination with respect to profile of *X. helleri.*

Amino Acid	*X. helleri*	65:35 FM:SM
Arginine	0,7	0,8
Histidine	0,3	0,3
Isoleucine	0,5	0,6
Leucine	0,9	1,0
Lysine	1,0	1,0
Phenylalanine	0,5	0,6
Methionine	0,3	0,3
Threonine	0,5	0,5
Valine	0,6	0,7
r^2 (%)		96,2

DISCUSSION

The high degree of similarity between the A/E and lysine ratios of the proposed protein combination and *X. helleri* demonstrated that it could be regarded as an "ideal" protein source for *X. helleri*, and as such was suitable for investigations into the protein and protein to energy requirements of this species (Chapter 3). Although only two protein sources were utilised, the combination chosen demonstrated a close correlation to the amino acid profile of *X. helleri* and that of the standard farm flake, despite the far broader base of source proteins used in the flake formulation. Furthermore, these results indicate that where fish meal and soya oil cake meal are used as the major protein sources in formulation of artificial feeds for *X. helleri* (and probably also other closely related poeciliid fish) the above inclusion ratio should be considered.

Lysine, and to a lesser extent arginine, appeared to be the primary limiting essential amino acids. The essential amino acids present in relatively low quantities in soya oil cake meal, including lysine and methionine, are complemented by the higher levels in fishmeal. Supplementation with crystalline arginine could be considered, but the practicality of this would have to be evaluated as crystalline amino acids are highly water soluble and thus leaching losses are likely to be high. However, this could be practically achieved by supplementing formulated feed with livefeeds such as *Daphnia* which are rich in lysine and arginine (Yurkowski and Tabacher, 1979; De la Noüe and Choubert, 1985). The comparison between the essential amino acid profiles of *I. punctatus*, *X. helleri* and the protein source indicates that tryptophan would not, in all likelihood, be a limiting amino acid.

CHAPTER 3

THE EFFECT OF DIETARY CRUDE PROTEIN AND DIGESTIBLE ENERGY ON THE GROWTH AND BODY COMPOSITION OF JUVENILE SWORDTAIL FISH.

INTRODUCTION

The success of intensive commercial aquaculture, whether it involves foodfish or ornamental fish species, relies to a large extent on supplemental feeding with nutritionally complete diets. In the case of ornamental fish farms which produce a wide range of species, the fish tend to be fed on a single dietary formulation, despite the quite different feeding ecology and nutritional requirements of many of the species.

As growth rate is dependent upon dietary protein level and protein generally constitutes the most expensive component of a commercial diet, it is important to establish for a specific group or species of fish, the optimal protein level in the diet. Previous research into both quantitative and qualitative protein requirements, as well as energy requirements and utilization, has traditionally been focused on food species, particularly salmonids. Very little information was available on the specific nutritional requirements of ornamental fish species, with no published reports on the requirements of *Xiphophorus helleri* in intensive aquaculture (Fernando *et al.*, 1991).

The optimal dietary protein requirements of a fish species are influenced by a number of factors, including the protein source, protein to energy ratio (P:E ratio) in the diet (Sargent *et al.*, 1989), size and age of individual fish, and ambient temperature. Increasing dietary protein in fish diets generally results in an increase in production (Nematipour *et al.*, 1992). At high levels of dietary protein, protein is used for both somatic growth and to satisfy maintenance energy requirements, thus partial replacement with relatively inexpensive energy sources such as carbohydrates and lipids may result in a degree of "protein sparing". However, increasing these components beyond a certain level may lead to excessive somatic lipid accumulation with resultant poor growth (Daniels and Robinson, 1986) and even pathological changes.

The quantity of food ingested is regulated according to the energetic requirements of a fish (Lee and Putnam, 1973), and thus optimal crude protein content of a diet cannot be determined without considering digestible energy (DE) content as well. The protein to energy ratio (g protein/kg diet to MJ digestible energy/kg diet) of a feed is a measure of the required balance, and clearly expresses the fact the feed intake is dependent upon energy requirements (Steffens, 1989).

In research involving tropical ornamental fish, Degani and Gur (1992), using practical feed ingredients including fish, wheat and corn meals, determined that the optimal dietary crude protein content for the pearl gourami (*Trichogaster leeri*) lies between 26 and 36 % (dry mass). In guppies (*Poecilia reticulata*), which are closely related to swordtails, it has been demonstrated that optimal growth occurs on diets containing a crude protein level of 47 % (Dahlgren, 1980).

The aim of the present experiment was to determine an optimal protein level for maximum growth in juvenile *X. helleri*, and to attempt to establish an optimal protein to energy ratio for the species. The soya oil cake and fishmeal combination developed in Chapter 2 was used in the feed formulations. The nutritional indices used to evaluate these factors included growth rate, final mass and length, feed conversion ratio, protein efficiency ratio and proximate whole body composition.

MATERIALS AND METHODS

Experimental Diets

Nine test diets were formulated to combine three protein levels (45, 38 and 30%) with three digestible energy levels (16,5, 14,5 and 12,5 kJ/gram) in a three by three factorial design (Table 3.1).

Digestible energy values of the various dietary components have not been quantified for *X. helleri*. Thus, on the basis of a review of the available literature, the "physiological fuel values" of 18,9 kJ, 16,8 kJ, and 37,7 kJ per gram feed for protein, carbohydrate and lipid respectively (Pike and Brown, 1967), were used to approximate the digestible energy content of the

experimental diets. These values have been used in similar studies on other species of warmwater fish (*Oreochromis niloticus*; Wang *et al.*, 1985 and *Clarias gariepinus*; Uys 1989). Pregelatinized corn starch was used as a primary source of carbohydrate, while a combination of 50% marine fish oil and 50% sunflower oil was used as a lipid source.

In preparation of the diets, the guidelines for experimental feed preparation described by Lovell (1989) were followed. All dry ingredients were thoroughly mixed, using a domestic food blender. Oils were then gradually added, while mixing constantly. Eighty five ml of water per 100 grams of feed was then slowly blended into the mix, resulting in a suitably textured dough. This was then further processed through a commercial cold-extrusion pasta-maker, to obtain thin spaghetti-like strips. Drying was carried out in a convection oven at 30 °C for 24 hours. The dry product was finally ground and sieved into a particle size of between one and two millimetres diameter. All feeds were stored at -20°C until required.

Experimental System

A closed, fully recirculating system incorporating both trickle and submerged types of biological filtration was used for this trial (Figure 3.1). Thirty 20 litre glass aquaria were arranged in three tiers, with three replicates for each treatment being allocated to tanks according to a randomized block design. Water temperature was controlled at a constant 27°C for the duration of the experiment. Heating was provided by a 2 kW element in the system sump tank, linked to a solid phase thermostat. Total volume of the system, including sump tank and filters was 1300 litres. Flow rate to the tanks was 1 litre per 90 seconds, resulting in 2 water changes per hour. Ambient air temperature in the room housing the aquaria was controlled by means of an air conditioner. Aeration was supplied via an airstone in each tank, coupled to a low pressure, sidechannel air blower. A 14 hour light : 10 hour dark photoperiod was maintained through fluorescent lighting controlled by an automatic timing device. All tanks were siphoned clean weekly, while water pH and total ammonia were tested at regular intervals.

TABLE 3.1. Formulation and proximate composition of experimental diets containing three digestible energy and three protein levels. Diets are labelled according to their protein percentage and energy content (H = 16,5 kJ/g; M = 14,5 kJ/g; L = 12,5 kJ/g). Soya OCM = Soya oil cake meal.

INGREDIENT	DIET								
(% Dry Mass)	30H	30M	30L	38H	38M	38L	45H	45M	45L
Fishmeal	30,5	30,5	30,5	39	39	39	45,5	45,5	45,5
Soya OCM	16,5	16,5	16,5	21	21	21	24,5	24,5	24,5
Fish Oil	4,5	2,5	1,5	4	2	1	3,5	1,5	0,5
Sunflower Oil	4,5	2,5	1,5	4	2	1	3,5	1,5	0,5
Starch	34	31	23	24	21	13	15	12	4
Mineral Premix	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
Vitamin Premix	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6
Vitamin C	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4
Cellulose	8,8	15,8	25,8	6,8	13,8	23,8	6,6	13,8	23,8
CALCULATED PROXIMATE COMPOSITION									
Crude Protein	30	30	30	38	38	38	45	45	45
Lipid	12	8	6	12	8	6	12	8	6
Carbohydrate	38	35	27	29	26	18	21	18	10
% Carbohydrate as pregel. starch	89,5	88,6	85,2	82,7	80,8	72,2	71,4	66,6	40
DE (kJ/gram)	16,5	14,5	12,5	16,5	14,5	12,5	16,5	14,5	12,5
P:E Ratio (mg/kJ)	18,2	20,7	24	23	26,2	30,4	27,3	31	36

Vitamin and Mineral Premix (per kg premix): Choline chloride 166g; Copper 0,05g; Mangenese 4g; Zinc 11g; Iodine 0,2g; Iron 0,5g; Vitamin A 1300000IU; Vitamin D3 200000IU; Vitamin E 10000IU; Vitamin K3 4500IU; Vitamin B1 1600mg; Vitamin B6 1350mg; Vitamin B12 4mg; Folic acid 580mg; Biotin 55mg; Calcium pantothenic acid 11g; Niacin 19g; Antioxidant 20g; Inositol 58g; Carophyll pink 3,4g. Vitamin C provided as "Stay C" (150mg Vitamin C per gram Stay C)

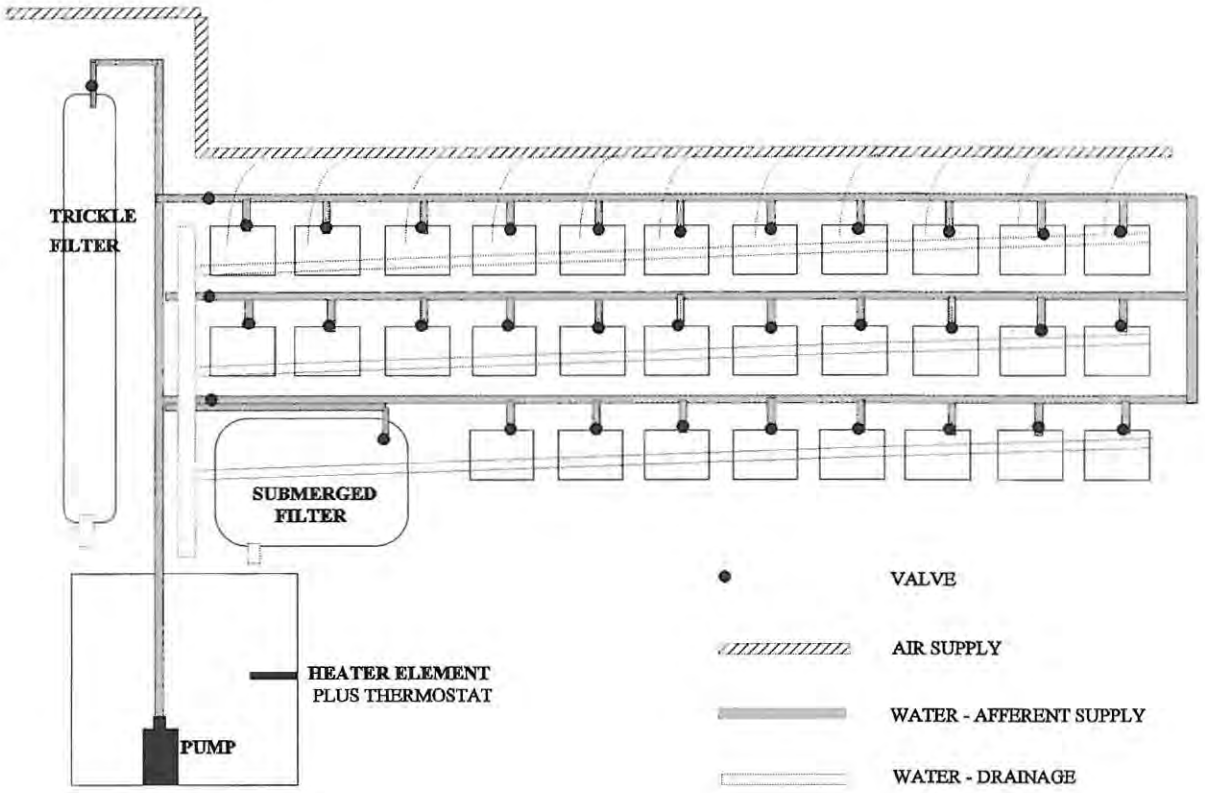


FIGURE 3.1. Indoor environmentally controlled aquarium system: Schematic Diagram - Side View.

Experimental Fish: Feeding and Handling

Juvenile (6-8 week old) *X. Helleri* (Red Victory strain) were obtained from Amatikulu Hatchery. These were isolated in quarantine tanks for a period of two weeks before being transferred to the experimental tanks. Eleven fish were allocated at random to each tank and were held for a further one week acclimation period, before the feeding trial was begun. At the beginning of the trial period, and every ten days thereafter, fish were weighed and measured. All fish were anaesthetized using a solution of 0,4 ml 2-phenoxyethenol per litre water, gently blotted using a paper towel to remove excess water, and individually weighed (using a Mettler 3000 Electronic Scale) and measured to the nearest millimetre (standard length). When feeding, fish were fed to satiation, without excessive wastage of food. This was done by direct observation of feeding, with food being provided twice daily in small amounts until it was observed that feeding activity

ceased. Fish were not fed on the days when weighing and measuring were performed. The trial was run for a period of 60 days.

Chemical Analyses

Proximate analyses of the feed ingredients (fishmeal and soya oil cake meal) were performed according to the methods of the Association of Official Analytical Chemists (AOAC, 1984). A random sample of twenty fish were sacrificed prior to beginning the feeding trial and were analysed for moisture content, ash, crude protein and lipid content as described in Chapter 2. At the end of the experimental period, fish from each treatment were sacrificed and analysed as described above. Due to the small size of the fish, replicates within each treatment were pooled in order to obtain a suitable sample size for analyses.

Data Processing and Statistical Analyses

The following nutritional indices were used to evaluate the performance of fish fed the experimental diets:

Food conversion ratio (FCR) :

FCR = Food Intake (Dry Weight) / Body Weight Gain (Wet Weight)

Protein efficiency ratio (PER) :

PER = Body Weight Gain (Wet Weight) / Protein Ingested (Grams)

Percentage protein deposited (PPD):

PPD = (Final Body Protein - Initial Body Protein)*100 / Protein Fed

Data on final weights, FCR, PER and feed, protein and DE consumption were analysed using ANOVA and Tukey's Multiple Range Test with significance declared at P = 0,05. Simple regression analysis was performed on growth data, with multiple regression and comparison of F-values employed to establish the degree of variance between replicates, and between treatments.

A one-tailed F-test based on an analysis of co-variance (Zar, 1984) was used. Data from the three replicates for each treatment was pooled, having established that no significant difference ($P< 0,05$) was present between slopes.

RESULTS

Water temperature was maintained at 27°C throughout the duration of the experiment. Total ammonia was measured weekly and remained below 0,01mg/l. pH was monitored regularly, and with exception of one reading on day 21 of the trial, remained at $7,3 \pm 0,2$. On day 21, the pH dropped to 6,0, probably as a result of the gradual acidification effect of the biological filtration system. Addition of oyster shell to the submerged filter resulted in normalisation of the pH within twenty four hours.

The growth rates for the fish fed all the 30 and 38 % protein diets did not differ significantly, however, the high and low energy 45% protein diets (16,5 and 12,5 kJ/g respectively) produced significantly more rapid growth (Table 3.2, 3.3; Figure 3.2). Furthermore, growth rates of fish fed the high and medium energy 45% protein diets (16,5 and 14,5kJ/g respectively) differed significantly, indicating that dietary energy content significantly affected growth within the 45% protein level. These trends were reflected in the final weights of the fish (Table 3.2).

No clear trend was apparent between feed consumption and P:E ratio. The fish fed the 45% protein diets consumed significantly more protein during the experiment, and the level of dietary DE had no apparent effect on this consumption. However, in the 38% protein treatments, fish fed the low DE diet, ingested significantly less protein than the medium or high DE groups. A similar pattern was recorded in the 30% protein treatments. Digestible energy intake was positively related to the DE content of the diets (Table 3.2).

Although significant differences were present in PER between treatments, no clear trends were apparent. As protein level in the diet increased, so the feed conversion ratio improved and those for the high protein diets 45H and 45L were significantly ($P < 0,05$) lower than for the other treatments (Table 3.2; Figure 3.4). Similarly, no clear trends in the PPD results, which ranged

from 23% for the 38M diet to 61,7 for the 45L diet, emerged.

TABLE 3.2 Mass and length parameters, feed, DE and protein consumption, feed conversion ratio, protein efficiency ratio, percentage protein deposited and mortality of swordtails fed diets with different protein to energy ratios for 60 days. Results represent the mean of 3 replicates (± SD). Diets are labelled according to their protein percentage and energy content (H = 16,5 kJ/g; M = 14,5 kJ/g; L = 12,5 kJ/g).

	DIET NUMBER								
PARAMETER	30H	30M	30L	38H	38M	38L	45H	45M	45L
Protein Content (% Dry Mass)	30	30	30	38	38	38	45	45	45
Digestible Energy (kJ/gram)	16,5	14,5	12,5	16,5	14,5	12,5	16,5	14,5	12,5
Initial Weight	0,52± 0,03g	0,52± 0,03g	0,5± 0,03g	0,51± 0,03g	0,52± 0,03g	0,51± 0,03g	0,54± 0,03g	0,52± 0,03g	0,51± 0,03g
Final Weight	$0,87^{a}$ ±,03g	$0,86^{a}$ ±,03g	0.81^{a} ±,03g	0.88^{a} ±,03g	$0,79^{a}$ ±,03g	$0,84^{a}$ ±,03g	$1,07^{bc}$ ±,03g	$0,91^{ab}$ ±,03g	$1,14^{c}$ ±,03g
Initial Standard Length (mm's)	25,70 ±0,35	25,97 ±0,35	25,67 ±0,35	25,58 ±0,35	26,15 ±0,35	25,79 ±0,35	26,27 ±0,35	25,97 ±0,35	25,61 ±0,35
Final Standard Length (mm's)	30,61 ±0,35	30,31 ±0,35	29,67 ±0,35	30,64 ±0,35	30,12 ±0,35	30,15 ±0,35	32,79 ±0,35	31,54 ±0,35	33,76 ±0,35
Feed consumed per fish (g dry mass)	$1,44^{bc}$	$1,48^{c}$	$1,26^{a}$	$1,38^{b}$	$1,42^{bc}$	$1,23^{a}$	$1,37^{b}$	$1,38^{b}$	$1,42^{bc}$
Protein consumed per fish (g)	$0,43^{b}$	$0,44^{bc}$	$0,38^{a}$	$0,53^{d}$	$0,54^{d}$	$0,46^{c}$	$0,62^{e}$	$0,62^{e}$	$0,64^{e}$
Total DE intake per fish (kJ)	$23,8^{f}$	$21,5^{d}$	$15,8^{a}$	$22,8^{ef}$	$20,6^{cd}$	$15,4^{a}$	$22,6^{e}$	$20,0^{c}$	$17,8^{b}$
FCR	$4,0^{bc}$	$4,4^{bc}$	$4,1^{bc}$	$3,9^{bc}$	$4,7^{c}$	$3,9^{bc}$	$2,7^{a}$	$3,6^{b}$	$2,5^{a}$
PPD*	37,7	41,1	38,2	34,5	23,0	39,4	42,1	41,1	61,7
PER	$0,85^{a}$	$0,7^{abc}$	$0,81^{ab}$	$0,7^{abc}$	$0,56^{c}$	$0,7^{abc}$	$0,85^{a}$	$0,62^{bc}$	$0,89^{a}$
Mortalities	1	0	0	0	0	0	1	1	0

Figures in the same row with different alphabetic superscripts differ significantly ($P < 0,05$).

* No statistical comparison of PPD values was performed.

Refer to Appendix for Anova statistics (dF, F- ratio and level of significance).

TABLE 3.3. Linear regression equations of mass data for each dietary treatment (3 replicates pooled) for 60 day experimental period. Equations in same column sharing a common superscript are not significantly different (P > 0,05).

Dietary Treatment	% Crude Protein	Digestible Energy (kJ/g)	Regression Equation
30H	30	16,5	$y = 0{,}530 + 0{,}005x^{a}$
30M	30	14,5	$y = 0{,}528 + 0{,}005x^{a}$
30L	30	12,5	$y = 0{,}514 + 0{,}005x^{a}$
38H	38	16,5	$y = 0{,}526 + 0{,}005x^{a}$
38M	38	14,5	$y = 0{,}551 + 0{,}004x^{a}$
38L	38	12,5	$y = 0{,}519 + 0{,}005x^{a}$
45H	45	16,5	$y = 0{,}573 + 0{,}008x^{ab}$
45M	45	14,5	$y = 0{,}554 + 0{,}006x^{bc}$
45L	45	12,5	$y = 0{,}487 + 0{,}011x^{a}$

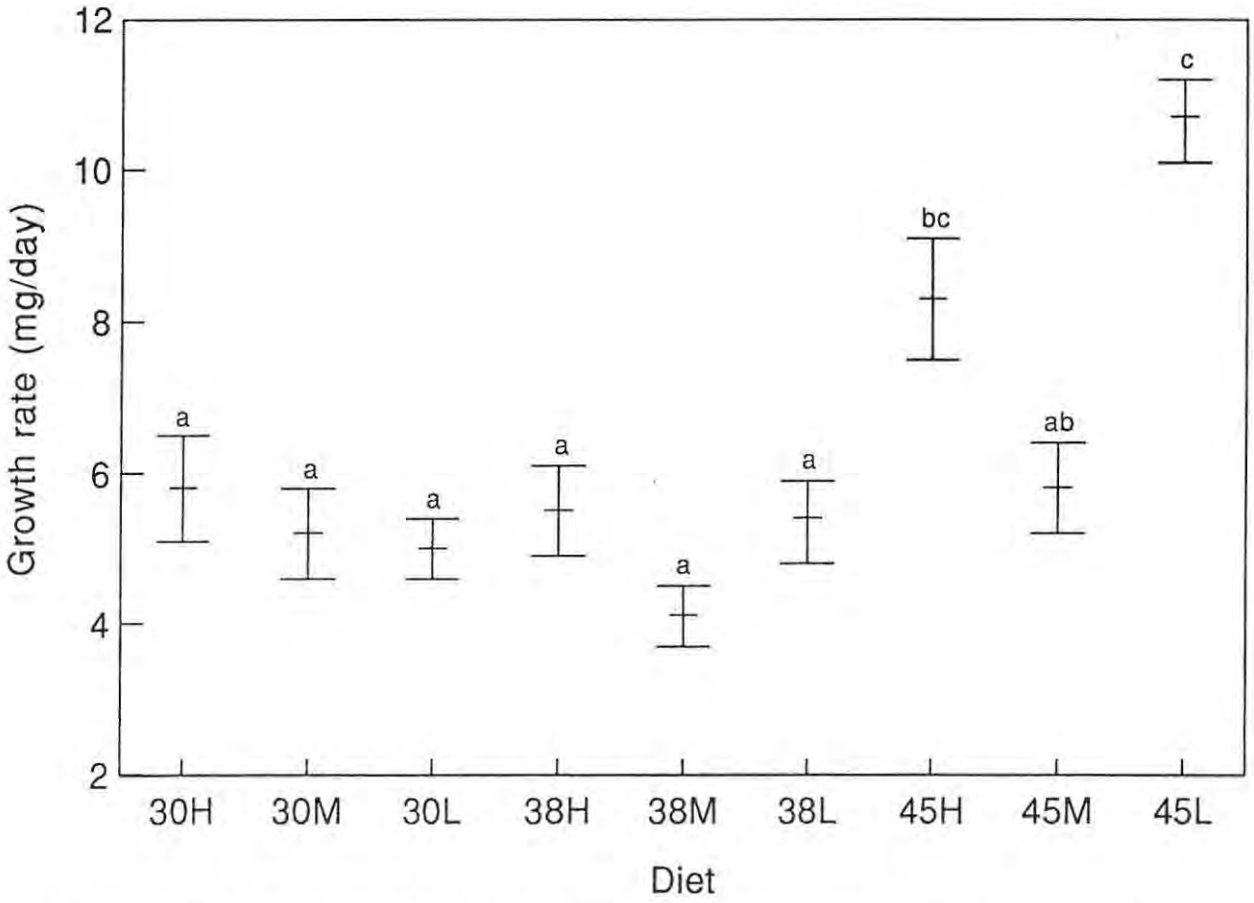


FIGURE 3.2. Rate of weight gain in *X. helleri* juveniles fed diets containing 3 crude protein and 3 apparent digestible energy levels over a 60 day period. Rates were derived from linear regressions. Bars represent Standard Error of slope. Points sharing a common superscript are not significantly different (P > 0,05).

Statistical testing of differences between treatments could not be calculated for the results of body composition analyses, as the quantities of tissue available for processing necessitated pooling the tissue from 3 replicates for each treatment. However, the following trends were apparent: within the 45 % protein treatments, the diet containing the highest energy level (diet 45H) resulted in greater fat deposition and a lower protein content than the other treatments in this group (Table 3.4). A similar pattern was evident in the 38 % protein treatments, with the low energy diet (38L) producing fish with relatively low total body lipids. Within the 30 % protein level, no obvious trends in final body composition were observed. No clear trends in ash or moisture content were evident.

TABLE 3.4. Initial and final whole body composition data expressed as a percentage of dry mass.

Component	Initial	Final Composition								
		30H	30M	30L	38H	38M	38L	45H	45M	45L
Moisture	75,20	72,24	73,09	73,03	73,33	75,17	73,40	71,90	73,66	74,58
Protein	51,07	49,14	51,99	49,43	50,31	49,38	52,46	50,16	57,29	57,44
Lipid	25,84	29,55	27,84	28,89	25,93	26,47	22,22	27,78	18,75	20,44
Ash	13,46	11,17	11,64	11,80	11,00	12,68	11,67	9,48	11,47	11,50

The final protein content of the fish was positively correlated to the dietary protein to energy ratio, while the final lipid content was negatively correlated (Figure 3.3).

Mortalities during the experiment were minimal (Table 3.2).

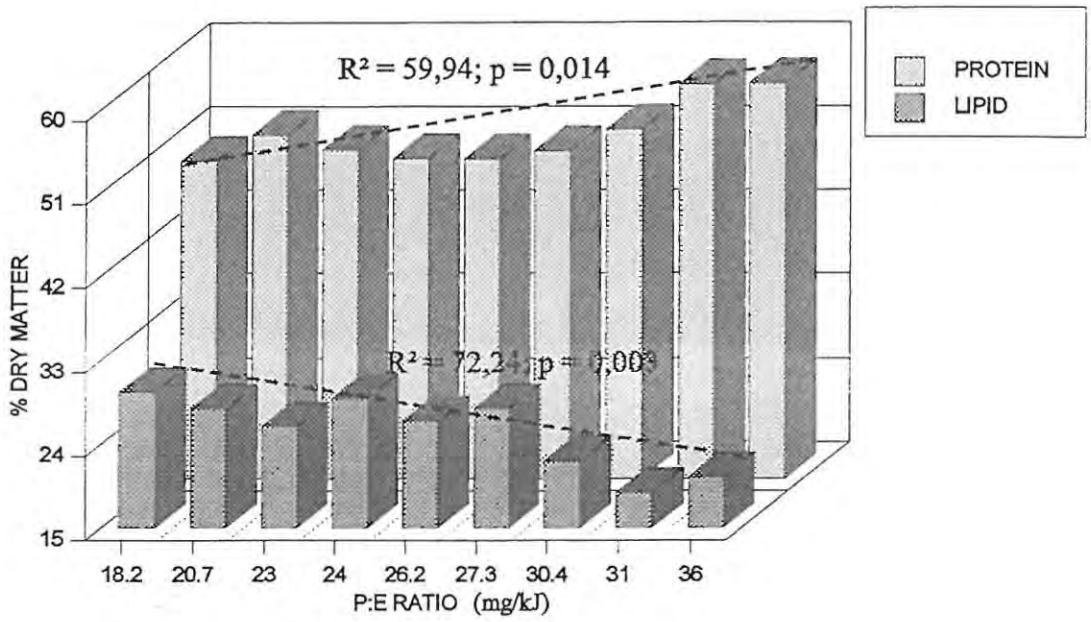


FIGURE 3.3. Relationship between the dietary protein to energy ratio and the final body protein and lipid composition of fish sampled from each treatment.

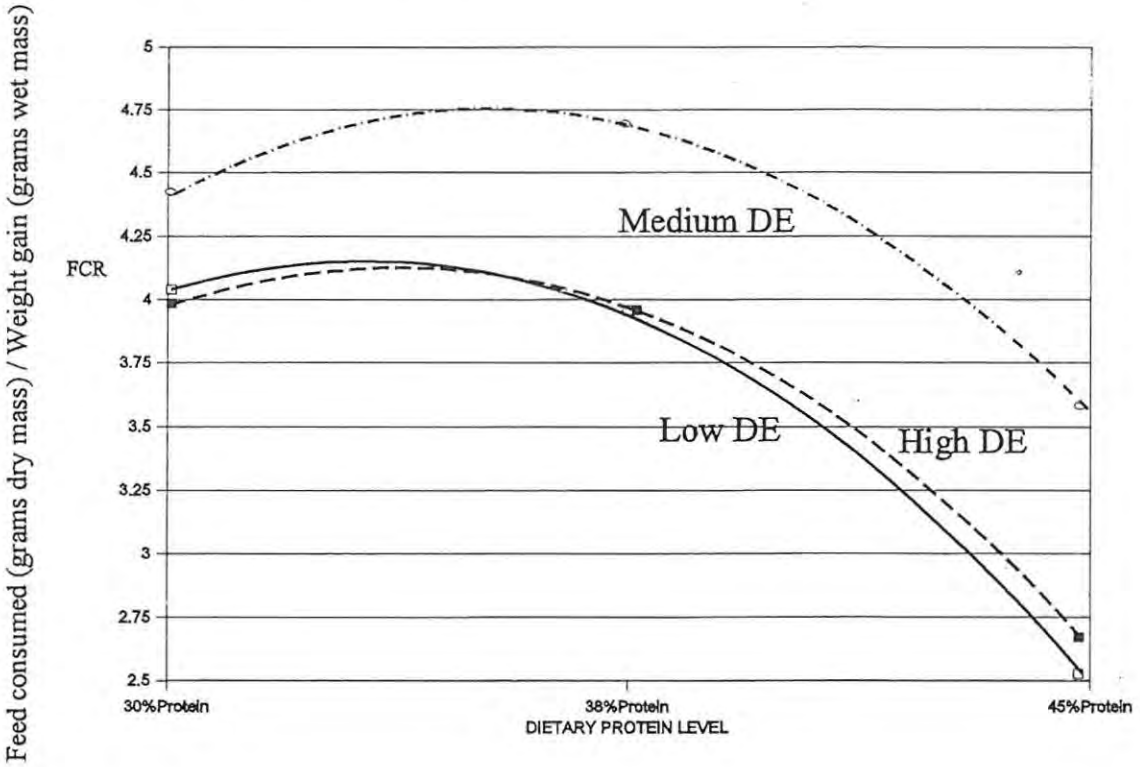


FIGURE 3.4. The effect of dietary protein level at three digestible energy levels on food conversion ratios of *X. helleri* after 60 days. Curves fitted by eye.

DISCUSSION

Although the protein to energy ratios of the experimental diets ranged from 18,2 to 36 mg crude protein/kJ DE, no clear relationship between growth rate and P:E ratio emerged. Nonetheless, it was clear that diets containing 45% protein (P:E ratio of ≥ 27) produced the fastest growth and most efficient feed conversion. Only at a 45% protein level did the dietary energy level significantly affect growth rate, and despite similar quantities of feed being consumed, the best growth rate was observed in the diets containing the highest P:E ratio (36 mg/kJ). This suggests that a relatively high P:E ratio promotes optimal growth in juvenile swordtails. Recommendations on the optimal dietary protein to energy ratios for other warmwater fish indicate a similar range (Table 3.5).

TABLE 3.5. Dietary protein to energy ratios for selected fish species (mg protein/kJ Digestible energy).

SPECIES	P : E RATIO	TEMPERATURE*	REFERENCE
Ictalurus punctatus	24 - 30	27°C	Steffens, 1989
Oreochromis aureus	31	31°C	Steffens, 1989
Morone chrysops	33	27°C	Nematipour *et al.*, 1992

* Water temperature at which research was conducted.

No depression in feed consumption was apparent in fish fed the high energy diets during this experiment. It is possible that satiation was determined by gut fullness, and those fed the higher protein diets simply consumed more protein and grew faster as a result. Feeding frequency may have restricted overall consumption, and it is possible that a more frequent feeding regime (three or four times daily) may have resulted in a more realistic pattern of satiation feeding, with the emergence of trends in the nutritional indices that could have been related to dietary energy content in the 38 and 30% protein treatments.

Efficiency of feed conversion improved with increasing dietary protein content, and this result, seen together with the high growth rates obtained on the 45% protein diets implies that a high protein diet is optimal in the grow out phase of *Xiphophorus* culture. This suggestion is supported by the findings of similar studies performed on other poeciliid fish (Dahlgren, 1980).

No clear trends were evident in PER's, suggesting that there was no significant "protein sparing effect" due to the extra lipid and carbohydrate in the 38 and 30% protein diets.

Final body protein and lipid composition was apparently correlated to the P:E ratio in this trial (Figure 3.3), and the increase in somatic lipid accumulation with increased total dietary energy content is consistent with the results of similar studies in other fish species (Garling and Wilson, 1976; Bromely, 1980; Nematipour *et al.*, 1992).

In conclusion, the results of this experiment indicate that, for optimal growth of juvenile *X. helleri*, a high protein diet (45% protein) with a protein to energy ratio of at least 27 mg protein / kJ DE is recommended.

CHAPTER 4

THE EFFECT OF FEED PROCESSING AND FORM ON GROWTH AND NUTRITIONAL INDICES OF SWORDTAILS.

INTRODUCTION

Various methods of feed processing are available for the preparation of fish feeds for commercial production of ornamental fish species. In South Africa, drum-dried flake diets manufactured for hobbyist use are commonly used on ornamental fish farms. Flakes are formed by exposing a pre-mixed slurry of finely ground ingredients to high temperature on revolving drums. A very thin layered product is produced, with a moisture content of below ten percent. The physical characteristics of the feed ingredients used, the type of machine employed, drum temperature and exposure time of the mixture to heat all play a role in determining the type and quality of flake produced.

Alternate methods used in production of dry artificial fish feeds include the process of cold extrusion, resulting in a pellet or a crumble type diet, and heat (steam) extrusion, to produce either floating or sinking pellets.

Advantages of flake-type foods over other types of preparations for ornamental fish include palatability, soft texture, buoyancy, a degree of water stability, and in the aquarium trade, an aesthetically pleasing appearance.

The major disadvantage is a high loss of nutrients, in particular of the water soluble vitamins such as ascorbic acid, through denaturation as a result of exposure to high temperature as well as via direct leaching during processing, and upon exposure to water during feeding. For example, in a study where flakes were prepared on a drum at 160 - 165°C with an exposure time of only 8 seconds, and a pre-drying slurry temperature of 90°C, seventy six percent of ascorbic acid activity was lost (Boonyaratpalin and Lovell, 1977). Although more heat stable forms of vitamin C are now commonly used, even these are susceptible to degradation under extreme environmental

conditions.

A great advantage of pellet or crumble type diets is the potential reduction in the loss of nutrients through leaching, due to the increased density of the feed particle, and the lower surface area to volume ratio in comparison to a flake.

Depending on species specific feeding habits and adaptations, certain ornamental fish will more readily accept a feed particle which floats, or sinks very slowly, such as a flake diet. It is thus important to establish, for each species under consideration, the acceptability and palatability of the feed particle used. In preliminary trials it was found that *X. helleri* readily consumed feed from both the water surface and tank bottom, provided the diet particle was small enough for ingestion.

Since no previous research has been performed examining the effect of the method of feed processing (temperature) and particle form on growth indices in ornamental fish, the present study was undertaken. The aim of this experiment was thus to compare, using growth rate, survival rate and incidence of ascorbic acid deficiency symptoms as test parameters, the nutritional value of identical dietary formulations exposed to different methods of feed processing. Vitamin C was selected as an indicator of nutrient deficiency as it is highly labile and is commonly implicated in nutritional deficiencies in fish.

Vitamin C is an essential nutrient for most fishes (Halver, 1989). It has the chemical formula $C_6H_6O_6$ and occurs in nature in three common forms: L-ascorbic acid, the biologically most active form; dehydroascorbic acid, the less active oxidized form and L-ascorbate 2-sulphate, a storage form of the vitamin which is synthesized in fish tissues. L-ascorbic acid is rapidly and easily oxidized to the inactive form by atmospheric oxidation, with this process being catalyzed by heat or exposure to copper, iron or several other metals. Ascorbic acid is also very water soluble, an important consideration in the manufacture of fish feed. Due to its labile nature, more stable forms of vitamin C are used in preparation of commercial feeds (Hilton *et al.*, 1977; Soliman *et al.*, 1987; Lovell, 1990).

L-ascorbic acid plays a role as a biological reducing agent for hydrogen transport, and forms an integral part of numerous enzyme systems. As such, it is involved in biochemical reactions of practically all groups of nutrients. In particular, vitamin C is required for many hydroxylation reactions, including hydroxylation of the lysyl and prosyl groups in procollagen, and synthesis of various amino acids. Due to its involvement with collagen formation and normal fibroblast function, ascorbic acid is essential for normal bone, cartilage, tooth and intercellular ground substance production, as well as for normal wound healing. Ascorbic acid, being a reducing agent, also plays a role synergistically with vitamin E and selenium, in the maintenance of intracellular antioxidant activity. Furthermore, vitamin C is required for normal absorbtion and metabolism of iron in the body, and as such, plays an important role in erythrocyte production and maturation. Further functions include involvement in steroid synthesis, various detoxification reactions (Mayer *et al.*, 1978; Yamamoto and Inoue, 1985; Mazik *et al.*, 1987) and a role in immunoglobulin production and function of phagocytes.

The most obvious clinical signs observed in cases of severe ascorbic acid deficiency include scoliosis and lordosis, leading to gross spinal abnormalities. This occurs as a result of reduced collagen formation in the vertebral column, with subsequent subluxation or luxation of vertebrae. These changes, once present, are of a permanent nature, even if dietary ascorbic acid levels are restored to normal. Other symptoms that have been noted include anorexia, reduced growth rates, fin malformation - in particular caudal fin abnormalities - lethargy, haemorrhagic exophthalmia, ascites, anaemia, intramuscular petechiation, poor wound healing (Ashley *et al.*, 1975) and depigmentation.

Very little published data dealing with the vitamin C requirements of ornamental fish is available, and the vitamin C inclusion levels used in the formulation of feeds for these species are based on studies performed on food fish species. Various researchers have shown that the quantitative requirement in fish is dependent upon a number of factors, including fish size, water temperature, environmental stress or exposure to pathogens or toxins (Hilton *et al.*, 1978; Dabrowski *et al.*, 1988; Dabrowski and Ciereszko, 1993).

Perhaps more important, in the context of commercial fish feeds such as the flake diets tested in

this trial, are the subclinical deficiency signs which may occur, especially immunosuppression via decreased antibody formation and inefficient phagocyte function (Durve and Lovell, 1982; Li and Lovell, 1985; Wahli *et al.*, 1986; Navarre and Halver, 1989).

Due to the suspected high losses of vitamin C and other water-soluble, heat labile nutrients during drum-drying and through leaching from flake feeds, doubt existed as to whether vitamin C specifications designed for other fish feeds would be adequate in ornamental flake feeds. Diets formulated from the same batches of raw ingredients, at identical inclusion levels, were prepared as drum-dried flakes, heat-treated crumbles, and cold-extruded crumbles, and evaluated in a growth trial with juvenile swordtails.

MATERIALS AND METHODS

Experimental Diets

Two dietary formulations, an open semi-purified formulation and a proprietary commercial formulation, were used to manufacture heat-treated flake and crumble diets as well as cold extruded crumbles (Table 4.1). Although the exact formulation of the commercial diet could not be disclosed, the major protein sources included fish meal and soya oil cake meal, while soya oil and a fish oil (Marinol-R) were used as a lipid source. Carboxymethylcellulose (CMC) was included in diets as a binding agent.

During production of the drum-dried flake feeds, the premix slurry was maintained at a temperature of 90°C, while the drum temperature ranged between 160°C and 170°C. Processing times were between 5 and 10 minutes as a slurry and 2 to 3 minutes on the drum.

The cold extruded diets were manufactured according to the method described by Lovell (1989). All dry ingredients (ground to the same particle size as used in the flake feeds), were thoroughly mixed, using a domestic food processor. The oils were then slowly added, while mixing constantly, after which 85 ml water per 100 grams of premix was added to obtain a suitably textured dough. Extrusion was then carried out using a commercial cold extrusion "pasta

machine". The resultant thin strips of food were subsequently dried in a convection oven at 30°C for 24 hours.

TABLE 4.1. Dietary formulations and proximate analyses (percentage dry matter). Formulations were used to manufacture heat treated flakes and crumbles as well as cold-extruded crumbles.

	Commercial Diet	Semi-Purified Diet
Fish Meal	Formulation supplied by manufacturer as complete premix. (oils, vitamin C and water added just prior to processing)	45,5
Soya Oil Cake Meal		24,5
Fish Oil ("Marinol-R")		3,5
Soya Oil		3,5
Pregelatinized Starch		18,6
Vitamin/Mineral Premix[1]		1,6
Vitamin C ("Stay-C")		0,8
Carboxymethylcellulose		2,0
	Proximate Composition (% Dry Mass)	
Crude Protein	41,8	45,0
Lipid	14,7	12,0
Carbohydrate	17,9	23,2

[1] Vitamin and Mineral Premix (per kg premix): Choline chloride 166g; Copper 0,05g; Mangenese 4g; Zinc 11g; Iodine 0,2g; Iron 0,5g; Vitamin A 1300000IU; Vitamin D3 200000IU; Vitamin E 10000IU; Vitamin K3 4500IU; Vitamin B1 1600mg; Vitamin B6 1350mg; Vitamin B12 4mg; Folic acid 580mg; Biotin 55mg; Calcium pantothenic acid 11g; Niacin 19g; Antioxidant 20g; Inositol 58g; Carophyll pink 3,4g.

To produce "heat-treated" crumbles, flakes produced from the two formulations were ground in a hammer mill, and extruded, using the technique described above. Seven test diets were produced from the two formulations using different processing techniques. (Table 4.2).

TABLE 4.2. Test diets prepared from commercial and semi-purified feed formulations.

1.	Commercial formulation *drum-dried* **flake** (excluding 2% carboxymethylcellulose).
2.	Commercial formulation *drum-dried* **flake** (including 2% carboxymethylcellulose).
3.	Commercial formulation *drum-dried* flake reprocessed as a **crumble**.
4.	Commercial formulation as *cold-extruded* **crumble**.
5.	Semi-purified formulation *drum-dried* **flake**.
6.	Semi-purified formulation *drum-dried* flake reprocessed as a **crumble**.
7.	Semi-purified formulation as cold-extruded **crumble**.

All diets were stored at -20°C until required for feeding. In order to ensure equal acceptability and palatability all diets were ground and sieved to the same particle size range before storage (200 to 400 μm diameter).

In this experiment "Stay-C®" (Roche Products), a powder form of L-ascorbyl-2-polyphosphate, containing a mixture of tri-, di-, and monophosphate esters of ascorbic acid, was used. This product has a vitamin C activity specification of fifteen percent. Vitamin C was added to all the above diets during mixing at a level of 8 grams Stay-C® per kilogram dry mix, equivalent to an inclusion level of 1200 mg/kg active ascorbic acid. After processing, samples of the heat-treated flake diets (1 and 4) and corresponding cold-extruded crumble diets (5 and 7) were sent to Hoffman-La Roche, Basel, Switzerland for ascorbic acid assay.

After conclusion of the experimental period, a sample of the remaining stored commercial drum-dried flake formulation (Diet 1) was used in a leaching study in order to quantify the degree of leaching of vitamin C which occurs upon contact with water. 250 g of the diet, prepared as fed in the trial, was immersed in water at 28°C for a period of 10 minutes. The sample was then recovered and immediately freeze-dried for a period of 48 hours. A control, unleached sample was simultaneously freeze-dried. The dried samples were then forwarded to Hoffman-La Roche for determination of vitamin C levels.

Experimental System

A closed, fully recirculating system as described in Chapter 3 was used in this experiment (Chapter 3: Materials and Methods, Figure 3.1). Twenty one 20 litre glass aquaria were arranged in three tiers, with three replicates for each treatment being allocated to tanks according to a randomized block design. Water temperature was controlled at a constant 28°C for the duration of the experiment.

All tanks were siphoned clean weekly, while water pH and total ammonia were tested weekly. pH was determined by means of an electronic pH meter (pHep1-Hanna Instruments), while total ammonia was tested using a commercial colour reagent test kit. A pH of 7,4 ± 0,1 was maintained throughout the 8 week period, while total ammonia never exceeded 0,001mg/litre.

Experimental Fish and Feeding Regime

All test fish originated from a *Xiphophorus helleri* breeding population maintained at the Department of Ichthyology and Fisheries Science, Rhodes University. 420 ten day old fish, born within a 24 hour period were selected at random from the broods of three females. These were randomly distributed into 21 tanks, 20 fish per tank, and held in the experimental system for a one week acclimation period, during which they were fed a commercial ornamental fish diet (Tetra-Min® "Baby Fish Food for Livebearers") three times daily.

Feeding during the course of the trial was performed three times daily, with the exception of days when weighing of fish was carried out, when feeding was twice daily. An initial feeding rate of 20% body weight feed (on a dry matter basis) was adopted, with the required quantity weighed at the beginning of the first week. As soon as it became evident that not all food was being consumed, the feeding rate was adjusted for all treatments. Recalculation of feed quantities was done weekly, based on the last weight measurement of each replicate. Feeding rates for the rest of the experiment were as follows: week 2 = 15%; week 3-5 = 10%; week 6 = 7,5%; week 7 and 8 = 5%.

Weighing of fish was performed weekly, using the technique described in Chapter 3 (Materials and Methods). During the process of weighing, all fish were carefully examined for any morphological abnormalities, or swimming defects. Where these occurred, such observations were recorded. Any mortalities during the preceding week were also noted at each weighing.

Where physical abnormalities were observed at the end of the feeding trial, affected fish were anaethetised using 0,4ml 2-phenoxyethenol/litre water, and abnormalities recorded by taking photographs and radiographs of these fish for comparison with normal specimens.

Statistical Analysis

One-way analysis of variance, with multiple range testing (Tukey's) was used to determine statistically significant differences between means of final mass and feed conversion ratios.

In order to ensure utilization of all data points when comparing growth curves, simple and multiple linear regression of slopes was carried out. Analysis of covariance (Zar, 1984), with the use of a one-tailed T-test for F-values was used to determine whether significant differences in slopes were present. For all treatments, analysis of the slopes of growth curves for the three replicates within a treatment was first performed, and only if no significant differences between replicates was present, were data from the replicates pooled.

RESULTS

Ascorbic Acid Levels

Analysis of the level of ascorbic acid activity remaining in the diets after processing indicated that approximately one quarter of the included vitamin C was rendered inactive during the drum-drying process in both the commercial and semi-purified formulations (Table 4.3). By contrast, in the cold extruded preparations, sixteen percent of activity was lost in the commercial formulation and less than ten percent in the semi-purified diet.

TABLE 4.3. Vitamin C activity measured in feed samples after manufacture.

Ascorbic Acid Levels (mg/kg dry feed)

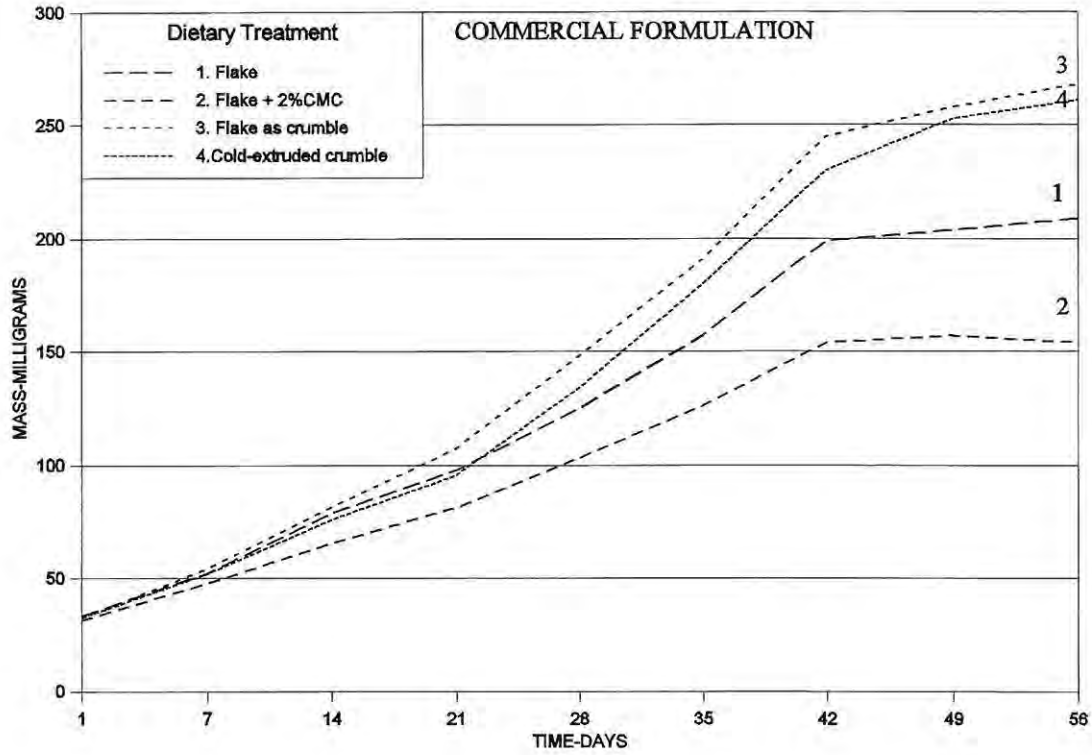
	Pre-processing Inclusion Level	Heat-Treated Flake	% Loss	Cold-Extruded Crumble	% Loss
Commercial Formulation	1200	866	27,84	1008	16
Semi-Purified Formulation	1200	910	24,17	1115	7,1

The vitamin C levels of the stored dietary samples used in the leaching study were 87 and 17 mg/kg for the unleached and leached samples respectively, indicating a loss of eighty percent of activity after ten minutes immersion in water. The low level of vitamin C measured in the control sample was attributed to losses which occurred during the storage period.

Growth Data

Growth data was compared separately for the commercial (Diets 1, 2, 3 and 4) and semi-purified formulations (Diets 5, 6 and 7).

Positive growth was recorded in all treatments but from day 42 of the experiment onwards growth rates were depressed (Figure 4.1). This corresponds with the stage at which the feeding rate was decreased to 5% body weight per fish per day, and indicates that this level was insufficient to provide adequate nutrition for maximum growth.



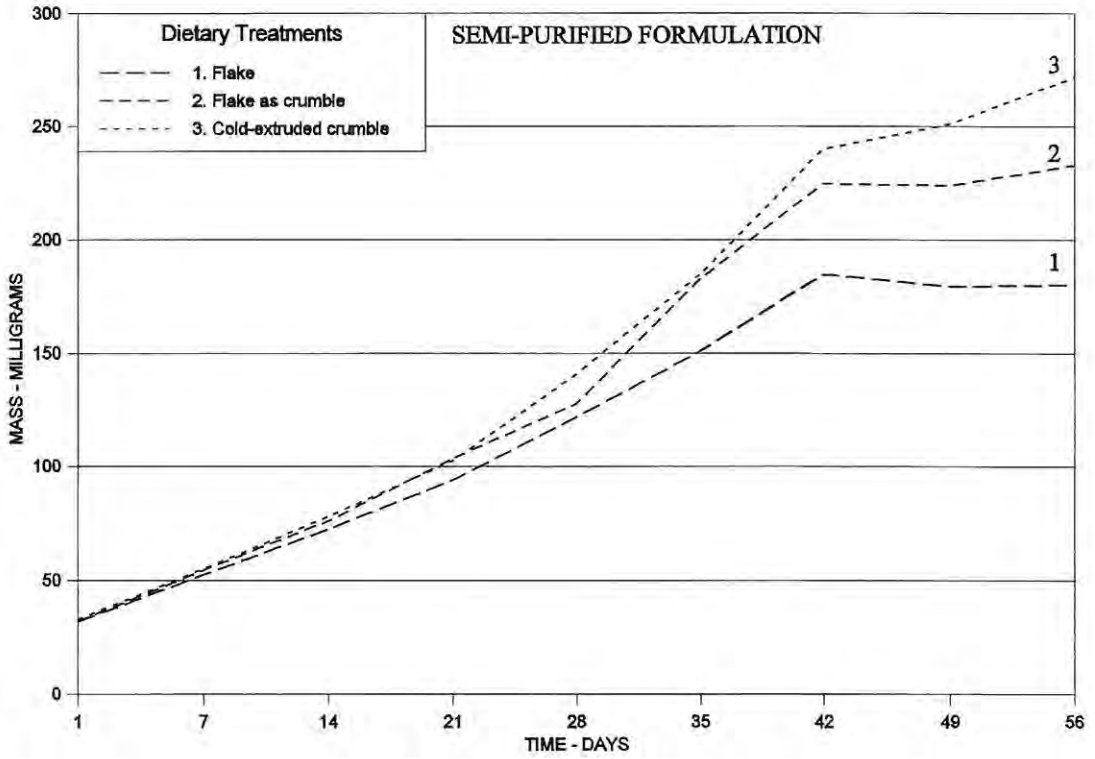


FIGURE 4.1. Rate of weight gain for *X. helleri* fed commercial and semi-purified formulations of drum-dried and cold-extruded diets, presented in flake and crumble forms. Each curve represents the pooled data from the three replicates for each treatment.

Since the growth curves became non-linear after day 42, regression analyses for the purpose of comparative analyses of slopes was performed on data only up to day 42. No significant differences ($P > 0,05$) were present when replicates within each treatment were compared, hence regression curves presented represent pooled data from three replicates for each treatment (Table 4.4).

TABLE 4.4. Linear regression equations of growth data for the seven experimental diets for the period day 1 to day 42. Treatments 1 to 4 = Commercial formulation; Treatments 5 to 7 = Semi-purified formulation.

Dietary Treatment	Regression Equation	R^2
Commercial Formulation		
1. Flake	$y = 22,876 + 3,925(x)^a$	97,15
2. Flake + 2% CMC	$y = 25,446 + 2,097(x)^b$	98,48
3. Flake as crumble	$y = 15,799 + 5,065(x)^c$	97,63
4. Cold-extruded crumble	$y = 14,937 + 4,702(x)^c$	92,71
Semi-purified Formulation		
5. Flake	$y = 23,938 + 3,667(x)^x$	98,12
6. Flake as crumble	$y = 17,599 + 4,648(x)^y$	96,53
7. Cold-extruded crumble	$y = 15,504 + 4,907(x)^y$	93,86

Equations in the column sharing a common superscript do not show a significant difference in slope ($P > 0,05$).

Comparison of the slopes of curves obtained for the crumble and flake diets prepared from the commercial formulation revealed significantly ($P < 0,05$) higher growth rates in the crumble formulations. Growth of fish fed the heat and cold treated crumbles did not differ significantly. However, fish fed the flake containing 2% CMC grew significantly slower than those fed the flake only.

In the second group (semi-purified diets), a similar trend emerged, with no significant difference ($P > 0,05$) between the crumble diets (diets 6 and 7), while the flake formulation (diet 5) yielded significantly lower growth rates ($P < 0,05$).

Results of analyses of final mass data paralleled those obtained by comparative analyses of regression curves .The average final mass of fish which were fed the flake diets (1, 2, and 5) were significantly lower ($P < 0,05$) than those fed the corresponding crumble diets (Table 4.5).
The crumble-type diets yielded significantly lower feed conversion ratios than the flake preparations for both the commercial and semi-purified diets ($P < 0,05$). No significant difference ($P > 0,05$) was apparent in feed conversion ratio between the heat and cold treated crumbles in the commercial formulation, however in the semi-purified group, feed conversion of the cold-extruded crumble was significantly lower than the heat treated crumble ($P < 0,05$) (Table 4.5).

TABLE 4.5. Initial and final mean masses, feed conversion ratios, percentage mortality and physical abnormalities recorded during the 8 week trial. Treatments 1 to 4 = commercial formulation ; Treatments 5 to 7 = Semi-purified formulation.

Treatment Number	Initial Mean Mass*(mg) ± SD	Final Mean Mass*(mg) ± SD	Mean FCR* (56 days) ± SD	Mortalities	Fish showing Scoliosis and/or Lordosis
1. Flake	31,3 ±0,57	209,6 ±11,0^{a}	3,02 ±0,23^{a}	18 %	18,4 %
2. Flake + CMC	32,6 ±2,30	154,3 ±12,6^{b}	3,72 ±0,11^{b}	10 %	14,8 %
3. Flake as crumble	32,6 ±1,52	268,6 ±14,6^{c}	2,61 ±0,15^{c}	5 %	0
4. Cold-extruded crumble	33,3 ±2,64	260,6 ±31,9^{c}	2,55 ±0,07^{c}	15 %	0
5. Flake	32,0 ±1,73	180,3 ±14,2^{d}	3,35 ±0,11^{d}	23	18,8
6. Flake as crumble	32,3 ±1,52	233,3 ±27,1^{e}	2,80 ±0,11^{e}	8	0
7. Cold-extruded crumble	33,0 ±2,64	271,6 ±21,4^{e}	2,50 ±0,12^{f}	17	0

* Means indicate average value for all fish in each treatment (3 replicates).
Values in the same column sharing a common superscript are not significantly different ($P > 0,05$).
Refer to Appendix for Anova statistics (dF, F- ratio and level of significance).

Mortalities occurred sporadically throughout the experimental period, increasing somewhat during the final 2 weeks, notably for the drum-dried flakes, treatments 1 and 5, where 60 % of the losses occurred during this period (Table 4.5). With the exception of the death of some fish showing

spinal deformities (treatment 1, 2 and 5), no diagnostic clinical lesions or aetiology of death could be ascertained.

Macroscopic spinal deformity was first observed in a few fish during the sixth week of the trial. (Figure 4.2; Figure 4.3a and 4.3b). Numbers affected increased rapidly during the final two weeks, but were confined to the flake diets (Table 4.5). In none of the crumble formulated diets (heat or cold treated) were any cases of scoliosis or lordosis observed (treatments 3, 4, 6 and 7).

FIGURE 4.2. Macroscopically visible scoliosis in juvenile *X. helleri* fed heat-treated flake diets. (Magnification 3 X)

Scoliotic fish Normal fish

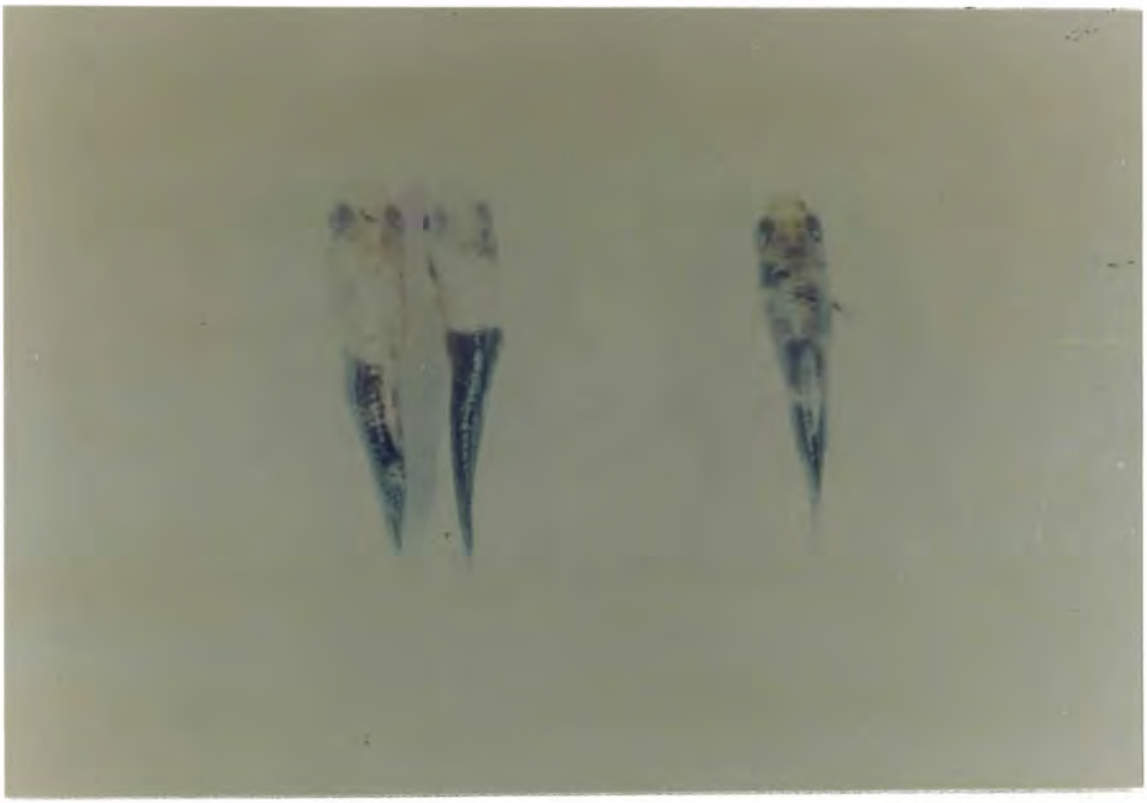


FIGURE 4.3a. Radiograph showing ascorbic acid deficiency induced vertebral subluxation in juvenile *X. helleri* fed heat treated flake diet. (Magnification 10 X).

FIGURE 4.3b. Radiograph comparing scoliotic with normal juvenile *X. helleri*. (Magnification 10 X).

DISCUSSION

Despite the inclusion of a relatively stable form of vitamin C, the drum-drying procedure employed in the production of the flake diets in this trial resulted in the loss of approximately one quarter of the included ascorbic acid. Previous research on formulated fish feeds has demonstrated even more marked losses, for example, Hilton *et al.* (1977) found that during heat extrusion of pellets, up to 70% of vitamin C activity was lost, with the onset of this process being initiated by the addition of water. Ten percent of these losses occurred within ten seconds of immersion of the dietary premix in water . Even where cold pelleting techniques are used, significant losses of vitamin C can occur. Sandnes *et al.* (1984) in preparation of experimental cold pelleted diets for trout, found that after processing and drying, ascorbic acid levels were reduced from an inclusion level of 1000 mg/kg dry feed to only 115 mg/kg.

The loss of 27,8 and 24,2 % vitamin C in the commercial and semi-purified flakes respectively still provided a final retention level of over 800 mg/kg dry feed in both diets, which was well in excess of the minimum vitamin C levels determined for other fish species. However, analysis of the commercial flake diet after completion of the trial revealed that almost 90 percent of vitamin C activity had been lost. This highlights the importance of potential nutrient losses which may occur during prolonged storage of fish feeds, and where feeds are manufactured in situ on commercial fish farms, production of large quantities of feed requiring storage should be avoided.

The poor growth rates, feed conversion ratios and increased incidence of vitamin C deficiency signs observed in fish fed the flake diets in comparison to those fed crumbles (either cold-extruded or reformulated from flakes) indicates that a substantial loss of nutrients occurred at the time of feeding, probably through leaching into the water. This hypothesis is further substantiated by the results of the leaching trial. Leaching of ascorbic acid into water during processing and at feeding thus appears to constitute a major cause of vitamin C loss. The rate of leaching is dependent upon a number of factors, including the solubility of the specific chemical form used, the duration of exposure, water temperature (Soliman *et al.*, 1987) and the physical binding properties and surface area to volume ratio of the individual feed particle. Yamamoto (1982) reported that, in his test diets, 99% of ascorbic acid activity was lost after only five minutes immersion in water.

In the present experiment, where flake feed was exposed to aquarium water at 28°C for 10 minutes, 80 % of the vitamin C activity was lost, further demonstrating the potential magnitude of nutrient losses which may occur in flake diets as a result of leaching. The physical deformities that occurred from the sixth week of the trial, including severe scoliosis and lordosis are typical of ascorbic acid deficiency in fish. In view of the vitamin C losses demonstrated in this experiment, both through heat treatment and more importantly via leaching from the flake diets, vitamin C deficiency was diagnosed as the aetiology for the spinal deformities. Perhaps as important as the macroscopic lesions observed, are the effects of vitamin C deficiency on the immunocompetence in fish, particularly in the context of large scale ornamental fish culture, where fish are subject to exposure to various environmental stressors and possibly high pathogen challenges. Although no measurements of immunocompetence were quantified in the present study, previous works (Navarre and Halver 1989; Durve and Lovell, 1982; Li and Lovell, 1985) suggest that dietary ascorbic acid level could be utilized as a practical management tool to minimize disease occurrence in aquaculture, especially under conditions of intensive production. Halver (1985) recommends that the minimum dietary vitamin C level for optimum growth of fish under ideal environmental conditions, be increased five to ten fold, where exposure to physical or environmental stressors, or potential toxins or pathogens occurs.

In commercial production of *X. helleri* and other tropical fish species, where fish are cultured in outdoor or plastic covered ponds exposed to sunlight, supplementation of dietary ascorbic acid is obtained through grazing on algae and aquatic fauna. However, where stocking densities are high enough to limit the availability of primary food, or where pond management is such that poor primary production is stimulated, through inadequate or no fertilization of ponds prior to stocking, subclinical or even clinical signs of ascorbic acid deficiency may occur. Lovell and Lim (1978) demonstrated that when channel catfish were held at high stocking densities in fertilized outdoor production ponds, additional supplementation of vitamin C was required in the diet in order to prevent the occurrence of scoliosis and lordosis in their experimental fish. Based on the results of the present experiment, where swordtails are kept in tanks providing little or no supplementary nutrition by way of primary production, a crumble-type diet is recommended, to ensure adequate vitamin C intake.

No apparent difference was observed between the heat and cold treated diets for the commercial formulation crumble diets, while in the semipurified diets, the cold-extruded type performed significantly better than the drum-dried form. This indicated that in this second group, the nutrient losses which occurred as a result of the heat processing were significant.

The ready consumption of the crumble diets by *X. helleri*, seen together with the significantly better growth rates, more efficient feed conversion and lowered incidence of deficiency signs, indicates that this form of presentation is superior to flakes in this species. On the basis of these results, a crumble diet, preferably produced via a cold-extrusion technique, offers a desirable alternative for commercial culture of swordtails, and other aquarium fish species which find this form of diet acceptable.

Where flake diets are used, additional vitamin C supplementation must be ensured, either through increased inclusion levels in the dietary formulation or via stimulation of primary production in the culture tanks. The use of a feeding strategy involving frequent, small feeds, ensuring rapid ingestion of feed may possibly also contribute to limiting nutrient losses through leaching.

CHAPTER 5

THE EFFECT OF LIVEFEED SUPPLEMENTATION ON GROWTH AND SURVIVAL OF JUVENILE SWORDTAILS.

INTRODUCTION

New born fry of livebearing fishes are relatively large and precocious in comparison to oviparous ornamental fish. As a result livebearers will readily accept artificial diets as a first feed, provided the particle size is small enough for ingestion, and the feed is palatable. In commercial livebearer production in South Africa, fish are commonly reared from birth to marketable age on flake feeds. In some instances a degree of primary production of phyto- and zooplankton is stimulated in grow out ponds by means of prior fertilization of pond water before stocking. This source of nutrition tends to be unpredictable, and does have associated disadvantages, for example, the attraction of predatory insects such as diving beetles and dragon flies to the green water, as well as large diurnal fluxes in pH and dissolved oxygen concentration caused by the algal bloom.

Young of oviparous fish such as the goldfish *Carassius auratus* are raised on livefeed including rotifers and brine shrimp during their early stages, and it is generally accepted amongst aquaculturalists that livefeed is vastly superior to artificial diets in young, rapidly growing fish of most species.

Beck (1978) stated that no single formulated artificial fish diet can provide equivalent survival or growth rates in comparison to livefeed, and despite the recent advancements in fish feed technology, livefeed organisms remain an indispensable feed supplement in the culture of many species. Shariff and Subasinghe (1992), in a discussion of the ornamental fish trade suggested that despite the use of so-called "complete" artificial formulations, the importance of livefeed in providing a suitable natural balance of vitamins and minerals could not be over emphasized. In recent research concerning the merits of artificial versus livefeed in juvenile fish, the growth performance of goldfish fry was notably improved when dry diets were supplemented with live brine shrimp (*Artemia salina*) (Mills *et al.*, 1993). Abi-Ayad and Kestemont (1994) also

demonstrated that goldfish fry fed a combination of artificial dry feed and brine shrimp exhibited better growth than did fish fed on either of the diets alone. Furthermore, their results indicated that the exogenous proteolytic enzymes obtained from the livefood played an important role in the digestion and assimilation of food, especially during the first three weeks of development. In the goldfish fry which received only dry feed, hepatic atrophy and later degeneration was evident.

The rationale behind the present experiment was to determine whether similar benefits to those described in goldfish fry would be manifested in *X. helleri* fry when fed on livefeed supplemented diets.

Daphnia sp. was selected as a representative livefeed organism for this experiment. *Daphnia* are already produced on many ornamental fish farms in South Africa and could thus be used practically in poeciliid culture. Furthermore, data on the nutritional value of cladocerans in general, and *Daphnia* in particular, is readily available. Preliminary trials revealed that swordtail young of thirty milligrams or more, readily caught and consumed *Daphnia* upon presentation. *Daphnia* have been shown to be more effective in promoting growth of young in certain freshwater fish species than other livefeeds such as *Artemia*. For example, in research on growth of young paddlefish (*Polyodon spathula*), another species capable of ingesting relatively large livefeed organisms at an early age, Webster *et al.* (1991) found *Daphnia pulex* to be the most effective livefood tested.

Colour is an important quality criterion in aquarium fish, as ornamental fish hobbyists tend to prefer fish exhibiting brighter and more clearly defined coloration. Fish obtain carotenoid pigments from their diet and crustaceans, including cladocerans such as *Daphnia* are known to constitute a rich source of carotenoid pigments (Hardy, 1989). Apart from their role in pigmentation, the exact physiological functions of carotenoids in fish have not been fully elucidated. However, it is thought that these pigments play an important role in protecting tissues against photodynamic damage and intracellular oxidation (Tacon, 1981).

The aim of this experiment was thus to evaluate the benefits of livefeed supplementation on growth rate, survival, degree of pigmentation and prevention of nutrient deficiency signs in

juvenile swordtails fed primarily on a dry artificial diet.

MATERIALS AND METHODS

Experimental Diets

Four dietary regimes were compared during the growth trial. Two drum-dried flake diets, one an in-house preparation formulated for general use on ornamental fish farms (referred to as the farm flake treatment) and Tetra Ruby®, a commercial flake designed for maintenance requirements and colour enhancement of ornamental fish (referred to as the commercial flake treatment), were fed as separate treatments. The third diet consisted of the farm flake supplemented daily with live *Daphnia* sp. (flake + *Daphnia* treatment), while in the fourth treatment, referred to as the Daphnia treatment, fish received *Daphnia* sp. exclusively.

The exact formulations of the flake diets were proprietary information, however the major protein sources of the farm flake included fish meal and soya oil cake meal, while soya oil and a fish oil (Marinol-R) were used as a lipid source. The specified proximate compositions of the farm and commercial flake diets are presented in Table 5.1. Both flake diets were milled and sieved to obtain an identical, readily ingestible particle size (200 - 400 μm diameter) and these were stored in a blast freezer at -20°C until required.

TABLE 5.1. Proximate composition of flake diets.

	Farm Flake	Tetra-Ruby®
Moisture Content	6,7 %	5,8 %
Crude Protein (Dry Matter)	41,8 %	45 %
Lipid (Dry Matter)	14,7 %	5 %
Vitamin C	1200 mg/kg Dry Feed[1]	>440 mg/kg Dry Feed[2]

[1] and [2] : L-ascorbyl-2-polyphosphate.

A series of four 3000 litre "portapool" plastic ponds were used for culture of *Daphnia* sp.. These were located outside under a cover of nylon shadecloth. Aeration was supplied to each pond.

Prior to filling with municipal tap water, ponds were sterilized with a dilute formalin solution, rinsed and allowed to dry completely. Once filled, the ponds were left standing, with vigorous aeration, for a period of four days, to ensure elimination of chlorine from the water. 400 ml of finely milled fishmeal was then mixed into the pond water as primary fertilization. Depending on water temperature, the pond was then allowed to stand for a further three to seven days, until a visible algal bloom was present. Seeding with *Daphnia* sp. from a pre-established pond was then carried out. Additional fertilization of cultures was performed every other day, using 30 ml ground fishmeal and 7 ml brewers yeast powder per tank.

Due to the unpredictable levels of production in the ponds, the cultures in the four tanks were initiated at one week intervals, thus ensuring a constant supply of sufficient *Daphnia* sp. throughout the experimental period.

Daily harvesting of *Daphnia* sp. was performed using a fine nylon aquarium net. The sample was then drained by blotting the net on paper towel for a period of 30 seconds in order to remove excess water.

Moisture content of the drained, concentrated *Daphnia* sp. was determined prior to the initiation of the feeding trial. Triplicate samples of 0,5 gram were dried to a constant weight in a convection oven (48 hours at 100°C), and the moisture content determined. A mean of these results indicated that the moisture content of the *Daphnia* sp., as fed in this experiment, was 93,6%. This is marginally higher than figures quoted in the literature (91,2% - Webster *et al.*, 1991; 90,73% - Watanabe *et al.*, 1983; 89,3% - Watanabe *et al.*, 1978) and was probably due to the adhesion of a small quantity of water between the clumped organisms.

Experimental System

A closed, fully recirculating system incorporating both trickle and submerged types of biological filtration was used for this trial. (See Figure 3.1). Twelve 20 litre glass aquaria were arranged in three tiers, with three replicates for each treatment being allocated to tanks according to a randomized block design. Two inflow pipes incorporating a flow control valve supplied each

tank, while the outflow port was covered with fine nylon gauze, in order to prevent escape of fish or wastage of food. Tanks were covered with plastic-laminated cardboard. Water temperature was controlled at a constant 26°C for the duration of the experiment. Heating was provided by a 2 kW element in the system sump tank, linked to a solid phase thermostat. Total volume of the system, including sump tank and filters was 1300 litres. Flow rate to the tanks was 1 litre per 90 seconds, resulting in 2 water changes per hour. Ambient air temperature in the room housing the aquaria was controlled by means of an air conditioner. Aeration was supplied via an airstone in each tank, coupled to a low pressure, sidechannel air blower. A 14 hour light : 10 hour dark photoperiod was maintained through fluorescent lighting controlled by an automatic timing device.

All tanks were siphoned clean weekly, while water pH and total ammonia were tested weekly. pH was determined by means of an electronic pH meter (pHep1-Hanna Instruments), while total ammonia was tested using a commercial colour reagent test kit. A pH of 7,4 ± 0,1 was maintained throughout the 7 week period, while total ammonia never exceeded 0,001mg/litre.

Experimental Fish and Feeding Regime

240 ten day old *Xiphophorus helleri* fry (red victory/red twinbar strain) were selected at random from the broods of three broodstock fish, all born within a 24 hour period. These were randomly distributed in the twelve test aquaria, 20 fish per tank. An acclimation period of one week was allowed, during which a commercially available aquarium fish diet (Tetra-Min® "Baby Fish Food for Livebearers") was fed to satiation three times daily .

Feeding during the course of the trial was carried out three times daily, with the exception of days when weighing was performed, when the first feed was omitted. An initial feeding rate of fifteen percent total body mass per tank per day was calculated and measured on a dry matter basis for the flake diets. Satiation feeding was practised, with small quantities of feed provided and fish monitored until active feeding ceased. Feed remaining at the end of each week was then weighed and the true feeding rate calculated. The feeding rate was thus readjusted on a weekly basis and the quantities of food (dry matter) fed per fish, expressed as a percentage of body weight

calculated.

Drained *Daphnia* sp. were measured into the test tanks using a 3 mm wide spatula, which allowed the accurate measurement of a constant mass of *Daphnia* sp. on a repeatable basis. The relatively slow feeding on the *Daphnia* sp. made it difficult to judge the point at which satiation was achieved, however sufficient livefeed was introduced to ensure that a few live organisms were still present in the tanks one hour after feeding.

In the flake + *Daphnia* treatment the first feed of the day consisted of livefeed, while in the *Daphnia* treatment all three feeds constituted *Daphnia* sp. only.

Weighing of fish was carried out weekly, using a Mettler A240 Electronic scale, reading to 3 decimal places. As the fish were too small to be accurately weighed on an individual basis without incurring injury from handling, collective weighing was performed. The technique used involved catching and counting all fish in a tank, decanting these into a small nylon net, and then drying the net for 30 seconds on paper towel. In this fashion most of the excess water on the fish was removed. The fish were then carefully transferred to a pre-measured beaker of water on the scale, and the total mass of fish recorded. An average mass for individual fish could thus be calculated. During the process of weighing, all fish were carefully examined for any morphological abnormalities, or swimming defects. Where these occurred, such observations were recorded. Any mortalities during the preceding week were also noted at each weighing.

For determination of differences in degree of skin pigmentation at the end of the trial period, the replicates were randomized, so that each row of tanks contained one replicate from each treatment. Thirty four people were then requested, having studied the fish under constant lighting conditions, to complete a questionnaire rating the fish in each row according to their perception of the degree of red pigmentation. A rating of 0, 1, 2 or 3 could be selected, where 0 indicated the least pigmented (palest red colour) and 3 represented the most pigmented (darkest red colour).

The experiment was continued for a period of seven weeks, with growth of fish, mortalities,

occurrence of physical abnormalities, observations on behaviour and degree of skin pigmentation recorded.

Statistical Analysis

One-way analysis of variance, with multiple range testing (Tukey's) was used to determine statistically significant differences between means, where required.

In order to ensure utilization of all data points when comparing growth curves, simple and multiple linear regression of slopes was carried out. Analysis of covariance (Zar, 1984), with the use of a one-tailed T-test for F-values was used to determine whether significant differences in slopes were present. For all treatments, analysis of the slopes of growth curves for the three replicates within a treatment was first performed, and only if no significant differences between replicates was present, were data from the replicates pooled.

Observations of degree of pigmentation were compared by means of contingency table analysis (Zar, 1984). A four by four contingency table was used to determine whether significant differences were present between treatments when all observations were considered. Two by four contingency tables were used to compare observations on pairs of treatments. The stated null hypothesis was that no significant differences in degree of pigmentation should be present between fish fed the four dietary treatments.

RESULTS

Livefeed supplementation had a positive effect on the growth rate of swordtails. The highest growth rates were observed in the fish fed on the farm flake + *Daphnia* and the *Daphnia* only diets (Figure 5.1).

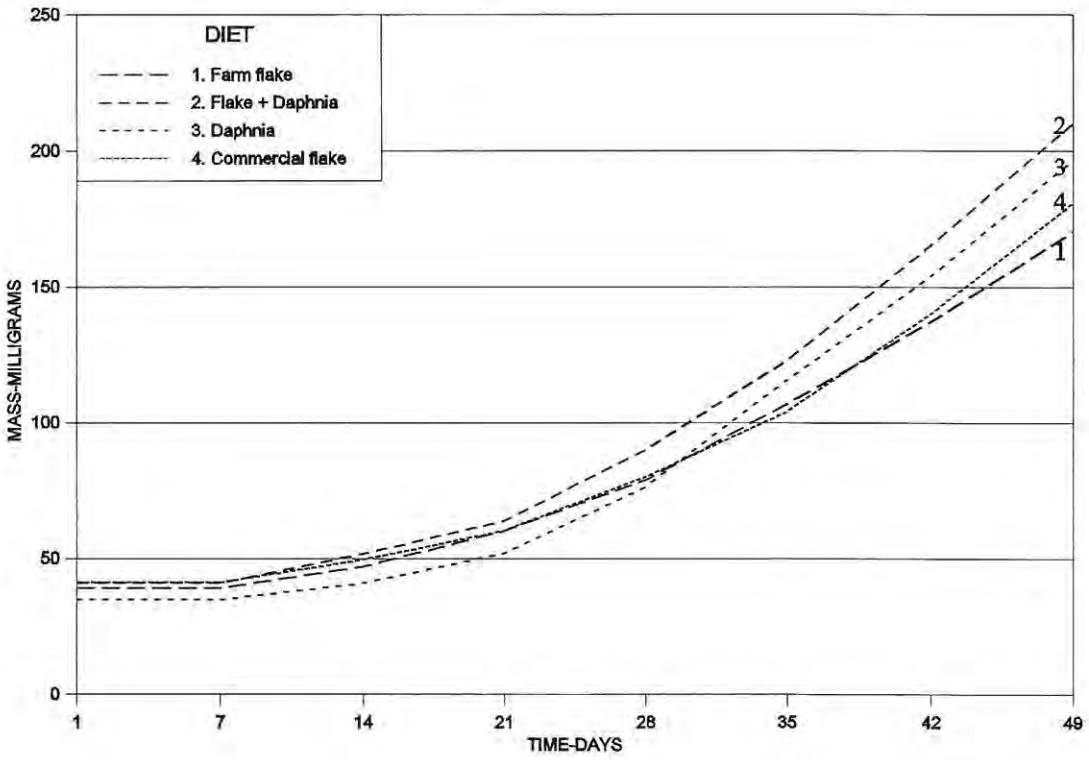


FIGURE 5.1. Growth curves (mass) of juvenile *X. helleri* fed four test diets over a seven week trial period.

In order to facilitate statistical analysis, mass data was log transformed and linear regression curves derived (Table 5.2).

TABLE 5.2. Linear regression equations for Ln transformed data for growth curves (mass) of each dietary treatment.

Dietary Treatment	Regression Equation	R^2
1. Farm flake	$y = 3,359 + 0,040(x)^a$	98,27
2. Flake + *Daphnia*	$y = 3,392 + 0,041(x)^b$	99,55
3. *Daphnia*	$y = 3,248 + 0,041(x)^b$	96,85
4. Commercial flake	$y = 3,410 + 0,035(x)^a$	99,18

Equations sharing a common superscript do not show significant differences in slope ($P > 0,05$).

The slope of the curves for the flake + *Daphnia* and *Daphnia* only treatments were significantly steeper than for the flake only diets ($P < 0,05$), confirming the more rapid growth rate achieved on the diets containing livefeeds. No significant differences were present when comparing the slopes of the two flake diets or those of the *Daphnia* supplemented diet and the *Daphnia* only diet.

Analysis of variance of means of final masses confirmed the observed trends in growth rate with the mixed diet (farm flake supplemented with *Daphnia*) yielding a significantly higher final average mass per fish than the farm flake alone ($P < 0,05$) (Table 5.3).

TABLE 5.3. Values for various parameters measured over a seven week trial period.

Dietary Treatment	Initial Mean Mass*(mg) ±SD	Final Mean Mass*(mg) ±SD	MeanFCR * (49days)	MeanPER* (49 days)	Mortalities (Mean of 3 replicates)	Scoliosis and/or Lordosis
Farm flake	29,6±2,9	$170,3±13,4^a$	$2,25^a$	$1,06^a$	$13,4\%^a$	13,5%
Flake + *Daphnia*	31,3±1,5	$210,1±5,7^b$	$1,89^b$	$1,07^a$	0^b	0
Daphnia	31,4±1,7	$196,4±24,6^{ab}$	$2,65^c$	$0,66^b$	$15\%^a$	0
Commercial flake	31,5±1,8	$180,0±11,9^{ab}$	$2,16^{ab}$	$1,03^a$	$8,4\%^a$	0

* Means indicate average value for all fish in each treatment (3 replicates).

Values in the same column sharing a common superscript are not significantly different ($P > 0,05$).

Refer to Appendix for Anova statistics (dF, F- ratio and level of significance).

The amounts of feed consumed per treatment (dry matter), expressed as a percentage of mean body mass are presented in Table 5.4. The fish receiving the *Daphnia*-supplemented diet consumed significantly less feed than did the other treatments.

TABLE 5.4. Quantities of feed (flake feeds and *Daphnia* sp.) fed over seven week experimental period.

Week	Farm flake		Flake + *Daphnia*				*Daphnia*		Commercial Flake	
	mg[1]	%[2]	Flake	mg[1] *Daphnia*	Total	%[2]	mg[1]	%[2]	mg[1]	%[2]
1	16,7	8,2	9,9	1,51	11,41	5,21	4,04	2,29	15,57	7,06
2	25,8	9,4	17,1	2,88	19,98	6,98	9,45	3,88	26,23	9,07
3	32,8	9,96	22,3	6,93	29,23	8,08	23,1	8,07	32,7	9,42
4	41,9	9,94	31,2	13,84	45,05	10,0	49,8	13,68	42,2	9,96
5	54,9	9,91	43,9	20,77	64,67	10,2	74,5	13,91	55,8	9,94
6	68,9	9,22	46,1	27,67	73,77	8,58	101,4	12,56	68,2	9,38
7	74,9	7,81	61,0	34,6	95,6	8,26	131,5	11,16	79,9	8,15
%(week 1-7)[3]		9,21[a] ±0,36				8,20[b] ±0,17		9,36[a] ±0,08		9,00[a] ±0,44

[1] Average mass feed (dry matter) ingested per fish, per week (milligrams).

[2] Feed fed per fish, per day ; expressed as a % of average body mass (beginning of week).

[3] Feed fed per fish, per day ; expressed as a % of average body mass ± S.D. (entire experimental period).

Values in the same row sharing a common superscript are not significantly different ($P > 0,05$).

Feed conversion ratio was significantly better (lower) for the mixed diet ($P < 0,05$) in comparison to the treatments receiving either farm flake or livefeed only. No significant difference in growth rate or feed conversion ratio was present between the two types of flake tested ($P > 0,05$). Although the feed conversion ratio of the fish fed the mixed diet was lower than those fed on the commercial flake, the difference was not statistically significant ($P > 0,05$).

The protein efficiency ratio over the seven week period did not differ significantly amongst all three treatments receiving the flake diets or the flake + *Daphnia* diet, however the fish receiving *Daphnia* only exhibited a significantly poorer (lower) protein efficiency ratio ($P < 0,05$) (Table 5.3).

Significantly less (zero) mortalities occurred in the fish receiving the *Daphnia* supplemented flake diet, when compared to the other three treatments ($P < 0,05$) (Table 5.3). The majority of mortalities occurred from the fifth week of the experiment onwards, with no obvious macroscopic aetiology visible.

By the end of the experiment, physical abnormalities indicative of ascorbic acid deficiency, including severe lordosis and scoliosis were present in 13,5 percent of the fish fed the farm flake only. In no other treatment did these symptoms occur. Other symptoms of vitamin C deficiency were observed in farm flake only treatment from week six onwards with fish in all three replicates becoming lethargic in their swimming action and exhibiting a depressed appearance with dorsal and anal fins held close to the body. As these symptoms progressed, some fish became anorexic, and tended to swim in a tail-down position, spending prolonged periods resting on the bottom of the tank. At this stage the spinal deformities began to appear.

Observations on colour of fish, after analysis using contingency tables, revealed that fish in treatment 3 (*Daphnia* only), were perceived to be significantly more pigmented (red) than the fish in the other treatments (Figure 5.2).

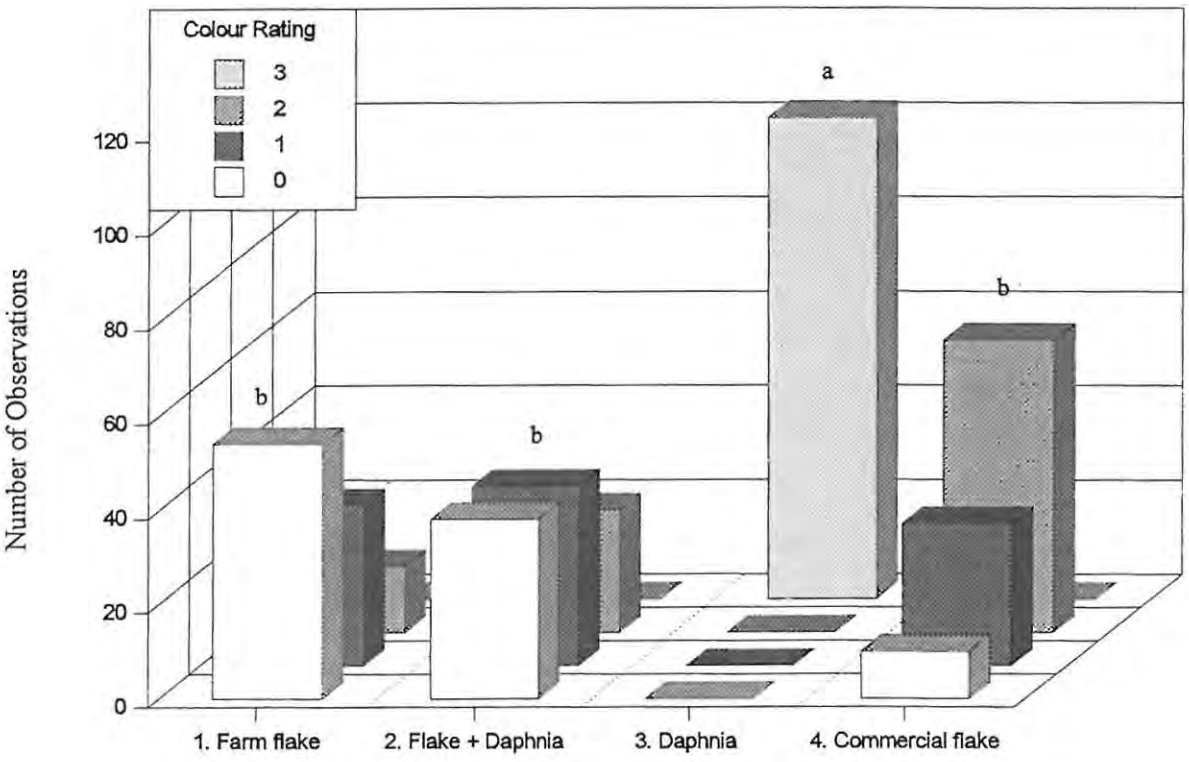


FIGURE 5.2. Distribution of observations of degree of red pigmentation in juvenile *X. helleri* fed one of four dietary treatments for 7 weeks. Rating of 0 indicates least (palest) red colour, while 3 represents most (darkest) red colour. Alphabetic characters denote statistically significant differences ($P < 0,05$).

DISCUSSION

The significantly higher growth rate and feed conversion ratio of swordtails fed dry feed supplemented with *Daphnia* sp. compared to those fed dry feed only is consistent with other studies on livefeed supplementation, and a number of possible explanations exist for this phenomenon. These include the effect of the protein and lipid composition of the livefeed organism, as well as a possible influence by exogenous proteolytic enzymes or micronutrients. A synergistic role between essential nutrients obtained from the livefeed and those supplied by the artificial diet is suggested.

The crude protein content of *Daphnia* sp. has been determined in a number of previous studies (Webster *et al.*, 1991; Fernando and Phang, 1985; Watanabe *et al.*, 1978). These values ranged

from 62,5 % to 65,6 % with a mean of 63,5 % on a dry matter basis. Considering the crude protein content of the commercial flake was 41,8 %, the supplementation of this diet with *Daphnia* sp. allowed the ingestion of quantitatively more protein, at similar rates of feeding. In previous trials examining the relationship of protein to energy in diets for swordtails (Chapter 3) the optimum dietary crude protein level was established to be around 45 %. Furthermore, the protein quality of livefeeds, including *Daphnia* sp. has been shown to be excellent, when compared to artificial feeds (De la Noüe and Choubert, 1985). The essential amino acid composition of *Daphnia pulex* is favourable for inclusion in fish feeds, as the organism is rich in arginine and lysine, two of the commonly limiting amino acids (Yurkowski and Tabacher, 1979). Watanabe *et al.* (1983) demonstrated that the net protein utilization (NPU) of *Daphnia* when fed to rainbow trout was very high. However, in the present experiment *Daphnia* sp. supplementation yielded no improvement in protein efficiency ratio and the PER recorded in the fish fed *Daphnia* sp. only was markedly poorer than in either of the flake diets or the mixed diet. It would appear thus that factors other than merely an increase in protein ingestion played a role in improving the performance of the fish receiving the livefood supplement.

The presence of essential fatty acids in livefeed organisms may play an important role in promoting growth when used as a dietary supplement in fish. Although essential fatty acid (EFA) requirements of *X. helleri* have not been documented, one can assume that these are similar to other fish species. In general fish require a dietary source of linolenic (18:3n3) acid (Sargent *et al.*, 1989). While certain species require long chain highly-unsaturated fatty acids in their diets (Webster and Lovell, 1990), others are capable of production of docosahexanoic (22:6n3) acid and eicosopentanoic (20:5n3) acid through elongation of linolenic acid. *Daphnia* are particularly rich in linolenic and eicosopentanoic acids, and thus constitute a rich source of EFA's (Watanabe *et al.*, 1983). An important consideration, when feeding livefeed is the elimination of the risk of oxidative destruction of EFA's through incorporation of the fatty acid within a living organism. The fatty acid content of the *Daphnia* will vary, to a certain extent, depending on the method of culture employed, particularly with respect to the fatty acid composition of the substrate used for fertilizing *Daphnia* cultures. It is possible that in the current experiment, the *Daphnia* sp. may have served as a useful source of essential fatty acids, thus enhancing growth where used as a supplement to flake feed.

Much research has focused on the role that proteolytic enzymes in livefeed play in the digestive process of fish, particularly in young fish. Mills *et al.* (1993) and Abi-Ayad and Kestemont (1994) demonstrated that in goldfish fry, dietary exogenous enzymes are critical for efficient protein digestion up to the age of 3 weeks. In *Corygonus* sp. (hybrid: *C. wartmanni* X *C. larvretus*) fed on cladocerans (*Moina* sp.), exogenous trypsin was found to be very important in digestion in fry up to 60 days old (>150 mg) (Lauff and Hofer, 1984). In juvenile *X. helleri*, although relatively more developed at birth than goldfish fry, it is possible that the improvement in growth rate and feed conversion seen in the livefeed-supplemented diet is, at least partially, a result of more efficient digestion and assimilation of protein due to the effects of exogenous enzyme activity. Dabrowski and Glogowski (1977) concluded that exogenous proteolytic enzymes are important for optimal digestion, not only in fish fry, but throughout the lives of many fish species, and suggested that where the feeding of livefood is impractical, proteolytic enzymes be incorporated into dietary formulations.

Daphnia sp., and other livefeed organisms, constitute a rich source of vitamins including ascorbic acid. Because the organisms are fed to the fish alive, the loss of these nutrients through oxidation or leaching, which can readily occur in artificial feeds, is prevented. For example, Grabner *et al.* (1981) demonstrated that substantial nutrient (enzyme and amino acid) losses occurred when cladocerans were freeze-dried.

The observation that no signs of ascorbic acid deficiency occurred in fish fed the *Daphnia*-supplemented diet (treatment 2), while 13,5 % of fish on the non-supplemented flake diet exhibited scoliosis and lordosis, indicates that a vital supplement of vitamin C was provided by the *Daphnia* sp.. A similar observation was made by Mills *et al.* (1993) who observed that 6,5 % of goldfish larvae receiving a dry diet exhibited morphological signs of ascorbic acid deficiency, while this was completely prevented by supplementation with brine shrimp. Young, fast growing fish are most susceptible to the effects of marginal nutrient deficiencies in their diets. It would appear, on the basis of these results, that a practical means of eliminating these risks, where diets are based on artificial dry feeds, is by daily supplementation of the diet with livefeed such as *Daphnia*.

In the present experiment, fish fed the *Daphnia* sp. supplemented diet showed a higher survival rate than any of the other treatments, including treatment 3, where livefeed only was provided. The mortalities recorded in fish fed the farm or commercial flake exclusively, or *Daphnia* only diets over a relatively short period of 7 weeks, indicates that very substantial losses may occur during the three to four months required to grow *X. helleri* young out to a marketable size. No mortalities were recorded in the mixed diet treatment, and from a commercial point of view this constitutes a strong argument in favour of using regular livefeed supplementation in intensive production situations.

Despite the inclusion of synthetic carotenoid pigments in the two flake diets, fish fed *Daphnia* sp. only exhibited significantly darker red pigmentation in comparison to all other treatments. This indicates that although *Daphnia* do provide an important source of carotenoid pigment, inclusion levels of livefeed would need to be higher than those used in the *Daphnia*-supplemented diet in this trial, in order to make a significant difference to the colour of the fish. It is possible though, that even at low inclusion levels, if the *Daphnia* supplement was fed for a long enough period, that benefits would be seen in pigmentation.

In conclusion, the results of this experiment demonstrate the significant beneficial effects of livefeed supplementation on growth and survival of ornamental fish cultured under intensive conditions.

CHAPTER 6

THE INFLUENCE OF LIVEFEED SUPPLEMENTATION ON GROWTH AND REPRODUCTIVE PERFORMANCE OF SWORDTAIL BROODSTOCK

INTRODUCTION

Many environmental factors, particularly the level and quality of available nutrition and ambient temperature play a role in determining the reproductive success of broodstock fish (Wootton, 1982). Large commercial producers of swordtail fish (*Xiphophorus helleri*) and other poeciliid aquarium fish in Singapore emphasize the importance of regular supplementation of formulated feeds with livefood, usually *Daphnia* sp. or tubificiid worms (Fernando *et al.*, 1991). On South African ornamental fish farms, poeciliid broodstock are fed primarily on formulated drum-dried flake diets.

Nutrition has been recognized to have a profound effect upon gonadal development and fecundity in fish (Watanabe, 1985). Although most previous work on broodstock has focused on endocrinological aspects of reproduction and the effects of environmental factors on breeding, manipulations of food quantity and quality can modify reproduction in captive broodstock fish to a significant degree (Luquet and Watanabe, 1986).

Within a bioenergetics model, dietary energy resources of a mature fish are partitioned between reproductive output, and somatic maintenance and growth requirements. Maintenance requirements must be satisfied before gonadal development can occur. In fish and other vertebrates, reproduction is one of the first physiological functions to become limited where nutrition is restricted or suboptimal. Correct nutrition of breeding fish becomes particularly critical when they are artificially maintained in a breeding cycle for long periods. In the present experiment this was achieved by artificially elevating the water temperature, using an insulating tunnel, combined with water and air heating. Cech *et al.* (1992) kept mosquito fish (*Gambusia affinis*) in a continuous breeding cycle using photostimulatory conditions. (This species is more sensitive to photoperiod than temperature - Milton and Arthrington, 1983). These authors

showed that supplementation of commercial flake diets with livefeed (tubificiid worms) resulted in increased survival and fecundity of breeding females.

Temperature is the primary controlling factor determining food consumption in fishes. Within the normal physiological temperature range of a species, the difference between maximum ration consumed and the maintenance ration increases with temperature, and thus the quantity of energy available for reproduction after somatic requirements have been fulfilled also increases (Wootton, 1982). In *X. helleri*, temperature has been shown to be more important than photoperiod in controlling reproduction, with cessation of reproductive activity at temperatures of below 15°C (Milton and Arthington, 1983).

Lifetime fecundity of a broodstock fish can be defined as the product of the fecundity per spawning, the number of spawnings per breeding season, and the number of breeding seasons. Minimum age at first reproduction is genetically determined within a species, with the exact age dependent upon environmental factors which affect rate of growth, particularly temperature and nutrition. For example, Vondracek *et al.* (1988) demonstrated that female mosquito fish (*Gambusia affinis*) maintained at high temperatures reproduced at an earlier age and larger size than did conspecifics held at lower temperatures. Furthermore, the fry from these females were larger at birth, suggesting a competitive advantage in survival. A quantitative reduction in ration in young fish may delay maturation and thus lower eventual lifetime fecundity (Scott, 1962; Hester, 1964). The effect of ration size on fecundity may be further modified by various environmental factors, for example, population density (Dahlgren, 1979). Information on the effect of food supply on spermatogenesis in male fish is scarce. In male goldfish fed a limited ration, a degree of testicular atrophy occurred, with a concurrent decrease in spermatogenesis (Clemens and Reed, 1967 - cited in Luquet and Watanabe, 1986).

Fecundity in fish is generally positively related to body size, and thus improved food availability and higher temperatures which produce faster growth, will result in larger and more fecund individuals (Bagenal., 1969; Wootton, 1973a and 1973b; Hislop *et al.*, 1978; Townsend and Wootton, 1984; Springate *et al.*, 1985). In *X. helleri* a linear relationship has been demonstrated between fecundity and size of female fish (Milton and Arthrington, 1983).

The effect of dietary crude protein level on fecundity varies between fish species (Luquet and Watanabe, 1986; Watanabe, 1984a). In work on another tropical ornamental fish, the dwarf gourami (*Colisa lalia*), both gonadosomatic index and fecundity were affected by the level of protein in the diet, with the highest fecundity obtained on a diet of 25 to 45 % protein (Shim *et al.*, 1989). No reference could be found relating dietary protein and fecundity in *X. helleri* specifically, but two studies have been carried out on the guppy, a generalized poeciliid with a similar reproductive strategy to the swordtail. Dahlgren (1980) found that guppies fed a high (47 %) protein diet had higher gonadosomatic indexes than those maintained on lower (31 and 15 %) protein diets, however no difference in fecundity was recorded. By contrast, Shim and Chua (1986), working on the protein requirements of the guppy evaluated diets containing graded levels of between 0 and 60 % crude protein. They found that fish fed a 40 % protein diet exhibited both the highest gonadosomatic index and significantly higher numbers of oocytes in the ovaries.

In lecithotrophic poeciliids such as *X. helleri*, reproductive energy demands are pulsed, with the highest demand occurring during the period of vitellogenesis, in particular the first 4 to 8 days after parturition of the previous brood. The ability of these fish to build up somatic lipid stores during the rest of the reproductive cycle varies between species, and there is some evidence of a reproductive lipid (energy) limitation when body lipid content is low (Meffe and Snelson, 1993). It is thus important to ensure an adequate dietary energy supply throughout the cycle.

Of the constituents of a broodstock diet, lipids are the chemical components which most greatly affect the egg composition (Mourente and Odriozola, 1990a). Lipids play a major role in membrane structure and function, as well as comprising the most important source of energy reserve in the developing fish embryo (Sargent *et al.*, 1989).

During the primary stages of vitellogenesis, lipids are synthesized from acetate in the ovarian tissues. Later, lipoproteins and vitellogenin contribute the majority of lipids laid down in the ova (Luquet and Watanabe, 1986). During the final stages, lipids are also diverted from somatic adipose tissue to be deposited in eggs.

Numerous researchers have shown that the fatty acid profile of lipids in a broodstock diet has a

direct effect on the fatty acid composition of the eggs and fry (Xu *et al.*, 1994 and Mourente and Odriozola, 1990a; 1990b). Essential fatty acid deficiencies have been shown to be detrimental to oögenesis in fish (Luquet and Watanabe, 1986; Watanabe *et al.*, 1984b; Watanabe, 1985). A possible concern in the exclusive use of drum-dried flake diets in *X. helleri* broodstock was the likelihood of a degree of oxidative degradation of essential fatty acids through heat processing and during storage. The resulting fatty acid deficiencies may have an adverse effect on the reproduction of ornamental fish fed flake diets. Dietary fat soluble vitamins, in particular vitamins E and A, are very efficiently transferred to developing eggs (Luquet and Watanabe, 1986), and deficiencies of these nutrients may further adversely affect oöcyte development (Watanabe, 1985).

Carotenoid pigment accumulation in gonads and eggs of many fish species is a well documented phenomenon. In *X. helleri* mature eggs are a bright amber to orange colour. These pigments are obtained primarily from dietary sources, while in salmonids, muscle reserves are mobilized and transferred to the eggs. Suggested functions of carotenoid pigments in reproduction include a role in spermatazoid motility, a respiratory function in ova (Mikulin and Soin, 1975), and a possible antioxidant role in eggs.

Ascorbic acid is essential in broodstock diets. Vitamin C is concentrated in gonadal tissue in fish and other vertebrates, where it has a probable role in steroidogenesis and is important for collagen synthesis in the ovarian structure. A number of studies have demonstrated the beneficial effects of adequate vitamin C provision in broodstock diets in fish (Soliman *et al.*, 1986; Sandnes *et al.*, 1984; Eskelinen, 1989). The importance of sufficient dietary vitamin C for optimal growth and normal development of juvenile *X. helleri* is discussed in Chapter 4.

The present experiment was designed to test the hypothesis that livefood supplementation, by counteracting marginal nutrient deficiencies present in flake diets, would improve growth and fecundity of swordtail broodstock fed a farm flake diet. The design of the experimental system duplicated, as far as possible, the culture conditions found in South African commercial aquaculture ventures which produce poeciliid species.

Breeding families of *X. helleri* were fed either on the farm flake diet only or on farm flake

supplemented with different quantities of livefeed for a period of three months. Besides recording information related to growth and reproductive performance of the test fish, this experiment was also used to quantify the reproductive output of swordtails under intensive production conditions. In particular, an attempt was made to quantify the role of parental cannibalism of newborn fry as a limiting factor in poeciliid production. Cannibalism is a widespread phenomenon in many fish species and constitutes a significant but often underestimated cause of mortality in fish hatcheries. Furthermore, parental cannibalism of young is well documented in many poeciliid species, particularly under aquarium or intensive aquaculture conditions.

MATERIALS AND METHODS

Experimental System

A large recirculating system was built which was completely enclosed in a plastic tunnel similar to those used in practice on commercial farms (Figure 6.1a and Figure 6.1b). This system provided effective passive solar heating and allowed utilisation of natural light. The experiment was conducted from February to April during which period an average of 13L:11D photoperiod was present. A series of sixteen square fibreglass tanks, each holding 275 litres of water were used to house broodstock fish. Shelter for newborn fry was provided by two mats of 64 nylon shadecloth strips (3 cm by 45 cm), suspended on 1000 cm^2 rectangular polystyrene floats in each tank. Exactly the same amount of cover in this form was provided in each of the nine tanks used during the feeding trial. Inflow into each tank was via an adjustable valve, while outflow was through a downpipe, covered with a stainless steel grid, situated in the centre of the tank. Two large (6400 l) concrete tanks served as holding and grow-out facilities for fry harvested during the experiment. All tanks drained, via closed PVC piping into a sump / settlement tank of 8800 l capacity, which in turn drained into the biological filter. The filter consisted of 4,8 m^3 of graded gravel, housed in a 9000l tank, through which water was drawn. Water was then returned to culture or holding tanks by means of a 1,1 kilowatt swimming pool pump.

Both air and water temperature in the tunnel were regulated. A 2 kilowatt fan heater was situated above the level of the culture tanks at one end of the tunnel. This was connected to a thermostat

and served to heat the air in the tunnel when air temperatures dropped to below 20°C, effectively reducing heat loss from the water through radiation. Conversely, on very hot days, cool air was blown through the tunnel, resulting in evaporative cooling. Water was heated by 6 kilowatts of thermostatically controlled heater elements, situated in a 350 l asbestos heater tank, linked to the filter. The probe for this thermostat was located at the outflow of the tanks into the sump.

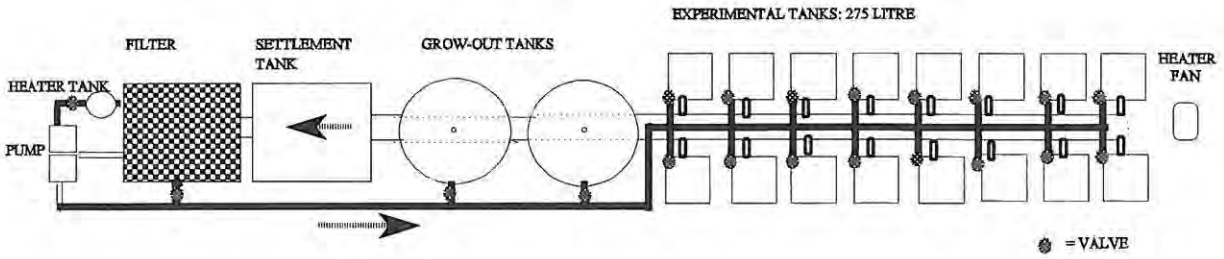
Water quality parameters measured during the trial period included temperature (minimum and maximum), pH, total ammonia and dissolved oxygen level. Flow rate in the culture tanks was checked on a daily basis and maintained at 1 litre per 10 to 15 seconds, ensuring a complete water change in each tank every threequarter to one and threequarter hours. All tanks were siphoned clean on a weekly basis.

Experimental Fish and Feeding Regime

Fish were fed on one of three dietary treatments. The control group (treatment 1) were maintained on a diet of farm flake only, while the two test groups received live *Daphnia* sp. supplementation, either on a daily or a once weekly basis. The flake diet used was the staple farm feed currently produced at Amatikulu Hatchery, and details of it's proximate composition are presented in Chapter 5 (Table 5.1). The flake feed was stored in a blast freezer at -20°C until required for feeding. Feeding with the flake diets was carried out twice daily (08h00 and 16h00), while in the treatments where livefeed was provided, this was supplied simultaneously with the first feed of the day. On days when weighing and measuring were performed, the morning feed was omitted.

An initial feeding rate of three percent body weight (dry weight feed) per day was fed for the flake diet, but it became apparent after the first week that this was excessive. For the rest of the experiment a feeding level of one percent body mass (dry weight feed) per fish per day was used. The exact level was recalculated every four weeks, based on the results of the previous weighing. In the feeding of livefeed, one gram of concentrated *Daphnia* sp. was fed daily in treatment 2, and once a week in treatment 3. To equilibrate the amount of feed fish received in the different treatments, the dry mass of the *Daphnia* sp. fed was taken into account in the livefeed supplemented treatments, and the weight of flake feed adjusted accordingly.

FIGURE 6.1a. Schematic diagram of experimental system (aerial and side views).



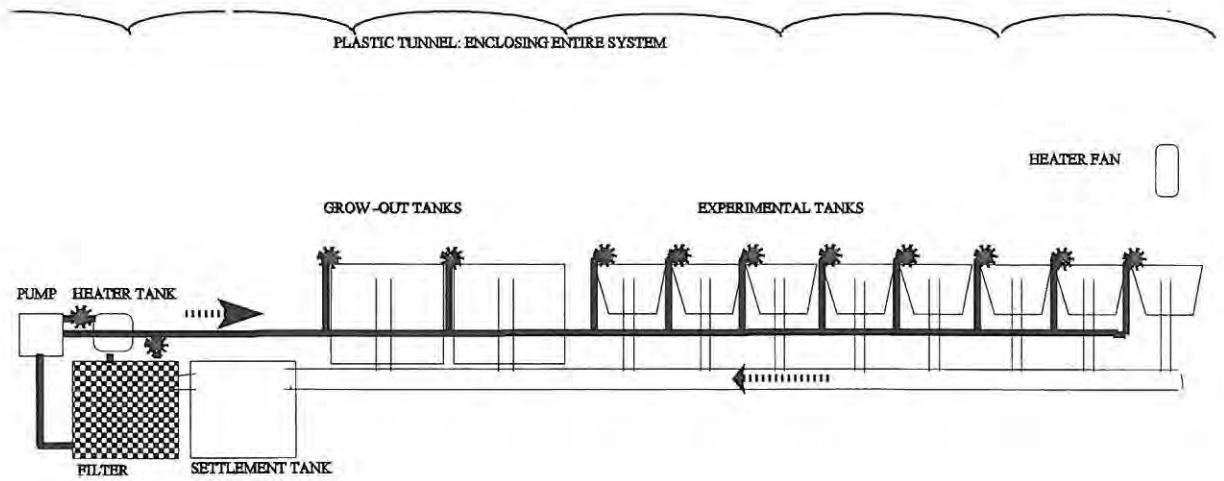


FIGURE 6.1b. Photographs of experimental system. External view of tunnel structure and layout of culture tanks within tunnel.

The culture, harvesting and technique used for concentrating the *Daphnia* sp. is described in Chapter 5 (Materials and Methods). A calibrated plastic spatula measuring one gram of drained *Daphnia* sp. was used to measure out the livefeed ration.

Eighty one mature (ten month old) female *X. helleri* (Red Victory/Red Twinbar strain) were distributed randomly (nine fish per tank) into nine culture tanks. Two mature male fish were added to each group. A tenth tank was stocked with females ranging in size from 34 to over 70 mm standard length. These were fed flake diet only, and maintained for purposes of determination of the relationship between fecundity and size.

The stocking density of breeding fish per litre was one per twenty five litres, similar to that used in commercial farming of swordtails in South Africa (pers. obs.). Three replicates were assigned to each of the three treatments, according to a randomized design. Fish were acclimated to their new environment for a period of two weeks before the feeding trial was initiated, during which they were fed the commercial flake diet to satiation twice daily.

Weighing and measuring of broodstock was performed on day one of the experiment and then every four weeks thereafter. The fish were anaesthetized using 0,4 ml 2-phenoxyethenol per litre water and, after blotting with paper towel to remove excess water, weighed on a Mettler 3000 electronic scale, reading to two decimal places. Standard length (in millimetres) was determined on a purpose designed glass measuring plate.

New born fry were removed from the tanks on a daily basis, prior to the first feed of the day, individually counted, and their numbers recorded. In order to ensure that no juveniles were overlooked, all shelter was briefly removed from the tanks.

At the end of the three month trial period, the absolute fecundity of fish in the three dietary treatments was quantified. Over a period of two weeks the first three fish in each replicate reaching late gestation (very swollen abdomen with developed gravid spot and eyes of embryos visible through body wall) were sacrificed by administration of an overdose of 2-phenoxyethenol. The following parameters were determined: total mass, standard length, ovarian mass,

gonadosomatic index and number of fully developed embryos in the ovary. The broodstock kept in the tenth tank were culled sequentially as they achieved late gestation, and processed as described above. In order to minimize the influence of size on fecundity the number of embryos was calculated per mm standard length.

The average interbrood interval during the experimental period was determined according to the method of Vondracek (1988) using the formula:

$$I = 7 \times \frac{1}{B/n}$$

where, I = average interbrood interval in days

B = number of broods per week

n = number of female fish

Statistical Analysis

One way analysis of variance (ANOVA) with Tukey's multiple range test was used to compare means of data where indicated, to determine whether results were statistically significantly different.

RESULTS

The tunnel system was effective at maintaining water temperatures within an acceptable range for survival and reproduction in swordtails (Table 6.1). Diurnal fluctuations were noted in water temperature as well as pH and dissolved oxygen readings. Filamentous algae growing in the filter and sump tanks was regarded as the major influence on pH and oxygen levels, with photosynthesis peaking in the mid to late afternoon, elevating the dissolved oxygen, and simultaneously affecting the water pH. Weekly testing for total ammonia using a Hach test kit and spectrophotometry revealed no trace of ammonia throughout the experimental period.

TABLE 6.1. Water quality parameters (Temperature, pH, dissolved oxygen and total ammonia) during a 3 month experimental period.

	Minimum	Maximum	Mean
Temperature (±SD)	24,6±1,8°C	28,5±1,4°C	26,5°C
pH	7,3	9,1	8,2
Dissolved Oxygen	7,4 mg/l	12,1 mg/l	7.7 mg/l
Total Ammonia	0	0	0

The decrease in quantity of feed fed between the first and second month (Table 6.2), is as a result of the adjustment in feeding rate due to incomplete consumption of the dry feed during the first month. The total dry mass of feed consumed by the fish in each treatment did not differ significantly ($P > 0,05$) over the experimental period (Table 6.2).

TABLE 6.2. Feed fed per fish (grams dry matter) over twelve week experimental period. Numbers in the same row sharing a common superscript are not significantly different ($P > 0,05$).

Week Number	Farm flake only	Flake + Daily *Daphnia*			Flake + Weekly *Daphnia*		
	Flake	Flake	*Daphnia*	Total	Flake	*Daphnia*	Total
1-4	1,41	1,39	0,16	1,55	1,41	,024	1,43
5-8	1,14	1,01	0,16	1,26	1,09	,024	1,11
9-12	1,31	1,34	0,16	1,50	1,28	,024	1,30
Total (1-12)	3,86[a]	3,74 +	0,48 =	4,22[a]	3,78 +	,072 =	3,85[a]

Fish receiving daily livefeed supplementation grew significantly faster than those receiving weekly supplementation or flake feed only ($P < 0,05$), the latter treatments showing no significant differences in either mass or standard length (Table 6.3; Figure 6.2).

TABLE 6.3. Initial and final mass, standard length and feed conversion ratios (FCR) for *X. helleri* broodstock fed flake feed or flake supplemented with daily or weekly *Daphnia* sp. over a 3 month period.

Treatment Number	Mean Initial Mass ± SD	Mean Initial Length ± SD	Mean Final Mass ± SD	Mean Final Length ± SD	Mean FCR
1. Flake food only	2,71±0,14 g	44,10±0,80mm	5,14±0,05 g[a]	54,37±0,49 mm[a]	1,60[a]
2. Flake + daily *Daphnia*	2,67±0,04 g	43,75±0,20mm	5,97±0,11 g[b]	56,16±0,64 mm[b]	1,33[b]
3. Flake + weekly *Daphnia*	2,60±0,22 g	42,59±1,10mm	5,01±0,27 g[a]	53,34±0,52 mm[a]	1,61[a]

Means indicate average value for all fish in each treatment (3 replicates).

Values in the same column sharing a common superscript are not significantly different (P > 0,05).

Refer to Appendix for Anova statistics (dF, F- ratio and level of significance).

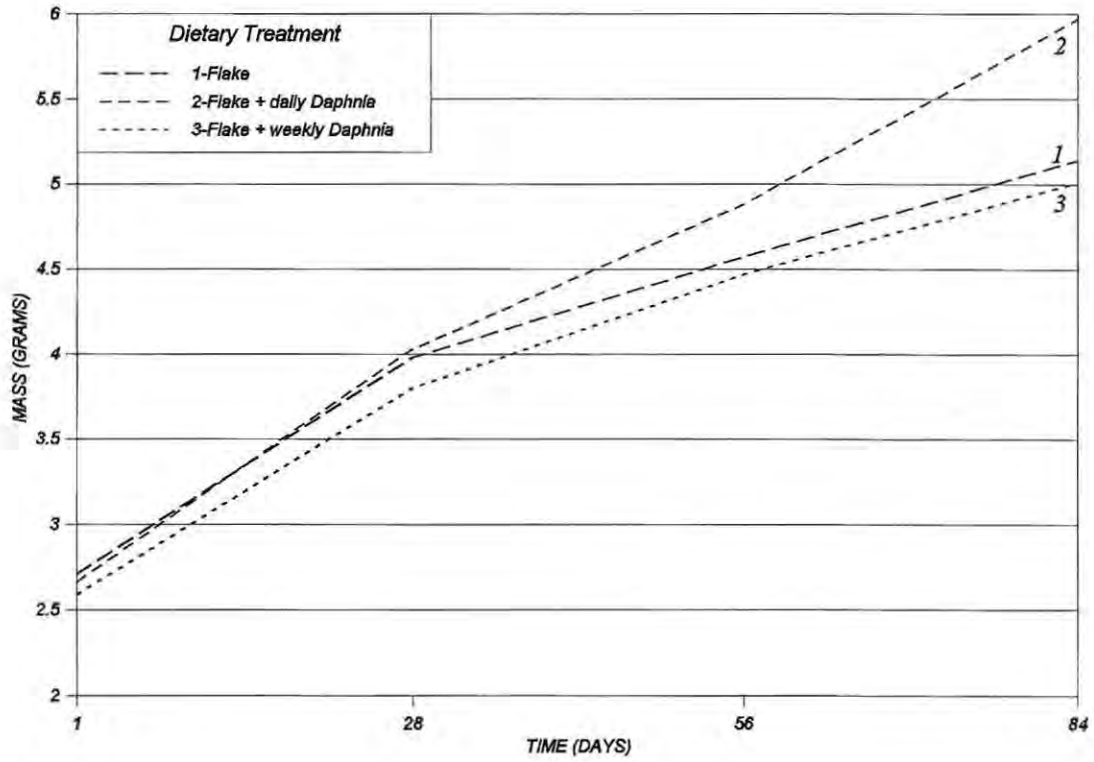


FIGURE 6.2. Growth curves (mass) of *X. helleri* broodstock fed 3 test diets over a 12 week experimental period.

When compared to fish on the flake diet only, or weekly *Daphnia* sp. supplemented diet, the fish receiving daily livefeed supplementation demonstrated a significantly better (lower) feed conversion ratio (Table 6.3).

Only one mortality was recorded amongst broodstock during the experimental period. This was a female which jumped out of its tank.

The average interbrood interval for females in all treatments was 5 weeks.

Daily totals of the number of young harvested were summed every 28 days, when weighing and measuring of broodstock took place (Table 6.4). A total of 11819 juveniles was collected over the 12 week experimental period, with the numbers harvested increasing monthly in each treatment. No significant difference between treatments was demonstrable when the average number of fry per female per month was compared ($P > 0,05$) (Table 6.4).

When absolute fecundity was determined in the fish sacrificed after completion of the trial, the fish on the daily livefeed supplemented diet had significantly higher numbers of developing embryos per female ($P < 0,05$) (Table 6.5).

Although the average mass and standard length of the culled fish did not differ significantly ($P > 0,05$) between treatments, the mean ovarian mass of fish receiving *Daphnia* sp. daily was significantly ($P < 0,05$) greater than those fed on the unsupplemented flake diet (Table 6.5). However, the gonadosomatic index and individual embryo mass did not differ significantly between treatments ($P > 0,05$) (Table 6.5).

Regression analysis of fecundity against standard length of female broodstock fish maintained on a diet of flake food only demonstrated a clear linear relationship (Figure 6.3).

TABLE 6.4. Number of newborn *X. helleri* fry harvested monthly from each replicate tank (9 female broodstock per tank). Values in the same column sharing a common superscript are not significantly different (P > 0,05).

	Replicate	Week 1-4	Week 5-8	Week 9-12	Total: Week 1-12	Average Number Fry/Female per Month
1. Farm flake	1	187	394	652	1233	
	2	244	535	733	1512	55[a]
	3	300	821	578	1699	
Total		731	1750	1963	4444	
2. Flake + Daily *Daphnia*	1	167	128	267	562	
	2	281	713	617	1611	42[a]
	3	224	463	514	1201	
Total		672	1304	1398	3374	
3. Flake + Weekly *Daphnia*	1	396	501	707	1604	
	2	178	375	438	991	49[a]
	3	237	441	728	1406	
Total		811	1317	1873	4001	
Grand Total					11819	

TABLE 6.5. Results of analysis of fecundity (counting of embryos at ovarian dissection) of culled late gestation *X. helleri* females .

Treatment Number	Mean Mass *	Mean Length*	Number of Fry *	Ovarian Mass	GSI	Mass of Embryo[2]
1. Flake food only	7,49 ± 0,78 g[a]	61,78 ± 2,11 mm[a]	133±16[a]	1,34 ± 0,27 g[a]	17,89[a]	10,18 g[a]
2. Flake + daily *Daphnia*	8,28 ± 0,93 g[a]	63,33 ± 2,23 mm[a]	190±30[b]	1,65 ± 0,20 g[b]	20,28[a]	8,81 g[a]
3. Flake + weekly *Daphnia*	7,95 ± 1,25g[a]	62,78 ± 3,63 mm[a]	140±30[a]	1,46 ± 0,25 g[ab]	18,58[a]	10,64 g[a]

* Means indicate average of measurements from 3 culled fish per replicate (3 replicates per treatment) ± SD.

GSI = Gonadosomatic index = (ovarian mass / total body mass) * 100.

[2] Calculated as: Ovarian mass/number of fry in ovary.

Values in the same column sharing a common superscript are not significantly different (P > 0,05).

Refer to Appendix for Anova statistics (dF, F- ratio and level of significance).

To obtain an indirect estimate of the rate of cannibalism of newborn fish, the number of juveniles harvested per mm female standard length was deducted from the number of embryos present in the sacrificed fish. Estimates of losses were extremely high, ranging from 50 to 70 percent of embryos (Table 6.6).

TABLE 6.6. Fecundity expressed as number of young per mm female standard length (standard length values measured at the beginning of each month).

Treatment Number	Number Fry Harvested per mm Female Standard Length	Number of Embryos per mm Standard Length.	% Difference (Theoretical Losses in Production)
1. Farm flake	1,08[a]	2,15[a]	49,76 %
2. Flake + Daily *Daphnia*	0.88[a]	3,02[b]	70,86 %
3. Flake + Weekly *Daphnia*	0.98[a]	2,22[a]	55,86 %

Values in the same column sharing a common superscript are not significantly different (P > 0,05).

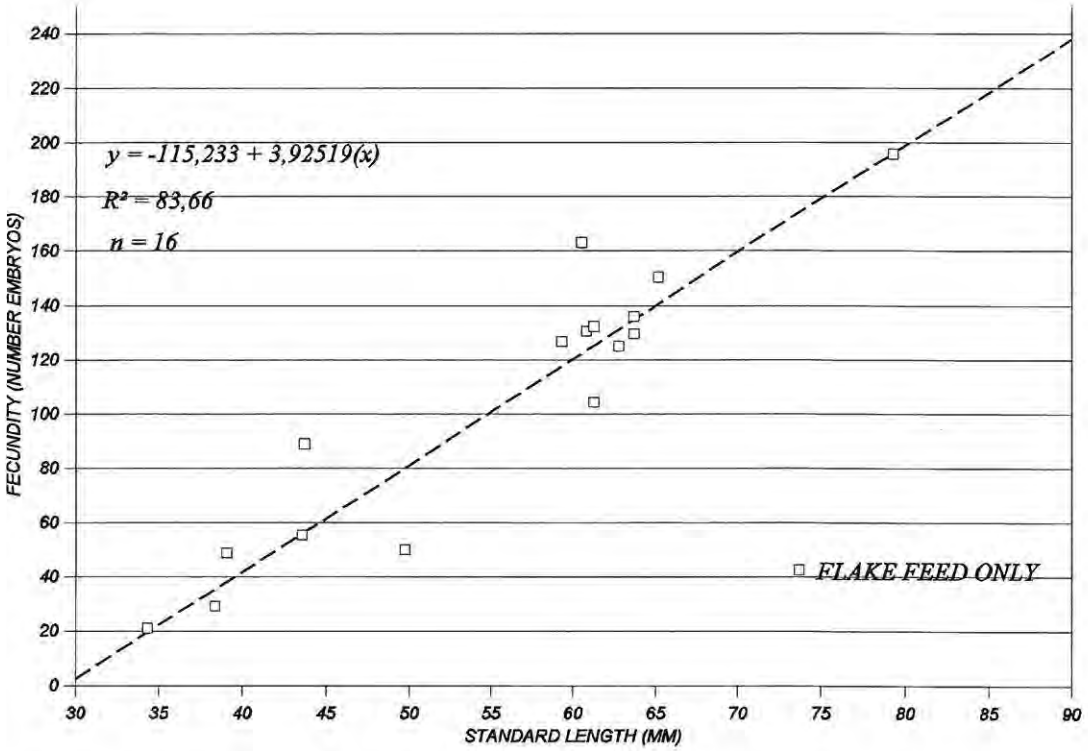


FIGURE 6.3. Linear regression of number of embryos per female standard length against female standard length in *X. helleri* females fed a diet of flake feed only.

DISCUSSION

The daily addition of *Daphnia* sp. as a livefood supplement led to a marked increase in the growth rate of broodstock swordtails fed on a commercial flake feed. Despite the relatively small quantity of livefeed provided (11,4% of daily dry matter intake), a significant improvement in feed conversion ratio was noted when compared to fish fed either flake feed only, or flake plus a weekly supplement of *Daphnia* sp.. Thus while the flake diet probably provided the bulk of the fishes nutritional requirements, the livefeed may have served to counteract marginal deficiencies present in the artificial dry diet. By contrast, weekly supplementation with *Daphnia* sp. provided no evident benefits in terms of growth, fecundity or feed conversion ratio.

A linear relationship between standard length (size) and fecundity was demonstrated in this

experiment. This trend in poeciliid fish reflects the findings of Krumholz (1948), cited in Meffe and Crump (1987) and Milton and Arthrington (1983), working on *Gambusia affinis* and *Xiphophorus helleri* respectively. Moreover, when the fecundity of fish of similar size from the different dietary treatments was compared, those fed the daily *Daphnia* sp. supplemented diet contained a significantly greater number of embryos. An increase of thirty percent in fecundity was recorded in the culled females examined, and from an economic viewpoint this may dramatically improve production, and hence profitability, provided cannibalism is controlled. It is postulated that improved utilisation of dietary nutrients through the presence of essential micronutrients provided in the *Daphnia* sp., or perhaps a direct effect of these additional nutrients on the gonad led to this effect. The GSI of the fish fed the daily supplemented diets was higher than the other treatments, but the difference was not significant. However, the mean ovarian mass of the fish fed the daily livefeed supplemented diet was significantly greater than in those fed flake feed only. This indicates a greater partitioning of nutrients into the gonadal tissue in these fish. In addition to the increased fecundity per mm standard length observed in the daily livefood supplemented group, the final size of the female fish in this group was significantly greater, thus further increasing fecundity in comparison to the fish fed flake diet only or those receiving weekly *Daphnia* sp. supplementation.

The disparity between absolute fecundity and harvested fry during the course of this trial indicates that losses through cannibalism of new-born young by adult fish may constitute a significant limiting factor in the intensive production of swordtail fish. The similar numbers of juveniles harvested from each treatment may indicate that a limited number of young could effectively take refuge in the shelter provided, leaving the remaining fish more susceptible to predation by adults. This hypothesis is supported by the observation that, with the exception of one brood where a few abnormal fry were found dead in the tank, no other mortalities of fry were recorded. Moreover, during the collection of juveniles, a number of attempts by adults (both successful and abortive) to prey on new born young were observed. The difference between fecundity of the culled females and number of new born juveniles collected indicates that the loss of production due to cannibalism was dramatic, ranging between fifty and seventy percent. In research examining the relationship between fecundity and nutrition in another poeciliid fish, *Gambusia affinis*, Vondracek *et al.* (1988) found that parental cannibalism of fry led to a significant underestimation of fecundity. A number of explanations for cannibalism in poeciliids have been postulated, for example, infantophagy was found to be related to population density (Breder and Coates, 1932 -

cited in Dahlgren, 1979) and was proposed as a mechanism for population control in poeciliid fish. A genetic basis for cannibalistic tendencies has also been demonstrated in poeciliid fish (Thibault, 1974). Hubbs (1991), however, suggested that parental cannibalism in *Gambusia* was simply an expression of normal predatory behaviour, and was exacerbated by the size difference between the predator and prey. Both stocking density and fish size have been shown to be important factors regulating the rate of cannibalism within a population. For example, in *Poeciliopsis occidentalis*, cannibalism of offspring was observed to increase at higher stocking densities, an observation with obvious significance for intensive culture of these species (Meffe, 1981). With reference to size, the relationship between the size of the cannibalistic individual and its potential prey is important, and in livebearers such as *X. helleri* where the female fish is much larger than the male, females tend to be involved to a greater extent. In the present experiment, at a stocking density of 1 broodstock fish per 25 litres water, cannibalism of fry was significant, and it may be concluded from these results that a lower stocking density in breeder tanks may be advisable unless alternative means to ameliorate the rate of cannibalism can be found.

The vast majority of broods were born during the night, and fry were observed to actively seek refuge in the shelter provided in the tanks as soon as they could swim efficiently. It is felt that if more refuge (perhaps a thick mat of nylon mesh/strips) had been provided, and if fry had been removed at first light, that losses through cannibalism could have been significantly curtailed.

Further factors which may influence the degree of cannibalism include the availability of food, and the provision of livefood. Although livefeed has been shown to alleviate cannibalism in fish under certain conditions (Hecht and Pienaar, 1993), it is unlikely that the livefeed provided in the present experiment would have played a role. *Daphnia* sp. was fed in the mornings and quickly consumed, so that no livefeed would have been available as an alternative prey to the new born fry in the early hours of the morning. Increasing the feeding rate and frequency to ensure optimal satiation feeding may possibly reduce the aggressive predatory behaviour leading to cannibalism, but further research on poeciliid behaviour is required.

A consideration stemming from the high levels of cannibalism in this study is the additional nutrition obtained by the breeding adults through ingestion of live young conspecifics. Meffe and Crump (1987) tested the hypothesis that cannibalism has nutritional benefits with respect to both growth and reproductive output in *G. affinis*. They suggested that intraspecific ingestion of

young was the equivalent of feeding an ideal "pre-packaged" feed item. They demonstrated that female *Gambusia* fed on a flake diet supplemented with tissue of conspecifics increased both growth and reproductive output, when compared to a control. Since the fish in the daily *Daphnia* sp. supplemented treatment apparently exhibited the highest level of cannibalism, this source of nutrients may have further enhanced their growth rate and fecundity.

Embryogenesis in the swordtail, as in other livebearing fish, is temperature dependent with the interbrood interval being determined by the length of time between the birth of a brood and subsequent fertilization of the next clutch of eggs - the yolk-loading period. The average interbrood interval for all females in this experiment was 5 weeks at 26,5°C, which corresponds with the results of Siciliano (1972) who measured the reproductive cycle of *Xiphophorus* sp. to be 35 days at 24 ± 3°C, with an actual gestation period of 27 days, and fertile eggs available in the ovary for 11 days.

In conclusion, this experiment has demonstrated that by feeding a daily supplement of *Daphnia* sp. as livefeed to *X. helleri* broodstock maintained on an artificial flake diet, a significant potential increase in production may be realised. This was due to an increase in fecundity as a result of more rapid growth and hence larger size of females, and a higher number of embryos per mm female standard length. A further benefit was the improved feed conversion produced by the livefood-supplemented flake diets. However, it was inferred that parental cannibalism of newborn fry was common, and in order to realise the benefits of increased fry production through a balanced diet, measures should be taken to eliminate this phenomenon. Suggested methods include:

- provision of sufficient and effective refuge for fry in breeder tanks
- regular monitoring of tanks and removal of new born fry, especially at first light
- frequent and, where possible, satiation feeding.

CHAPTER 7

CONCLUDING DISCUSSION AND RECOMMENDATIONS

The specific nutritional requirements of ornamental fish species have long been neglected, and only recently, with the recognition of the role of nutrition in coloration, health and longevity of these fish, have nutritional studies been undertaken.

Aquaculture research principles and techniques, adapted from studies performed on food fish such as trout, salmon and catfish, were applied to the swordtail, *Xiphophorus helleri*, in an attempt to identify the nutritional requirements of this species under intensive aquaculture conditions. In many ways this project was exploratory and tested whether these methods could be successfully extrapolated to ornamental fish species.

In view of the trend towards intensification in ornamental fish farming in South Africa, and the feeding of "complete" artificial diets, a great deal of the nutritional technology and theory developed for aquaculture of food fish species is directly applicable to ornamental fish. However, the criteria upon which the market judges the quality of ornamental and food fish differs in certain key aspects which have implications regarding nutrition, and are illustrated by the present results. As feed costs constitute a major proportion of overall production costs in food fish aquaculture, emphasis is on maximum growth rate in the shortest possible time, with optimal efficiency of feed conversion. While used as an indicator of the nutritional quality of fish diets in the present study, feed conversion ratio is not an important economic indicator in ornamental fish culture. In ornamental fish, while size is important for marketing, more important is the quality of fish in terms of aesthetic appearance, in particular colour, as well as health and ability to survive transport stress. As feed forms a relatively small part of production costs, after water, labour and transport, this project demonstrated that it may be practical, in ornamental fish culture, to include more expensive dietary ingredients in dry feeds. This together with greater utilization of livefeeds, may serve to improve production, and enhance the final quality of the marketable fish.

In both food and ornamental fish species, knowledge of their dietary essential amino acid and

protein requirements is essential in order to formulate diets which promote optimal growth using conventional feed ingredients . In the present study, using a protein source balanced in terms of its amino acid composition with respect to *X. helleri*, juvenile fish achieved optimal growth on a high protein diet (45% dry matter) with an estimated protein to energy ratio of over 27 mg protein per kJ digestible energy. This finding corresponds to requirements previously determined for other warmwater omnivorous fish. Although a clear relationship between P:E ratio and growth rate did not emerge in the present study, the "protein sparing" effect is not as important in ornamental fish farming as it is in food fish as protein costs constitute a relatively small percentage of overall operating expenses.

Although aquarium fish have traditionally been fed on drum-dried flake diets, due to their palatability, buoyancy and aesthetically pleasing appearance, the present study demonstrated that growth, feed conversion and survival of juvenile swordtails were all improved on a crumble type feed, similar to that used in the rearing of juvenile food fish. Significantly, vitamin C deficiency symptoms, which occurred in the fish fed flake diets, were absent in fish fed crumbles. Although heat treatment during drum drying of flake feeds resulted in the destruction of heat labile nutrients, including vitamin C, these losses were relatively insignificant when compared to the leaching losses which occurred upon immersion of flake feed in water.

The feeding of livefeeds is generally not considered cost effective in food fish culture beyond the early juvenile stages. Due to the small size of poeciliids and other ornamental species, the nutritional advantages of livefeed supplementation may outweigh the costs involved, and the present study has shown that livefeed supplementation may be justified in ornamental fish culture throughout the production cycle. Where one in three meals of flake diet per day was replaced with live *Daphnia* sp., significantly improved growth and survival of juvenile swordtails was observed. Furthermore, vitamin C deficiency signs were prevented in the fish receiving *Daphnia* sp.. While the bulk of the fishes energetic and protein requirements were apparently satisfied by the artificial diet, it is suggested that the provision of vitamin C and other essential micronutrients, essential amino acids and possibly essential fatty acids played a synergistic role in enhancing utilisation of the diet. Much research has been carried out demonstrating the role of adequate dietary vitamin C on the immunocompetence of fish, particularly where these are reared

intensively. Resistance to handling stress, diseases and parasites is depressed in fish fed vitamin C deficient diets, and in view of these observations, the use of livefeed supplementation is further justified. The intensity of pigmentation, often a deciding factor in the choice of an aquarium fish, was also markedly enhanced in those fish fed exclusively on *Daphnia* sp., however it would not be practical to feed only livefeed to intensively cultured ornamental fish.

As in any type of livestock farming enterprise, the breeding individuals form the backbone of the entire operation, and as such the value of broodstock fish should be recognized. Within the scope of the present project the only aspect of broodstock management addressed was nutrition, which was shown to play a significant role in productivity. Daily supplementation of dry feed with *Daphnia* sp. in broodstock fish significantly improved growth and fecundity, and a direct correlation was demonstrated between fecundity and size of female fish, demonstrating a major potential improvement in fish yield. Furthermore, parental cannibalism of newborn fish was shown to be an important limitation in production. More intensive management of broodstock, including the use of smaller breeder tanks, increased refuge for newborn, and immediate removal of young from tanks may help restrict these losses.

The results of this research demonstrate the significant production benefits derived from correct nutritional balance and presentation of artificial dry feed, together with regular supplementation of diets with livefeed. As *X. helleri* is typical of the poeciliid fish commonly produced, it is likely that these recommendations may be successfully extrapolated to other livebearer species. It may thus be concluded that by optimising nutrition, the production and profitability of farms engaging in intensive culture of *X. helleri* and other poeciliids could be markedly enhanced. The production of a crumble-type diet specifically for poeciliids and the provision of a regular supply of *Daphnia* for supplementary feeding should be considered a matter of priority on existing commercial farms in South Africa. A feasibility study focusing on the manufacture of a suitable crumble diet, and production of *Daphnia,* is recommended. This should include a comprehensive cost / benefit analysis and take into account the capital expenditure, labour, training and production costs involved. Direct benefits of optimal nutrition such as increased growth and fecundity / production of fish should be considered as well as indirect effects, including improved health status and coloration.

To date, lack of scientific research and development within the ornamental fish industry in South Africa has hampered rapid progress in terms of number of species, quantity and quality of fish produced. With increasing awareness within the South African aquaculture community of the importance and potential of the ornamental fish trade, an increase in both funding and the number of specialists working in this discipline is inevitable. The success of the present project in defining effective nutritional management techniques for *X. helleri*, using research methods adapted from established food fish studies, will hopefully serve to stimulate further work in this field. Considering the number and variety of ornamental fish species currently produced, and the number of potential species, huge scope exists for further research into nutrition and reproduction.

APPENDIX

ANOVA STATISTICS

Table Number	Parameter	dF	F - ratio	Significance Level
3.2	Final mass	8	5,729	0,001
	Feed consumed	8	9,149	0,0001
	Protein consumed	8	999,99	0,0000
	DE consumed	8	72,409	0,0000
	FCR	8	7,463	0,0002
	PER	8	3,751	0,0094
4.5	Final mass	3	22,223	0,0003
	FCR	2	40,482	0,0003
5.3	Final mass	3	3,804	0,0380
	FCR	3	14,614	0,0013
	PER	3	35,433	0,0001
	Mortalities	3	8,167	0,0081
6.3	Final mass	2	27,643	0,0009
	Final length	2	19,620	0,0023
	FCR	2	9,831	0,0128
6.5	Number of fry	2	12,411	0,0002
	Ovarian mass	2	3,721	0,0391
	GSI	2	1,044	0,3675
6.6	Fry per mm	2	0,299	0,7521
	Embryos per mm	2	11,850	0,0003

REFERENCES

Abi-Ayad, A. and Kestemont, P., 1994. Comparison of the nutritional status of goldfish (*Carassius auratus*) larvae fed with live, mixed or dry diet. Aquaculture, 128: 163-176.

Arthrington, A.H. 1989. Diets of *Gambusia affinis holbrooki, Xiphophorus helleri, X. maculatus* and *Poecilia reticulata,* (Pisces: Poeciliidae) in streams of Southeastern Queensland, Australia. Asian Fish. Sci. 2: 193-212.

Ashley, L.M., Halver, J.E. and Smith, R.R., 1975. Ascorbic acid deficiency in rainbow trout and Coho salmon and effects on wound healing. In Pathology of Fishes (Ribelin, W.E. and Migaki, G., Editors) 769-785. University of Wisconsin Press.

Association of Official Analytical Chemists (AOAC), 1984. Official Methods of Analysis, 14th edn. AOAC, Washington, DC.

Bagenal, T.B., 1969. The relationship between food supply and fecundity in brown trout *Salmo trutta* (L.). J. Fish Biol., 1: 167-182.

Beck, A.D., 1978. Recommended procedures for comparative evaluation experiments of artificial and live diets for marine finfish. Proc. World Symp. on Finfish Nutrition and Fishfeed Technology, Hamburg 20-23 June,1978. Vol. II. Berlin 1979.

Benitez, L.V., 1989. Amino acid and fatty acid profiles in aquaculture nutrition studies. Asian Fish. Sci. Spec. Publ. 4: 23-35.

Bleher, H., 1993. Current trends in the international aquatic trade. Aquarama '93, Conference Proceedings, Singapore.

Boonyaratpalin, M. and Lovell, R.T., 1977. Diet preparation for aquarium fishes.

Aquaculture, 12: 53-62.

Britz, P.J. and de Kock, S. (eds.), 1994. The South African Ornamental Fish Trade. Proceedings of the Aquaculture Association of Southern Africa, 3. 48pp.

Bromley, P.J., 1980. Effect of dietary protein lipid and energy content on the growth of turbot (*Scophthalmus maximus* L.). Aquaculture, 19: 359-369.

Cheong, L., 1993. The ornamental aquatic industry in Singapore. Aquarama '93, Conference Proceedings, Singapore.

Cech, J.J., Schwab, R.G., Coles, W.C. and Bridges, B.B., 1992. Mosquitofish reproduction: effects of photoperiod and nutrition. Aquaculture, 101: 361-369.

Dabrowski, K. and Glogowski, J., 1977. Studies on the proteolytic enzymes of invertebrates constituting fish food. Hydrobiologia, 52(2-3): 171-174.

Dabrowski, K., Hinterleitner, S., Sturmbauer, C., El-Fiky, N. and Wieser, W., 1988. Do carp larvae require vitamin C? Aquaculture, 72: 295-306.

Dabrowski, K. and Ciereszko, A., 1993. Influence of fish size, origin and stress on ascorbate concentration in vital tissues of hatchery rainbow trout. Prog. Fish. Cult. 55: 109-113.

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.

Dahlgren, B.T., 1980. The effects of three different dietary protein levels on the fecundity in the guppy, *Poecilia reticulata* (Peters). J. Fish Biol., 16: 83-97.

Daniels, W.H. and Robinson, E.H., 1986. Protein and energy requirements of juvenile red drum (*Sciaenops ocellatus*). Aquaculture, 53: 243-252.

De la Noüe, J. and Choubert, G., 1985. Apparent digestibility of invertebrate biomasses by rainbow trout. Aquaculture, 50: 103-112.

Degani, G. and Gur, N., 1992. Growth of juvenile *Trichogaster leerii* (Bleeker, 1852) on diets with various protein levels. Aquaculture and Fisheries Management, 23: 161-166.

Durve, V.S. and Lovell, R.T., 1982. Vitamin C and disease resistance in channel catfish (*Ictalurus punctatus*). Can. J. Fish. Aquat. Sci. 39: 948-951.

Eskelinen, P., 1989. Effects of different diets on egg production and egg quality or Atlantic salmon (Salmo salar L.). Aquaculture, 79: 275-281.

Fernando, A.A., Phang, V.P.G and Chan, S.Y., 1991. Diets and feeding regimes of poeciliid fishes in Singapore. Asian Fish. Sci. 4: 99-107.

Fernando, A.A. and Phang, V.P.E., 1985. Culture of the guppy, *Poecilia reticulata*, in Singapore. Aquaculture, 51: 49-63.

Garling, D.L. and Wilson, R.P., 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus*. J. Nutr., 106: 1368-1375.

Grabner, M., Wieser, W. and Lackner, R., 1981. The suitability of frozen and freeze-dried zooplankton as food for fish larvae: a biochemical test program. Aquaculture, 26: 85-94.

Halver, J.E., 1985. Recent advances in vitamin nutrition and metabolism in fish. In Nutrition and Feeding of Fish (Cowey, C.B., Mackie, A.M. and Bell, J.G. Editors.) 415-429. Academic Press, London.

Halver, J.E., 1989. The Vitamins. In Fish Nutrition (Halver J.E. Ed.) 31-109. Academic

Press, New York.

Hardy, R.E., 1989. Diet preparation. In Fish Nutrition (Halver, J.E. Ed.) 475-548. Academic Press, New York.

Hecht, T. and Britz, P.J., 1990. Aquaculture in South Africa: History, Status and Prospects. The Aquaculture Association of South Africa, Pretoria.

Hecht, T., Britz, P.J. and Uys, W., 1992. Aquaculture in South Africa. World Aquaculture, 23(1): 7-19.

Hecht, T. and Pienaar, A.G., 1993. A review of cannibalism and its implications in fish larviculture. Journal of the World Aquaculture Society, 24(2): 246-261.

Hester, F.J., 1964. Effects of food supply on fecundity in the female guppy, *Lebistes reticulatus* (Peters). J. Fish. Res. Board Can., 21: 757-764.

Hilton, J.W., Cho, C.Y. and Slinger, S.J., 1977. Factors affecting the stability of supplemental ascorbic acid in practical trout diets. J. Fish. Res.Board. Can. 34: 683-687.

Hilton, J.W., Cho, C.Y. and Slinger, S.J., 1978. Effect of graded levels of ascorbic acid in practical diets fed to rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board. Can. 35:431-436.

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

Hubbs, C., 1991. Intrageneric "cannibalism" in *Gambusia*. The Southwestern Naturalist, 36(2): 153-157.

Ketola, H.G., 1982. Amino acid nutrition of fishes: requirements and supplementation of diets. Comp. Biochem. Physiol., 73B(1):17-24.

Lauff, M. and Hofer, R., 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. Aquaculture, 37: 335-346.

Lee, D.J. and Putnam, G.B., 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr., 103: 916-922.

Li, Y. and Lovell, R.T., 1985. Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J. Nutr. 115: 123-131.

Lovell, R.T. and Lim, C., 1978. Vitamin C in pond diets for channel catfish. Trans. Am. Fish. Soc. 107(2): 321-325.

Lovell, T., 1989. Fish feeding experiments. In Nutrition and Feeding of Fish (T.Lovell, Ed.) 129-138. Von Nostrand Reinhold, New York.

Lovell, T., 1990. New sources of vitamin C for fish feeds. World Aquaculture, 21(2): 59.

Luquet, P. and Watanabe, T., 1986. Interaction "nutrition-reproduction" in fish. In Fish Physiology and Biochemistry, 2(1-4): 121-129. Kugler Publications, Amsterdam.

Mayer, F.L., Mehrle, P.M. and Crutcher, P.L., 1978. Interactions of toxaphene and vitamin C in channel catfish. Trans. Am. Fish. Soc. 107(2): 326-333.

Mazik, P.M., Brandt, T.M. and Tomasso, J.R., 1987. Effects of dietary vitamin C on growth, caudal fin development, and tolerance of aquaculture-related stressors in channel catfish. Prog. Fish. Cult. 49: 13-16.

Meffe, G.K., 1981. Density-dependent cannibalism in the endangered Sonoran topminnow

(*Poeciliopsis occidentalis*). The Southwestern Naturalist, 29(1): 500-503.

Meffe, G.K. and Crump, M.L., 1987. Possible growth and reproductive benefits of cannibalism in the mosquitofish. The American Naturalist, 129(2): 203-212.

Meffe, G.K. and Snelson, F.F., 1989. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall inc., New Jersey.

Meffe, G.K. and Snelson, F.F., 1993. Annual lipid cycle in Eastern Mosquitofish (*Gambusia holbrooki*: Poeciliidae) from South Carolina. Copeia 3: 596-604.

Mertz, E.T., 1972. The protein and amino acid needs... . In Fish Nutrition (Halver J.E. Ed.) 105-143. Academic Press, New York.

Mikulin, A.Ye. and Soin, S.G., 1975. The functional significance of carotenoids in the embryonic development of teleosts. Journal of Ichthyology 749-759.

Mills, D., Stone, D., Newton, P. and Willis, S., 1993. A comparison of three diets for larval goldfish rearing. Austasia Aquaculture, 7(1): 48-49.

Milton, D.A. and Arthrington, A.H., 1983. Reproductive biology of *Gambusia affinis holbrooki* Baird and Girard, *Xiphophorus helleri* (Günther) and *X. maculatus* (Heckel) (Pisces; Poeciliidae) in Queensland, Australia. J. Fish Biol., 23: 23-41.

Mourente, G. and Odriozola, J.M., 1990a. Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). Fish Physiol. and Biochem., 8(2): 93-101.

Mourente, G. and Odriozola, J.M., 1990b. Effect of broodstock diets on total lipids and fatty acid composition of larvae of gilthead sea bream (*Sparus aurata* L.) during yolksac stage. Fish Physiol. and Biochem., 8(2): 103-110.

Navarre, O. and Halver, J.E., 1989. Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. Aquaculture, 79: 207-221.

Nematipour, G.R., Brown, M.L. and Gatlin, D.M., 1992. Effects of dietary energy : protein ratio on growth characteristics and body composition of hybrid striped bass, *Morone chrysops* ♀ X *M. saxatilis* ♂. Aquaculture, 107: 359-368.

Ogata H., Arai , S. and Nose T., 1983. Growth responses of cherry salmon *Oncorhynchus masou* and amago salmon *O. rhodurus* fry fed purified casein diets supplemented with amino acids. Bull. of Jap. Soc. Sci. Fish. 49: 1381-1385.

Pike, R.L. and Brown, M. L., 1967. Nutrition: an Integrated Approach. John Wiley and Sons, Inc., New York, N.Y., 542pp.

Rosen, E.R. and Bailey, R.M., 1963. The poeciliid fishes (Cyprinidontiformes), their structure, zoogeography, and systematics. Bulletin of the American Museum of Natural History, Volume 126: Article 1.

Sandnes, K., Ulgenes, Y., Braekkan, O.R. and Utne, F., 1984. The effect of ascorbic acid supplementation in broodstock feed on reproduction of rainbow trout (*Salmo gairdneri*). Aquaculture, 43: 167-177.

Sargent, J., Henderson, R.J. and Tochar, D.R., 1989. The Lipids. In Fish Nutrition (Halver J. E. Ed.) 153-217. Academic Press, New York.

Scott, D.P., 1962. Effect of food quantity on fecundity of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board. Can., 19: 715-730.

Shariff, M. and Subasinghe, R., 1992. Aquarium fish health - do we really care? Infofish International, 6: 43-47.

Shim, K.F. and Chua, Y.L., 1986. Some studies on the protein requirement of the guppy, *Poecilia reticulata* (Peters). Journal of Aquariculture and Aquatic Sciences, 4(4): 79-84.

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

Siciliano, M.J., 1972. Evidence for a spontaneous ovarian cycle in fish of the genus *Xiphophorus*. Biol. Bull., 142: 480-488.

Simpson, R. J., Neuberger, M.R. and Liu, G.Y., 1976. Complete amino acid analysis of a protein from a single hydrolysate. J. Biol. Chem., 251: 1936-1940.

Snelson, F.F., Wetherington, J.D. and Large, H.L., 1986. The relationship between interbrood interval and yolk loading in a generalized poeciliid fish, *Poecilia latipinna*. Copeia, 2: 295-304.

Soliman, A.K., Jauncey, K. and Roberts, R.J., 1986. The effect of dietary ascorbic acid supplementation on hatchability, survival rate and fry performance in *Orechromis mossambicus* (Peters). Aquaculture, 59: 197-208.

Soliman, A.K., Jauncey, K. and Roberts, R.J., 1987. Stability of L-Ascorbic acid (vitamin C) and its forms in fish feeds during processing, storage and leaching. Aquaculture, 60:73-83.

Springate, J.R.C., Bromage, N.R. and Cumaranatunga, P.R.T., 1985. The effects of different ration on fecundity and egg quality in the rainbow trout (*Salmo gairdneri*). In Nutrition and Feeding of Fish (Cowey, C.B., Mackie, A.M. and Bell, J.G. Editors) 371-393. Academic Press, London.

Steffens, W., 1989. Principles of Fish Nutrition. John Wiley and Sons, Inc., New York, N.Y.

Stevens, S., 1983. Crossing swords? Tropical Fish Hobbyist, Vol XXXII(1): 54-57.

Tacon, G.J., 1981. Speculative review of possible carotenoid function in fish. Progressive Fish Culturalist, 43(4): 205-208.

Thibault, R. E., 1974. Genetics of cannibalism in viviparous fish and its relationship to population density. Nature, 251: 138-140.

Thibault, R.E. and Schultz, R.J., 1978. Reproductive adaptations among viviparous fishes (Cyprinidontiformes: Poeciliidae). Evolution, 32(2): 320-333.

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

Uys, W., 1989. Aspects of the nutritional physiology and dietary requirements of juvenile and adult sharptooth catfish *Clarias gariepinus* (Pisces: clariidae). Phd Thesis, Rhodes University.

Vondracek, B., Wurtsbaugh, W.A. and Cech, J.J., 1988. Growth and reproduction of the mosquitofish, *Gambusia affinis*, in relation to temperature and ration level: consequences for life history. Env. Biol. of Fishes, 21(1): 45-57.

Wahli, T., Meier, W. and Pfister, K., 1986. Ascorbic acid induced immune-mediated decrease in mortality in *Ichthyopthirius multifiliis* infected rainbow-trout (*Salmo gairdneri*). Acta Tropica, 43: 287-289.

Walton, M.J., Cowey, C.B. and Adron, J.W., 1984. British Journal of Nutrition, 52: 115.

Wang, K., Takeuchi, T. and Watanabe, T., 1985. Optimum protein and energy levels in diets

for *Tilapia nilotica*. Bull. Jpn. Soc. Sci. Fish., 51: 141-146.

Watanabe, T., Arakawa, T., Kitajima, C., Fukusho, K. and Fujita, S., 1978. Proximate and mineral compositions of living feed used in seed production of fish. Bull. Jap. Soc. Sci. Fish, 44(9): 979-984.

Watanabe, T., Kitajima, C. and Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: A review. Aquaculture, 34: 115-143.

Watanabe, T., Itoh, A., Kitafima, C. and Fujita, S., 1984a. Effect of dietary protein levels on reproduction of Red Sea bream. Bull. Jap. Soc. Sci. Fish., 50: 1015-1022.

Watanabe, T., Takeuchi, T., Saito, M. and Nishimura, K., 1984b. Effect of low protein-high calory or essential fatty acid deficient diet on reproduction of rainbow trout. Bull. Jap. Soc. Sci. Fish, 50: 1207-1215.

Watanabe, T., 1985. Importance of the study of broodstock nutrition for further development of aquaculture. In Nutrition and Feeding of Fish (Cowey, C.B., Mackie, A.M. and Bell, J.G. Editors) 395-414. Academic Press, London.

Webster, C.D., Mims, S.D., Tidwell, J.H. and Yancey, D.H., 1991. Comparison of live food organisms and prepared diets for paddlefish, Polyodon spathula (Walbaum), fry. Aquaculture and Fisheries Management, 22: 155-163.

Webster, C.D. and Lovell, R. T., 1990. Response of striped bass larvae fed brine shrimp from different sources containing different fatty acid compositions. Aquaculture, 90: 49-61.

Wilson, R.P., 1985. Amino acids and protein requirements. In Nutrition and Feeding of Fish (Cowey, C.B., Mackie, A.M. and Bell, J.G. Eds.) 1-16, Academic Press, London.

Wilson, R.P. and Poe, W.E., 1985. Relationship of whole body and egg essential amino acid

patterns to amino acid requirements in channel catfish, *Ictalurus punctatus*. Comp. Biochem. Physiol., 80B: 385-388.

Wilson, R.P. 1989. Amino acids and proteins. In Fish Nutrition (Halver J.E. Ed.) 112-151, Academic Press, New York.

Wootton, R.J., 1973a. The effect of size of food ration on egg production in the female three-spined stickleback, *Gasterosteus aculeatus* (L.). J. Fish Biol., 5:89-96.

Wootton, R.J., 1973b. Fecundity of the three-spined stickleback, *Gasterosteus aculeatus* (L.). J. Fish Biol., 5: 683-688.

Wootton, R. J., 1982. Environmental factors in fish reproduction. In Reproductive Physiology of Fish: Proceedings of the International Symposium on Reproductive Physiology of Fish, Wageningen, The Netherlands (Richter,C.J.J. and Goos, H.J.Th., Compilers) 210-219. Pudoc, Wageningen.

Xu, X.L., Ji, W.J., Castell, J.D. and O'Dor, R.K., 1994. Influence of dietary lipid sources on fecundity, egg hatchability and fatty acid composition of Chinese prawn (*Penaeus chinensis*) broodstock. Aquaculture, 119: 359-370.

Yamamoto, S., 1982. Examination concerning some synthetic diets for the larvae of ayu fish *Plecoglossus altivelis*: the stability of a supplemental vitamin C in the diets and its effects for the larvae. Can Trans. Fish. Aquat. Sci., No. 4850, 12pp.

Yamamoto, Y. and Inoue, M., 1985. Effects of dietary ascorbic acid and dehydro-ascorbic acid on the acute cadmium toxicity in rainbow trout. Bull. Jap. Soc. Sci. Fish. 51(8): 1299-1303.

Yurkowski, M. and Tabacher, J.L., 1979. Proximate and amino acid compositions of some natural fish foods. In Proceedings of the World Symposium on Finfish Nutrition and

Fishfeed Technology, Hamburg, June 1978 Vol. I, Berlin: 20-23.

Zar, J.H., 1984. Biostatistical Analysis. Second Edition. Prentice-Hall Inc., New Jersey.