

A STUDY ON
THE APPROPRIATE FOOD PARTICLE SIZE, FEEDING FREQUENCY AND
FEASIBILITY OF USING PREPARED FOOD FOR WALKING CATFISH,
Clarias macrocephalus, LARVAE

BY

RAKPONG PETKAM

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Zoology
University of Manitoba
Winnipeg, Manitoba

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Abstract

Studies on the effects of food particle size, feeding frequency and prepared food on the growth and survival of *Clarias macrocephalus* larvae with an initial size of 0.69 ± 0.05 cm in total length and 0.46 ± 0.12 mg in dry weight over a 21 day experimental period were conducted. Larvae were reared in glass aquaria and plastic tubs under aerated conditions at water temperatures from 25 to 29°C. Live food (*Artemia*) was given to excess at different size ranges and feeding schedules. In addition larvae were fed using combinations of live (*Artemia*) and prepared food; larvae were switched to prepared food on day 6, 11 and 16 after receiving *Artemia* from the start of exogenous feeding. Larvae fed on *Artemia* attained a high growth and survival. There was evidence artificial food was acceptable at the start of exogenous feeding but relatively poor growth and survival resulted. *Artemia* in a size range from 160 to 315μ is more suitable for *C. macrocephalus* larval rearing than sizes smaller than 160μ . A feeding schedule of one feeding per day is acceptable if feeding is to excess. Live food (*Artemia*) is necessary at the start of exogenous feeding to improve survival. *Clarias macrocephalus* larvae fed on *Artemia* at the start of exogenous feeding until day 11, followed by prepared food until the end of the experiment, showed significantly higher growth and acceptable survival compared to those fed on other food combinations, *Artemia*, or prepared food alone.

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Lastly, I wish to dedicate this thesis to my parents "Kesorn and Ruringsak Petkam" and my sisters for their endless love and eternal support.

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1. Introduction

In the past thirty years drastic population increase in Southeast Asia has led to overfishing, water pollution, and therefore deterioration of wild fish populations. The demand for fish has been rising rapidly. Fish farming has become widespread. Relative to alternatives, fish is a good protein source, in terms of texture and cholesterol content, moreover fish is comparatively cheaper. All these factors lead to a high demand.

Walking catfish (*Clarias* sp.) is one of the dominant fish genera cultured in Thailand. Among the Southeast Asian countries, Thailand is well known for the successful culture of catfish. *Clarias* sp. can be cultured at high density, in saline, and poorly oxygenated water, and will produce a high harvest per unit area over a short culture cycle, farming of these species is expanding widely throughout the country (Leenanone, 1981; Areerat, 1987). There are five *Clarias* sp. found in Thailand but only two native species are farmed, *Clarias batrachus* and *C. macrocephalus*. In addition, *C. gariepinus* which is an introduced species is widely farmed because it has the advantage of superior growth. *Clarias macrocephalus*, however, is preferred by consumers and regarded as the most delicious catfish because of its tender flesh and better taste. It is therefore the most valuable species of catfish on the market (Diana et al., 1985; Areerat, 1987).

During the early stages of catfish culture development, catfish larvae were traditionally collected from the wild, particularly the paddy fields which are preferred spawning areas of *C. macrocephalus*. As both intensive and extensive *C. macrocephalus* farming have gradually expanded, natural brood stock has decreased, and seedling supplies from natural sources and government facilities have become inadequate. Consequently, although a high demand for seed has resulted in increased seed production, the current demand for seed (larvae) has exceeded the supply available from natural sources and government facilities. The high demand has resulted in cultured larvae production becoming a major source of seedling supply.

Although *Clarias macrocephalus* is the catfish preferred by Thai consumers, its production is still limited in comparison to that of *C. batrachus* and *C. gariepinus*. The limited availability of *C. macrocephalus* larvae has held back the production of this species relative to that of *C. batrachus* and *C. gariepinus*. Additionally, *C. macrocephalus* larvae have a high mortality and the growth rate is slower than that of the other two species.

The early stage of transition from endogenous to exogenous feeding is considered the most crucial period in *C. macrocephalus* larval rearing. Traditionally, larval rearing of many species is based on zooplankton which can be collected from ponds (Thakur and Das, 1986). As larval production has

expanded throughout Thailand, harvestable live food can barely meet the demand. The problem is made worse by major environmental changes which have affected the production of natural live food. In addition, infectious diseases are difficult to control when wild plankton is used as food. All these circumstances have led to a shift to the culture of live food for the catfish larvae, particularly where a large number of larvae are produced. However production of both natural and cultured live food depends on seasonal and partially uncontrolled conditions. There may thus be considerable unpredictable variation in the supply of live food. Therefore there is risk in relying on those types of food, especially for intensive production. Good quality foods such as *Artemia* have been introduced for many types of larval production but such food can be too costly if the rearing period is prolonged.

Recently, there has been increasing use of prepared (artificial) foods for rearing larvae of several species. As a result of a short digestive tract and initial inactivity of certain digestive enzymes, the understanding of larval nutrient requirements is still unclear which causes many difficulties in formulating a suitable diet. The feasibility of using prepared foods depends on quality of foods, larval nutrient requirements, which in turn depend on the anatomy and physiology of the digestive tract, and larval feeding behaviour. Prepared food has been successfully used to rear

larvae of some species. However the combination of live food and prepared food is often necessary for larvae of other species.

Larvae of *C. macrocephalus* have a very small mouth gape and a short gut; the appropriate food particle size and the frequency of feeding are thus critical. The feasibility of using prepared food in this species in order to shorten the period of dependence on live food is unknown.

The objective of this study was to determine if methods could be found by which live and prepared food could be presented to *C. macrocephalus* so as to improve larval survival and growth. The following issues were examined:

- Appropriate food particle size for *C. macrocephalus* larvae.
- Appropriate feeding frequency for *C. macrocephalus* larvae.
- The feasibility of using prepared food for rearing *C. macrocephalus* larvae.

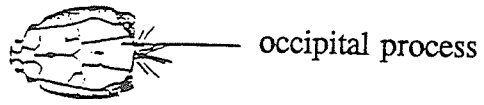
2. Review of the Literature

2.1 Biology of *Clarias macrocephalus*

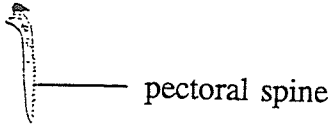
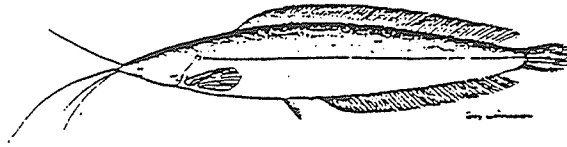
Clarias macrocephalus, locally known as Pla Duk Oui, is a member of the Family Clariidae, Order Siluriforms, Class Osteichthyes (Nelson, 1984; Suvatti, 1981; Smith, 1945). *Clarias macrocephalus* has neither scales nor adipose fin. Both pectoral fins are preceded by a spine with a saw-like edge which cause a painful wound if fish are handled carelessly. These spines are also used to move overland when it is raining or it is too dry hence the common name "walking catfish" (Figure 1). *Clarias macrocephalus* is able to survive outside the aquatic environment for a very long period by means of an auxiliary breathing organ. This organ is dendritic in form and located in the supra-brachial chamber. There is also some degree of respiration through the skin which is covered by a very thick layer of mucous. The body colour is normally black or dark grey and partly yellow in the abdominal area (Areerat, 1970; Leenanone, 1981).

The three catfish species farmed in Thailand are easily differentiated by the shape of the occipital process. *Clarias macrocephalus* has a rounded occipital bone with its base three to five times wider than its length, unlike *C. batrachus* which has a sharp occipital bone, with a width twice its length and *C. gariepinus* which has not only a bigger head but also a more elongate occipital bone (Figure 1) (Areerat, 1970).

Figure 1. Differentiating characteristics of the three dominant *Clarias* catfish species cultured in Thailand (Areerat, 1987; Balon, 1974).

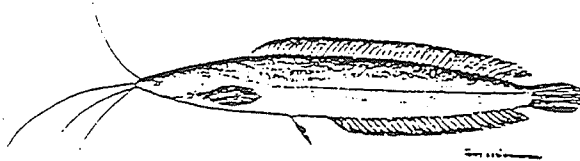
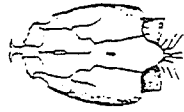


occipital process

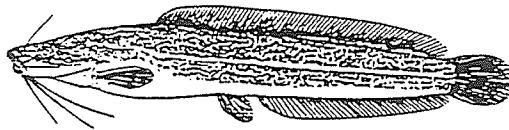
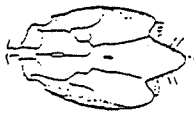


pectoral spine

Clarias macrocephalus Gunther.



Clarias bratrachus Linnaeus.

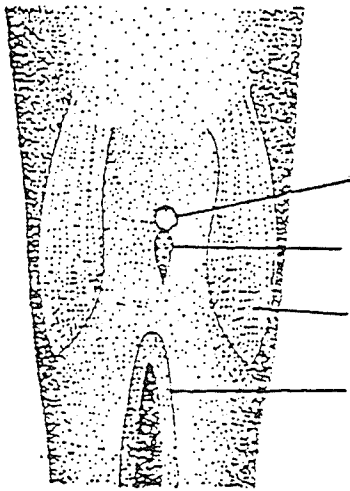


Clarias gariepinus.

The accessory air breathing organ allows *C. macrocephalus* to be found naturally in all types of freshwater bodies, even almost dry pools (Smith, 1945). It can also live in brackish water. The larvae feed on detritus and zooplankton. Although a benthic species sometimes classified as a scavenger, it can be trained to take food at the water surface. Adults are often fed trash fish or pellets composed of fish meal, rice bran and broken rice. The use of dry pelleted food is becoming more common on catfish farms (Areerat et al., 1982).

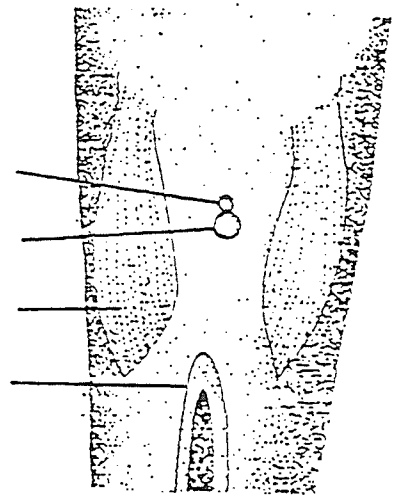
In nature, *C. macrocephalus* reaches maturity at the age of 8-12 months. At this age the sexes can be distinguished by an anal papilla on the male which is elongated relative to that of the female which is oval in shape (Figure 2). In addition, during the spawning season, the female has distended belly and is bigger than the male. Spawning time occurs during the rainy season (May - October). The female makes a small, round, hollow nest, about 30cm in diameter and 5 to 8cm deep, in the grassy bottom in shallow water. The eggs are deposited in the nest and adhere to the grass. The male finally guards the nest after spawning while the female stays nearby (Sidthimunka, 1971). *Clarias macrocephalus* does not seem to spawn in the confined water of fish ponds (Pillay, 1990). Artificial propagation using hormone injections is thus the best method of obtaining gametes. This method can be practised from February until October and can be repeated as often as 10 times per year. In culture, *C. macrocephalus* reaches

Figure 2. Differentiation of male and female of *C. macrocephalus* adults (Leenanone, 1981).



Male

Anus
Genital Papila
Pelvic Fins
Anal Fin



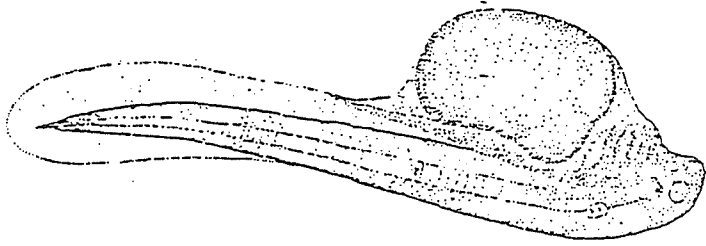
Female

150g in approximately 7-8 months. Fecundity of *C. macrocephalus* has not been determined but using *C. batrachus* as a reference, $F = 189.5W^{1.223}$ where F is thousands of eggs and W is body weight in kg. Spawning individuals weigh from 50-185g. On average eggs are 1.2mm in diameter and hatching occurs within 24-30h after fertilization. Larvae start feeding 3 days after hatching and are generally 5-8mm in length depending on each population and the size of the parent (Figure 3 and 4). Limitation on year-round culture of this species relates to poor larval survival rather than egg laying or hatching (Diana et al., 1985).

In rearing ponds, larvae supplied with adequate food reach an average length of 2cm with 30-35% survival within 2 weeks. Early growth of larvae approaches 45% of the body weight (BW) per day over the first 2 weeks of life, then, declines to 3-5% of BW per day for the duration of culture (Diana et al., 1985).

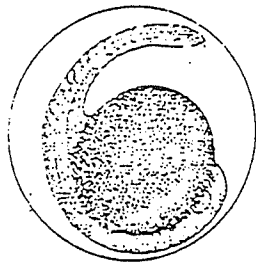
Growth occurs in the temperature range from 25 to 33°C. (Britz and Hecht, 1987). Optimal pH is 6.5-8.5. *Clarias macrocephalus* can be cultured at a high density. Suggested optimal rearing density is 15 fingerlings per litre. Although they can survive in poorly oxygenated water, adult feeding is reduced when dissolved oxygen (DO) falls below 0.5 mg/L. If DO is lower than 1 mg/L, growth of *C. macrocephalus* larvae is reduced. Mortality of larvae occurs when DO is less than 0.2 mg/L.

Figure 3. Development of *C. macrocephalus* from fertilized egg to yolked larvae (free embryo): 3a. Somite stage, 3b. Advance somite stage, 3c. Eyed stage, 3d. Free embryo stage (Leenanone, 1981).



38

3c



3b

3a

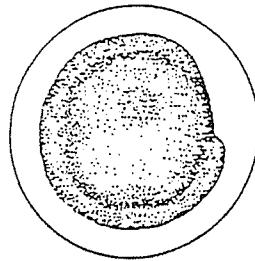
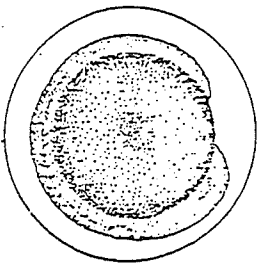
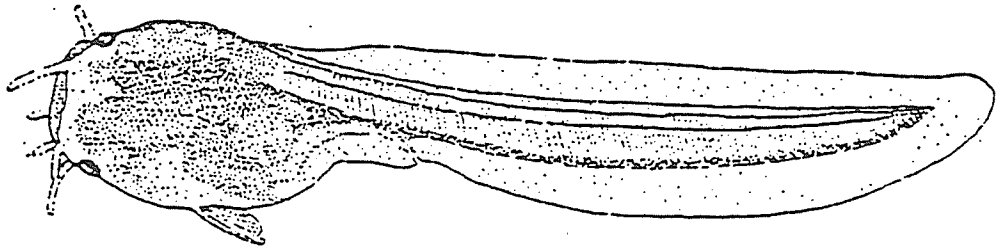
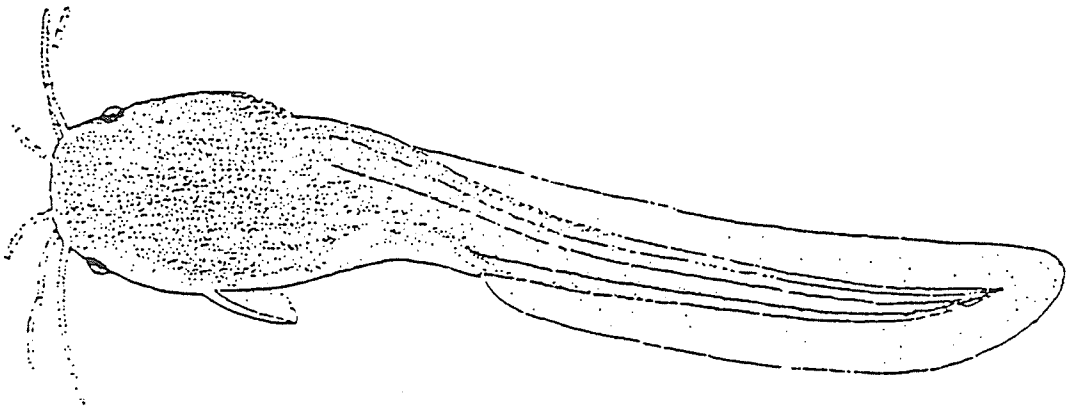


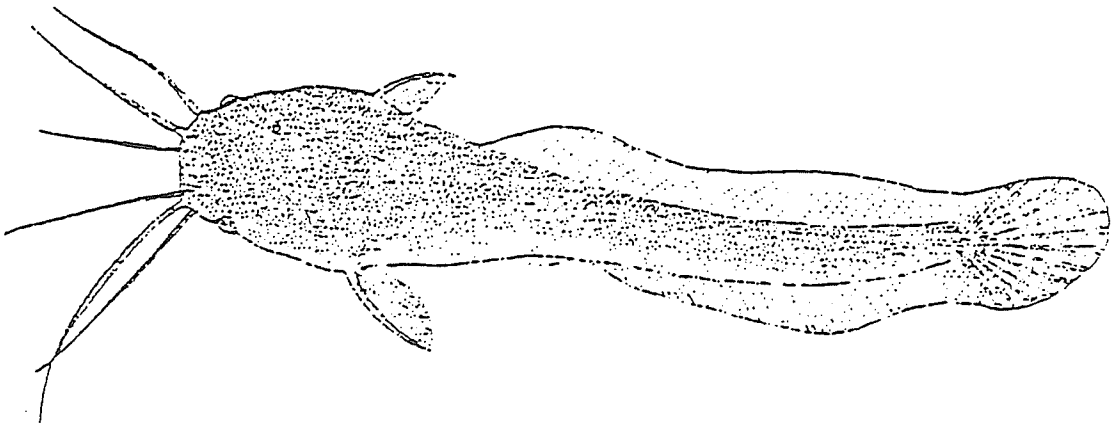
Figure 4. Development of *C. macrocephalus* larvae from approximately 2 to 8 days post hatching at 25°C: 4a. 2 days after hatched (mean = 5.5 mm.), 4b. 4 days after hatched (mean = 7 mm.), 4c. 8 days after hatched (mean = 10 mm.) (Leenanone, 1981).



4a.



4b.



4c.

2.2 Food Particle Size

Larval fishes usually have a very small, toothless mouth and limited mobility (Hyatt, 1979). These characteristics limit their ability to utilize a wide range of natural and prepared foods. Food particles should be smaller than the mouth width and should be located near the fish. The suitable size of food probably also depends on composition or species of food and changes with larval size. The particle size of decapsulated and dried *Artemia* cysts (200-250 μ) may be a more appropriate feed for *C. gariepinus* larvae than larger *Artemia* nauplii (470-550 μ) (Verreth et al., 1987). A particle diameter of 2.2% of the total length of *C. gariepinus* larvae was found to be optimal by Uys and Hecht (1985).

2.3 Prepared Food

The abundance of both natural live food and cultured food is seasonally dependent and good quality live food such as *Artemia* is relatively expensive. There has been, therefore, considerable interest in the feasibility of using prepared feed to rear *Clarias* sp. Uys and Hecht (1985) found that larvae fed on optimally formulated dry feed grew significantly better than those fed on plankton. Appelbaum and Damme (1988) similarly suggested that feeding *C. gariepinus* larvae exclusively with a formulated high quality dry feed is feasible. Artificial feed has advantages over live food in terms of the potential to eliminate disease organisms through

sterilization and the possibility of controlling nutrient composition and energy content, all of which may increase survival rate. Alternatively, *C. lazera (gariepinus)* larvae can be reared successfully on live *Artemia* and zooplankton (Hogendoorn, 1980). Selection of specific cyst sources may result in the continued use of *Artemia* cysts in aquaculture according to Vanhaeke and Sorgeloos (1983). In Southeast Asia, mashed boiled egg yolk is used to rear *Clarias* (Viveen et al., 1990). A mixed diet of *Artemia* plus a dry artificial feed for *C. macrocephalus* produced a higher specific growth over the first 14 days than a diet of either item alone (Fermin and Bolivar, 1991).

2.4 Feeding Frequency

Because *Clarias* larvae have a very short, small gut, the frequency of feeding must be high enough to maintain their metabolic requirements. Feeding twice a day was found to be economically optimal for 2 week old (0.3g) *C. macrocephalus*, whereas more frequent feeding with proportionately restricted amounts of prepared food (9% BW) did not affect growth but feed conversion ratio increased (Tabthipwon, 1990). Feeding three times a day with live food (*Moina*) has been suggested for rearing *C. macrocephalus* larvae (Mollah and Tan, 1982). Feeding more frequently may be preferred in the case of *C. gariepinus* with an application every 4 h suggested by Uys and Hecht (1985). Continuous feeding for 24 h per day gave the

fastest growth of *C. lazera* (*gariepinus*) larvae (0.5 g.)
according to Hogendoorn (1981).

3. Materials and Methods

3.1 Brood Stock Supply

A *C. macrocephalus* brood stock (totalling 80kg) was purchased from a private catfish farm located in Northeast Thailand. The brood stock was originally collected from a river the previous year. The average weight of individual fish was between 80 and 250g. The brood stock was transferred to the experimental site, temporarily deprived of food and kept separately in an earthen pond, 10m wide, 25m long and 1.5m deep. The pond was enclosed with a 30cm high net to prevent fish from escaping by "walking". Feeding to satiation took place twice a day, in early morning and the evening, using a dry pelleted diet.

3.2 Larval Supply

Clarias macrocephalus larvae were obtained by artificial propagation using a hormone injection method. Brood stock, kept separately in earthen ponds, were collected using a gill net, and transferred to cement tanks at the hatchery in the early morning before feeding. Spawning readiness of males was determined on the basis of general vigour, the presence of a pointed genital papilla, and a weight of 100-150g. Females were selected if they weighed 100-200g and had an extended reddish genital papilla. Weights were individually recorded and pairs of males and females were kept separately in the

tanks. Oxygen was monitored and water was sprayed on the water surface to imitate the rainy season (Spraying water is thought to improve hormone levels and there is some evidence that *Clarias sp.* may spawn at any time if subjected to sudden freshwater flows or sudden temperature changes according to Areerat et al. (1982)). A Luteinizing Hormone Releasing Hormone Analogue (LH-RH Analogue) was utilized for both males and females at a dosage of 10 μ g per kg of BW in the first injection, however, the dosage is arbitrary depending on a spawning readiness of the brood (a soft extended belly and reddish genital papillae). The second injection was done 6h later using 20 μ g per kg of BW in the male and 15 μ g per kg of BW in the female. Males received more because *C. macrocephalus* males normally have low volumes of milt. Six hours after the second injection the female was checked for readiness and then the eggs were stripped into a dry plastic bowl. The male was then sacrificed and the testes removed and minced, the milt was immediately mixed with the eggs using a feather. Fertilization took place within 1-2 minutes of sacrifice. Water was poured onto the eggs in order to facilitate the movement of sperm to the eggs. This procedure is similar to that used for *C. gariepinus*. The fertilized eggs were then removed to the hatching tanks and spread over netting which was submerged in each tank. Oxygenation and the water spray were monitored at all times. The eggs hatched within 24h of fertilization. All unfertilized eggs were removed. Two days

after hatching, the larvae were carefully transferred to the experimental units. Larvae from two parents were pooled and an average of 600 were randomly sampled by means of a siphon. Finally, exactly 600 larvae were removed with a table spoon and randomly allocated to the treatment groups, except in experiment 1, 1992, in which three hundred and twenty larvae were allocated in each experimental unit to yield a density of 20 larvae/L. (because of limited supply of larvae.) One day was allowed for recovery and complete yolk absorption for all experiments in 1991 and 1992. Dead larvae were replaced to maintain a density of 20 larvae/L before experiments started. Larvae generally started exogenous feeding at the age of 4 days after hatching. (All subsequent references to larval or juvenile ages refers to days after hatching.) All experiments began at the onset of exogenous feeding.

3.3 Food Sources

Artemia nauplii cysts were hatched in 8L glass cylinders at the ambient temperature of 25°C and a salinity of 30ppt. Air stones circulated the cysts and water at all times. The cysts hatched within 36h and were separated and held in culture medium until fed to the larvae. Prepared food used in this experiment was a commercial Thai food in a powder form. The chemical composition of *Artemia* and prepared food was generally similar except for water content (Table 1).

Table 1. Chemical composition of prepared food and *Artemia* nauplii used in experiments of 1991 and 1992.

	<i>Artemia</i>	Prepared Food
Protein (%DM)	51.3	≥ 40
Lipid (%DM)	16.6	≥ 10
Ash (%DM)	9.8	≤ 8
Moisture (%)	92.2	≤ 12

Source: Damme et al.(1990) and C.P. Ltd.(Thailand).

3.4 Rearing Tanks and Experimental Location.

In the summer of 1991, experiments 1 and 2 were conducted in glass aquaria having a total volume of 40L (30cm wide, 45cm long and 30cm high; Figure 5). containing of 30L city water. The aquaria were illuminated by natural light. Treatments and replications were randomly assigned to the aquaria.

In the summer of 1992, all three experiments were conducted in circular blue plastic tubs (50cm in diameter and 20cm high; Figure 6) filled to a capacity of 30L with city water. In experiment 1 (1992) the tubs were filled to a capacity of only 16L of water per tank in order to maintain the desired larval density of 20 larvae/L. This change from glass aquaria to the plastic tubs was prompted by difficulties encountered in 1991, in rearing the larvae and cleaning the glass aquaria. In the 1992 experiments, 80% of the water was replaced every second day and 30% was replaced on the intervening days to maintain acceptable water quality (between the optimal ranges at a temperature of 25-30°C and pH 6.5-8.5).

City (tap) water was utilized for both experiments in both summers after storage in an open tank for at least 24h to avoid the effect of chlorine.

The experiments in both 1991 and 1992 were conducted at the Hatchery Section, Department of Inland Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen Province, Thailand (Figure 7). In 1991 experiments were conducted inside

Figure 5. Glass aquaria used in the 1991 experiments.

Figure 6. The tanks used in experiments in the summer of 1992.

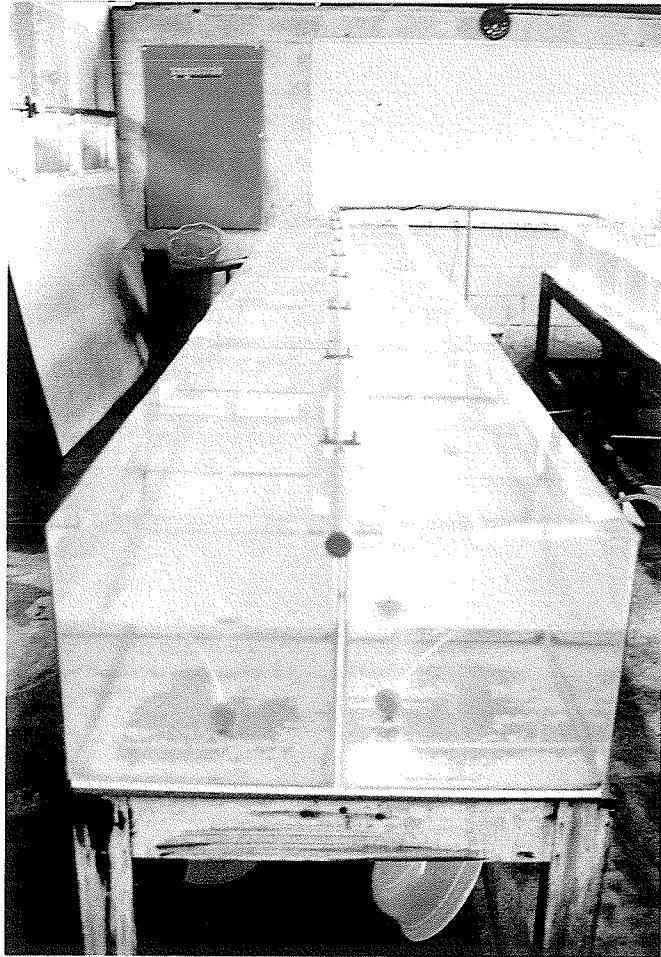
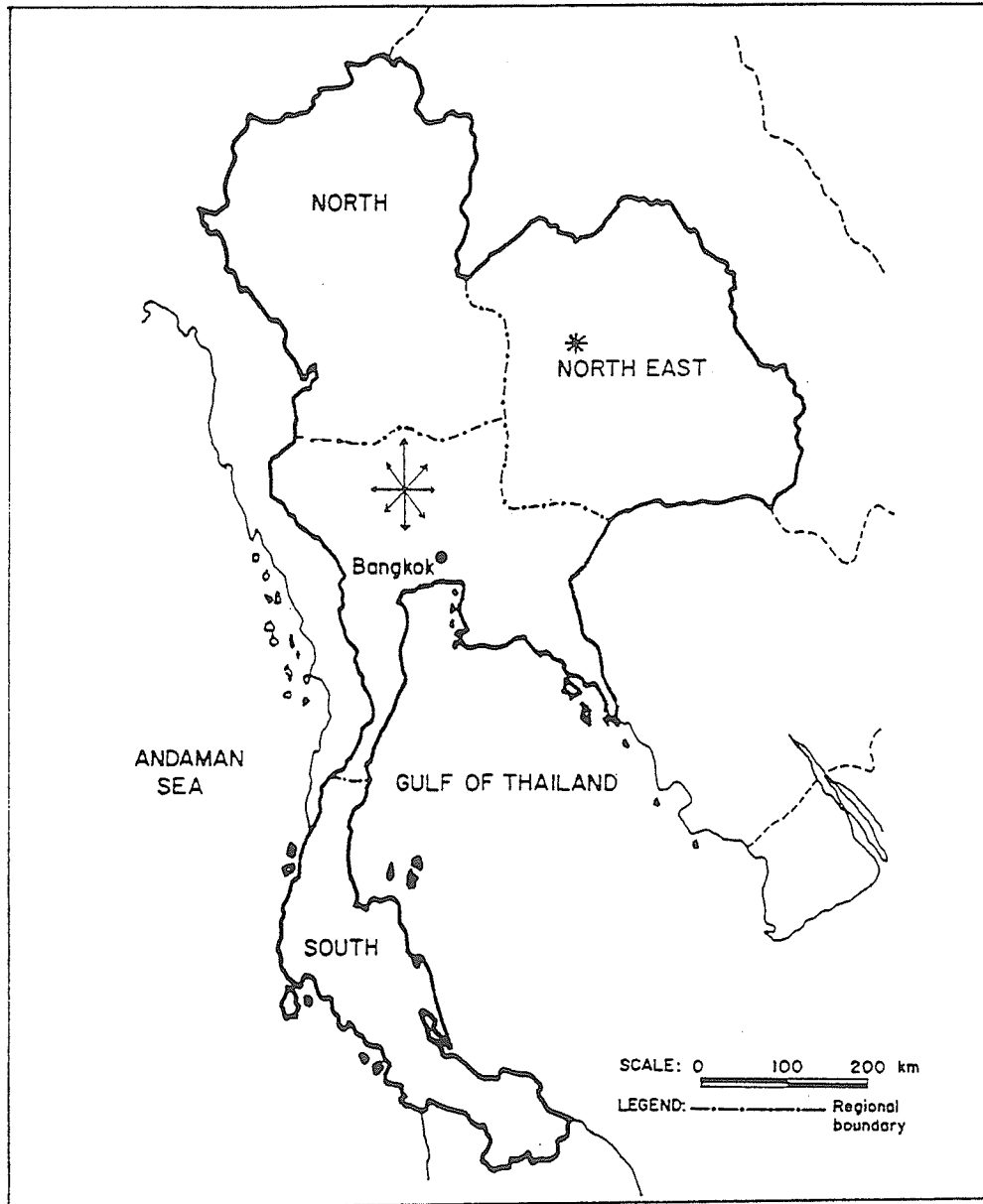


Figure 7. Map of Thailand indicating the experimental location (*) and the distribution of major catfish farms (arrows).



The Hatchery Building. Experiments in 1992 were conducted in the CIDA/ILP (UM & KKU) Building. The experimental areas were shaded but exposed to outdoor temperature. Water temperatures in the tanks and tubs ranged from 25 to 29°C over all experiments. Oxygen was monitored at all times. Tanks were cleaned regularly before the first feeding time each day. Dead larvae were removed daily and cause of death determined where possible.

3.5 Sampling and Water Quality Measurements

Samples for each experiment were obtained by randomly removing 30 larvae from each aquarium or tub on each sampling date. Ten larvae were preserved in a formalin solution for 24h. Their total lengths were then recorded using a stereo microscope. Larvae were preserved in 10% formalin in 1991. In 1992, 5% formalin was used to reduce length shrinkage caused by preservation in formalin (Glenn and Mathias, 1987).

The remaining 20 larvae from each sample were used to estimate dry weight. In 1991, larvae were placed in a drying oven set to 100°C for 24h. In 1992, the oven temperature was reduced to 80°C to avoid excessive loss of volatile fatty acids. After 24h samples were removed from the oven and allowed to cool for about 5 minutes in a desiccator. They were then weighed (as a group of 20) on a balance in an air-conditioned laboratory.

Water temperature (°C) and pH were measured using a

digital meter (Orion pH meter, SA 250). Dissolved oxygen (DO, mg/L) and ammonia concentration (mg/L) were measured using a Hach kit. Three random water samples were also analyzed for ammonia concentration by standard methods (American Public Health Association, Inc., 1965) to ensure the accuracy of the Hach kit readings.

3.6 Feeding Experiments

Experiment 1, 1991.

Artemia nauplii were graded by means of sieves so that equal numbers of food items were available over the following size ranges: $\leq 1\%$, 1-2%, 2-3%, and 3-4% of larval initial total length. Six hundred, two day old *C. macrocephalus* larvae were randomly sampled and allocated to 30L glass aquaria. The experiment utilized a completely randomized design with 5 treatments and 3 replications. The experimental lay-out is shown in Appendix 1.1. The treatments applied were as follows:

- treatment 1, larvae received unsieved *Artemia*
- treatment 2, larvae received *Artemia* $\leq 59\mu$
- treatment 3, larvae received *Artemia* 59-183 μ
- treatment 4, larvae received *Artemia* 183-205 μ
- treatment 5, larvae received *Artemia* 205-295 μ .

The food was given to excess every 4h throughout a 15 day experimental period. Thirty larvae were randomly sampled from each experimental unit on days 1, 3, 5, 7, 9, 11, 13, and 15.

The individual total length and dry weight were then recorded. Growth and survival were calculated.

Experiment 2, 1991.

The experiment utilized a completely randomized design with 4 treatments and 4 replications. Treatments consisted of prepared food pressed through sieves in the following size ranges:

- treatment 1, larvae received unsieved prepared food
- treatment 2, larvae received prepared food $\leq 183\mu$
- treatment 3, larvae received prepared food 183-205 μ
- treatment 4, larvae received prepared food 205-295 μ .

The experimental design is shown in Appendix 1.2.

Six hundred two-day old larvae were randomly sampled and allocated to 30L glass aquaria. Thirty larvae were randomly sampled from each experimental unit on days 1, 3, 5, 7, 9, 11, and 13. The individual total lengths and dry weights were then recorded. Prepared food (supplied in excess) was given every 4h. Growth and survival were calculated.

Experiment 1, 1992.

The experiment utilized a completely randomized design with four treatments and three replications. Three hundred and twenty, two day old *C. macrocephalus* larvae were randomly allocated to each tank. The 12 tanks were filled with 16L of water and a density of 20 larvae/L. The lay-out of the

experiment is shown in Appendix 1.3.

The treatments consisted of size graded *Artemia* nauplii. Nylon mesh sieves were used to separate nauplii into three size groups. Food density for each size of *Artemia* was evaluated by counting under a microscope. Larvae were then supplied with equal numbers of *Artemia*. Four treatments were applied each with three replicates:

- treatment 1, larvae received unsieved *Artemia*
- treatment 2, larvae received *Artemia* $\leq 160\mu$
- treatment 3, larvae received *Artemia* 160-214 μ
- treatment 4, larvae received *Artemia* 214-315 μ .

Thirty larvae were sampled from each experimental unit on days 1, 6, 13, and 21. Total length and dry weight were then measured. Feed was given equally for all treatments to excess every 6h. Growth and survival were calculated.

Experiment 2, 1992.

The experiment utilized a completely randomized design with 6 treatments and 3 replications. Six hundred, two day old larvae were randomly allocated to each tank. The lay-out of the experiment is shown in Appendix 1.4.

The six treatments applied in this experiment were as follows:

- treatment 1, larvae were fed on *Artemia* nauplii through the entire 21 day experimental period.
- treatment 2, larvae were fed on *Artemia* nauplii for the

first fifteen days and fed prepared food in powdered form for the remaining six days.

- treatment 3, larvae were fed on *Artemia* nauplii for the first ten days and fed prepared food in powdered form for the remaining eleven days.

- treatment 4, larvae were fed on *Artemia* nauplii for the first five days and fed prepared food in powdered form for the remaining sixteen days.

- treatment 5, larvae were fed prepared food in powdered form throughout the 21 day experimental period.

- treatment 6, larvae were fed prepared food in a paste-like form throughout the 21 day experimental period.

Larvae were fed to excess at 6h intervals each day depending on the treatment. Thirty larvae were randomly sampled on days 1, 5, 6, 10, 11, 15, 16 and 21 for length and dry weight measurements. The extent of stomach fullness was classified as "empty", "half-full" or "full" on the basis of a subjective examination under a dissecting microscope. The sample data were used to calculate growth, survival and degree of stomach fullness in each treatment group.

Experiment 3, 1992.

The experiment was conducted using a completely randomized design with 5 treatments and 3 replications. The dependent variables were growth and survival.

Six hundred, two day old larvae were randomly allocated

to 30L tanks resulting in a density of 20 larvae/L. The lay-out of the experiment is shown in Appendix 1.5. Five treatments were applied in this experiment as follows:

- treatment 1, larvae were fed once at the beginning of each day (7AM)
- treatment 2, larvae were fed once at the end of each day (7PM)
- treatment 3, larvae were fed twice at 7AM and 5PM
- treatment 4, larvae were fed three times at 7AM, 12AM, and 5PM
- treatment 5, larvae were fed four times at 7AM, 10AM, 2PM, and 5PM.

Larvae were fed using equal numbers of *Artemia* for all treatments depending on a assigned schedule of treatment. Thirty larvae were randomly sampled on day 1, 5, 9, 13, 17 and 21 for total length and dry weight measurements. Ten larvae were individually measured for a total length and twenty larvae used to obtain a pooled dry weight. Tanks were cleaned regularly at dusk, except for tanks used in treatment 2 which were cleaned at dawn to control food availability during day and night. Growth and survival were calculated.

3.7 Statistical Analysis

Analysis of variance (ANOVA) was used to compare the differences in treatment effects on the tested parameters, total length and mean dry weight. When statistically

significant differences existed at a Probability level <0.05 , Duncan's Multiple Range test was utilized to compare treatment means. Hartley's Test was used to test the Homogeneity of variance (Neter et al., 1990). Percent survival at the end of an experiment was normalized by an arcsin transformation and analyzed by means of one-way ANOVA. The correlation between mean length and survival among the replicates within an experiment was analyzed to determine whether differences in growth could be attributed to differences in survival as well as differences among treatments. Specific growth rate, relative growth rate and increment of both total length and dry weight were tested by means of one-way ANOVA (SAS Institute Inc., 1987, Neter et al., 1990 and Schulman, 1992). These parameters were calculated as follows (Ricker, 1975):

Total Length (TL)

Specific growth rate in total length, % per day:

$$= [(\ln \text{ final TL} - \ln \text{ initial TL})/\text{time}] * 100$$

Relative growth rate of total length, % per day:

$$= [((\text{Final TL} - \text{Initial TL})/\text{Initial TL})/\text{time}] * 100$$

Increment of total length, cm:

$$= \text{Final TL} - \text{Initial TL}.$$

Dry Weight (DW)

Specific growth rate in dry weight, % per day:

$$= [(\ln \text{ final DW} - \ln \text{ initial DW})/\text{time}] * 100$$

Relative growth rate of dry weight, % per day:

$$= [((\text{Final DW} - \text{Initial DW})/\text{Initial DW})/\text{time}] * 100$$

Increment of dry weight, mg:

$$= \text{Final DW} - \text{Initial DW}.$$

Survival, %.

$$= [\text{number of surviving larvae} / (\text{initial number of larvae} - \text{sampled larvae})] * 100.$$

4. Results

Effect of Particle Size on Growth and Survival: *Artemia* (1991).

A one-way analysis of variance indicated food particle size had a highly significant affect on both total length and dry weight of larvae ($F=73.64$, $Pr>F=0.0001$; $F=12.80$, $Pr>F=0.0006$ respectively). Treatment 4 (183-205 μ) yielded the maximum total length (1.67cm, Table 2). Larvae which received the smallest *Artemia* ($\leq 59\mu$, Treatment 2) attained the smallest size (0.96cm). Maximum dry weight of 4.72mg resulted when larvae received the largest *Artemia* (205-295 μ , Treatment 5, Table 2). The lowest dry weight (1.03mg) resulted when larvae received the smallest *Artemia* (Treatment 2). Differences among treatments 5, 4, and 1 were not significant. Treatments 2 and 3 generally resulted in poor growth throughout the 15 day experimental period (Figures 8 and 9).

There were no detectible significant differences in survival because very high variation occurred within treatments ($F=2.62$, $Pr>F=0.0987$; survival in each treatment: T1=68.33%, 55.00%, 78.89%; T2=20.83%, 4.17%, 18.89%; T3=41.39%, 1.67%, 43.33%; T4=52.78%, 51.94%, 14.17%; T5=50.00%, 14.44%, 60.00%). The best survival resulted when larvae received unsieved *Artemia* (Treatment 1, 67.4%, Table 2). Larvae which received the smallest nauplii ($\leq 59\mu$,

Treatment 2) showed the poorest survival. There was no significant correlation between growth and survival in the 15 tanks. Differences in specific growth rates, relative growth rates and increments of both total length and dry weight are shown in Table 3. There were significant differences among the specific growth rates of total length and dry weight ($F=17.44$, $Pr>F=0.0002$; $F=12.73$, $Pr>F=0.0006$ respectively). Treatment 4 (6.42%) and 5 (5.84%) gave the best specific growth rate of total length. The best specific growth rates of dry weight were found in treatment 1 (16.89%), treatment 4 (16.84%) and treatment 5 (16.72%).

Table 2. A comparison of final total length, dry weight and survival of larvae fed unsieved and size graded *Artemia* nauplii for 15 days after hatching. (Experiment 1, 1991; Means¹ ± SE²)

Treatments	Total Length ³ (cm)	Dry Weight ⁴ (mg)	Survival(%)
T1,unseived	1.372 ^c ± 0.040	4.473 ^a ± 0.615	67.41 ^a ± 6.91
T2, ≤59μ	0.969 ^e ± 0.014	1.030 ^b ± 0.087	14.63 ^a ± 5.26
T3,59-183μ	1.240 ^d ± 0.026	2.093 ^b ± 0.397	28.80 ^a ± 13.57
T4,183-205μ	1.666 ^a ± 0.039	4.690 ^a ± 0.318	44.54 ^a ± 7.83
T5,205-295μ	1.546 ^b ± 0.032	4.720 ^a ± 0.719	35.37 ^a ± 13.28

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

³after preservation in 10% formalin for 24h.

⁴after drying at 100°C for 24h.

Figure 8. Experiment 1, 1991. The effect of food particle size on the total length (cm) of larvae. T1 unsieved nauplii, T2 nauplii $\leq 59\mu$, T3 nauplii $59-183\mu$, T4 nauplii $183-205\mu$ and T5 nauplii $205-295\mu$. The vertical bars are the standard error of the estimate.

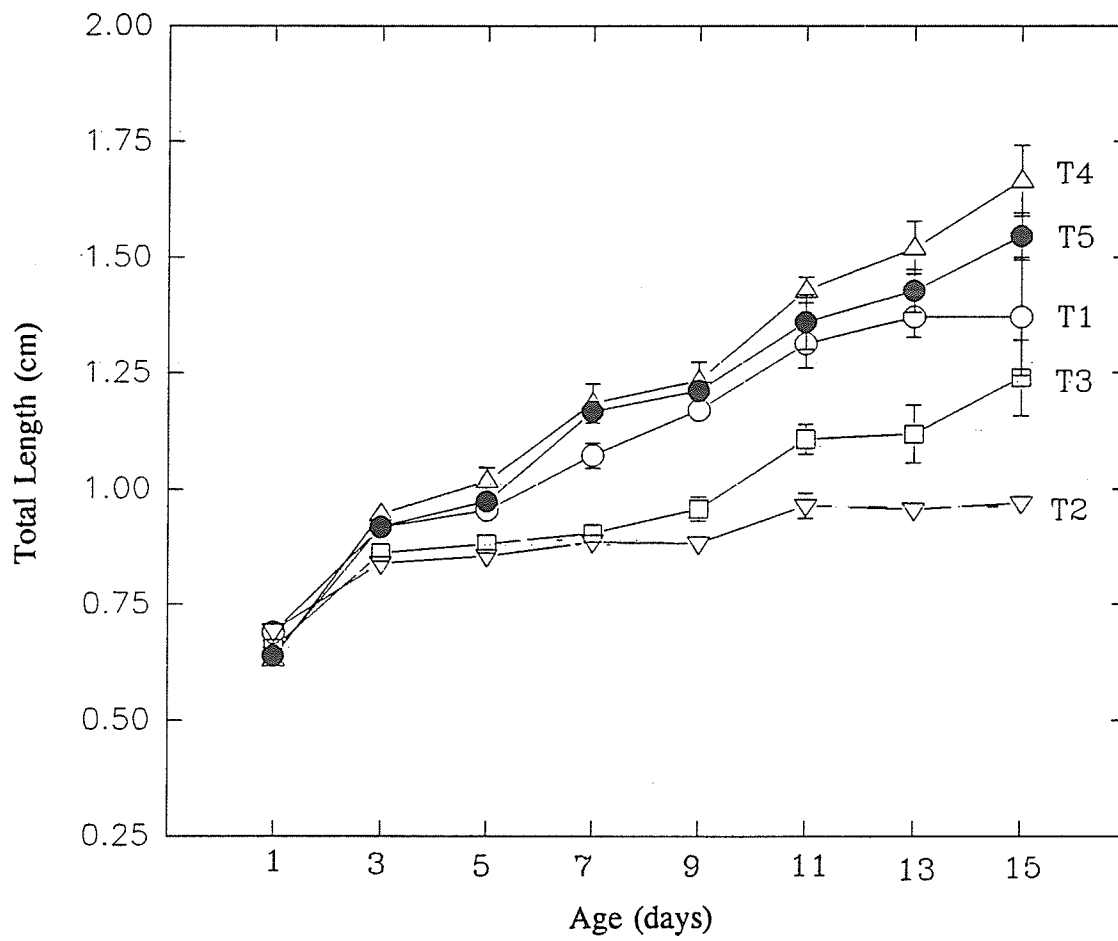


Figure 9. Experiment 1, 1991. The effect of food particle size on the dry weight (mg) of larvae. T1 unsieved nauplii, T2 nauplii $\leq 59\mu$, T3 nauplii $59-183\mu$, T4 nauplii $183-205\mu$ and T5 nauplii $205-295\mu$. The vertical bars are the standard error of the estimate.

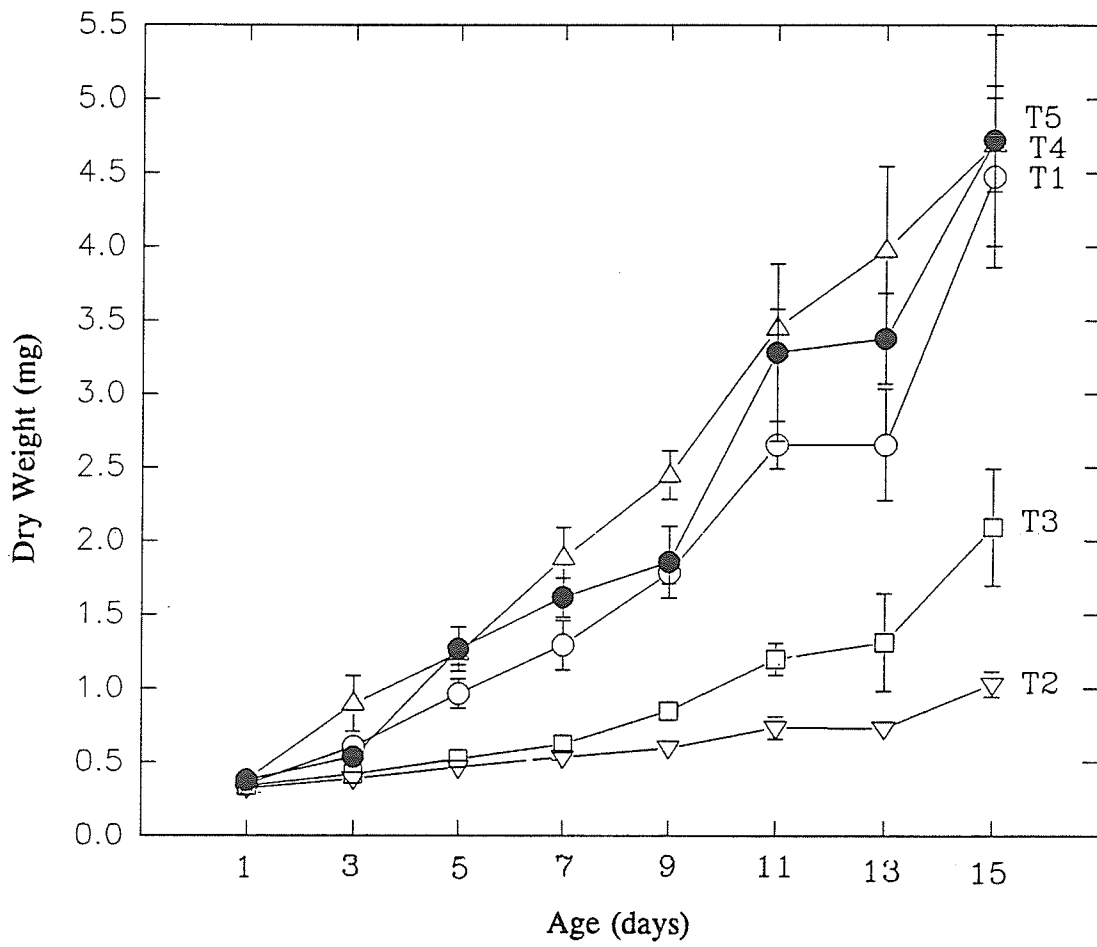


Table 3. A comparison of specific growth rates, relative growth rates and increments for total length and dry weight of larvae fed unsieved and size graded *Artemia* nauplii for 15 days after hatching. (Experiment 1, 1991; Means¹ ± SE²)

Treatments	Total Length		
	Specific Growth	Relative Growth	Increment
T1,unsieved	4.50 ^b ± 0.45	6.50 ^b ± 0.91	0.68 ^{bc} ± 0.11
T2, ≤59μ	2.24 ^c ± 0.30	2.69 ^c ± 0.43	0.28 ^d ± 0.03
T3,59-183μ	4.24 ^b ± 0.45	5.99 ^b ± 0.86	0.59 ^c ± 0.08
T4,183-205μ	6.42 ^a ± 0.41	10.87 ^a ± 1.04	1.03 ^a ± 0.09
T5,205-295μ	5.84 ^a ± 0.29	9.38 ^a ± 0.71	0.90 ^{ab} ± 0.06

Treatments	Dry Weight		
	Specific Growth	Relative Growth	Increment
T1,unsieved	16.89 ^a ± 0.90	78.77 ^a ± 10.95	4.12 ^a ± 0.60
T2, ≤59μ	7.68 ^c ± 0.45	14.52 ^b ± 1.42	0.71 ^b ± 0.08
T3,59-183μ	11.95 ^b ± 1.20	34.66 ^b ± 7.18	1.76 ^b ± 0.38
T4,183-205μ	16.84 ^a ± 1.51	80.84 ^a ± 18.13	4.31 ^a ± 0.38
T5,205-295μ	16.72 ^a ± 1.39	78.62 ^a ± 16.31	4.34 ^a ± 0.73

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

Effect of Food Particle Size on Growth and Survival: prepared food in powder form (1991).

Particle size of prepared food had no significant affect on total length (based on a one-way ANOVA, $F=1.78$, $Pr>F=0.1531$). Maximum total length (1.05cm) was found in treatment 1 (unsieved prepared food) and minimum total length (0.99cm) was found in treatment 3 (prepared food $\leq 183\mu$, Table 4). However, there were highly significant differences in dry weight ($F=10.69$, $Pr>F=0.001$). Maximum dry weight (1.08mg) was found in treatment 1 (unsieved prepared food).

Treatment 3 resulted poor growth throughout the 13 day experimental period (Figures 10 and 11). There were significant differences in specific growth rates of dry weight ($F=5.89$, $Pr>F=0.0104$) but no significant difference was found for specific growth rate of total length ($F=0.64$, $Pr>F=0.6056$, Table 5). The best specific growth rate of dry weight was found in treatment 1 (7.82%) and treatment 4 (7.64%).

Differences in survival were not significant ($F=1.96$, $Pr>F=0.1740$). Low survival occurred in all treatments. The best survival was only 18.9%, in treatment 1; the poorest survival, 3.3% was found in treatment 2 (whose larvae received the smallest size of prepared food, Table 5). There was no significant correlation between growth and survival in the 16 tanks.

Table 4. A comparison of final total length, dry weight and survival of larvae fed unsieved and size graded prepared food for 13 days after hatching. (Experiment 2, 1991; Means¹ ± SE²)

Treatments	Total Length ³ (cm)	Dry Weight(m ⁴ g)	Survival(%)
T1, Unsieved	1.052 ^a ± 0.022	1.080 ^a ± 0.053	18.85 ^a ± 6.34
T2, ≤183μ	1.031 ^a ± 0.015	0.865 ^{bc} ± 0.012	3.27 ^a ± 1.85
T3, 183-205μ	0.991 ^a ± 0.020	0.768 ^c ± 0.026	15.58 ^a ± 5.21
T4, 205-295μ	1.034 ^a ± 0.020	0.970 ^{ab} ± 0.056	16.67 ^a ± 3.46

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

³after preservation in 10% formalin for 24h.

⁴after drying at 100°C for 24h.

Figure 10. Experiment 2, 1991. The effect of food particle size on the total length (cm) of larvae. T1 unsieved prepared food, T2 prepared food $\leq 183\mu$, T3 prepared food $183-205\mu$ and T4 prepared $205-295\mu$. The vertical bars are the standard error of the estimate.

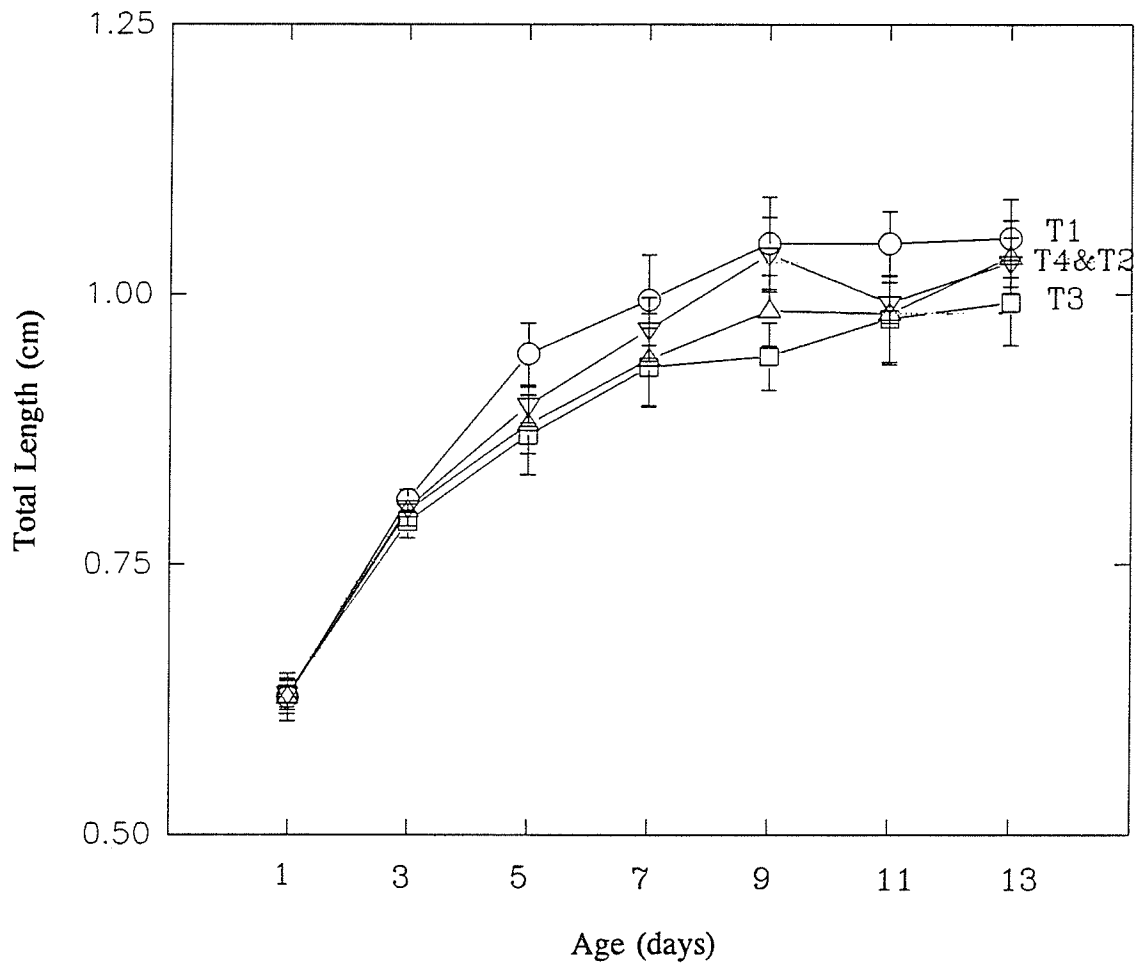


Figure 11. Experiment 2, 1991. The effect of food particle size on the dry weight (mg) of larvae. T1 unsieved prepared food, T2 prepared food $\leq 183\mu$, T3 prepared food $183-205\mu$ and T4 prepared $205-295\mu$. The vertical bars are the standard error of the estimate.

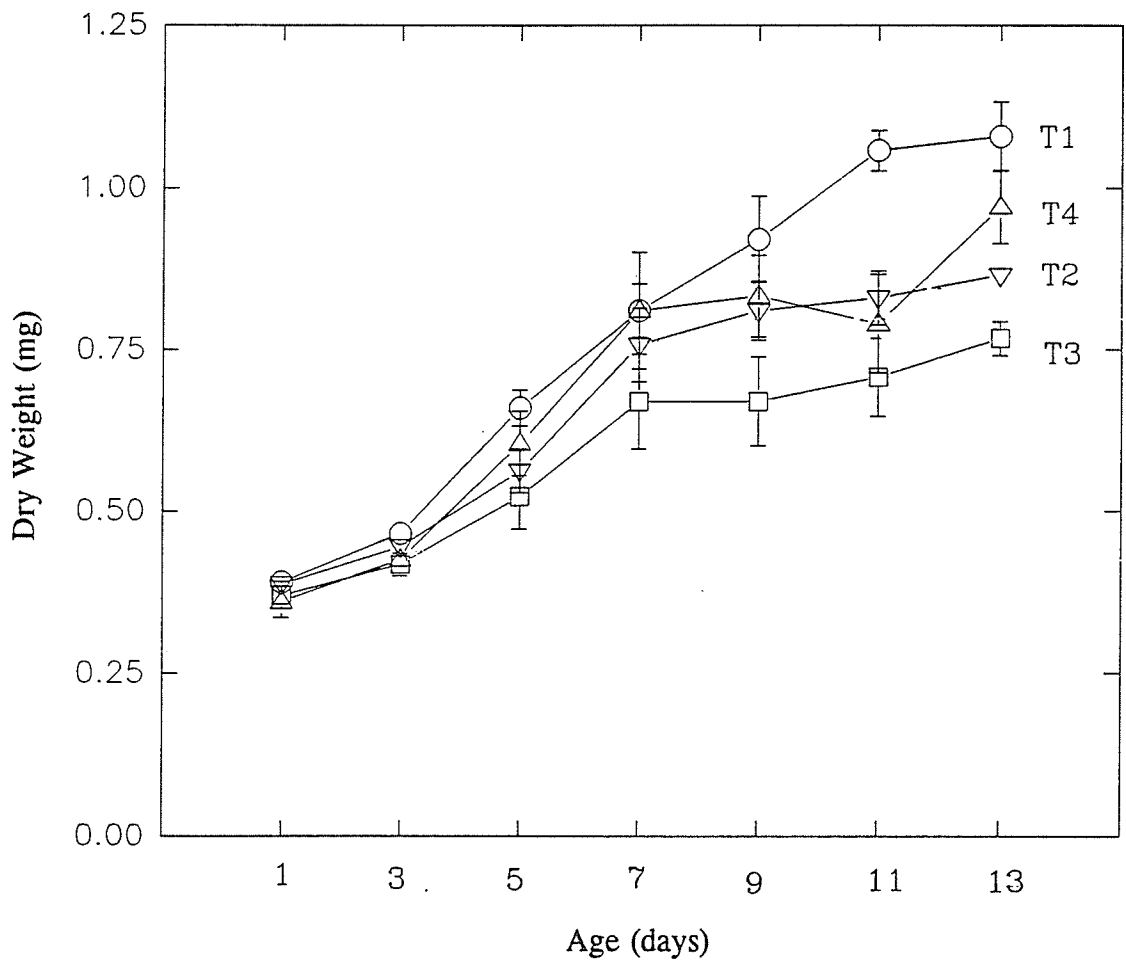


Table 5. A comparison of specific growth rates, relative growth rates and increments for total length and dry weight of larvae fed unsieved and graded prepared food for 13 days after hatching. (Experiment 2, 1991; Means¹±SE²)

Treatments	Total Length		
	Specific Growth	Relative Growth	Increment
T1,unsieved	3.98 ^a ± 0.42	5.27 ^a ± 0.68	0.43 ^a ± 0.05
T2, ≤183μ	3.78 ^a ± 0.17	4.89 ^a ± 0.27	0.40 ^a ± 0.02
T3,183-205μ	3.48 ^a ± 0.18	4.41 ^a ± 0.28	0.36 ^a ± 0.03
T4,205-295μ	3.84 ^a ± 0.20	5.00 ^a ± 0.33	0.41 ^a ± 0.03

	Dry Weight		
	Specific Growth	Relative Growth	Increment
T1,unsieved	7.82 ^a ± 0.37	13.63 ^a ± 1.03	0.69 ^a ± 0.05
T2, ≤183μ	6.19 ^b ± 0.31	9.55 ^b ± 0.71	0.48 ^b ± 0.02
T3,183-205μ	5.64 ^b ± 0.69	8.50 ^b ± 1.35	0.40 ^b ± 0.05
T4,205-295μ	7.64 ^a ± 0.27	13.10 ^a ± 0.74	0.61 ^a ± 0.04

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

Effect of Food Particle Size on Growth and Survival: *Artemia* (1992).

A one-way ANOVA indicated that treatment 4 in which larvae fed on *Artemia* (214-315 μ), and treatment 3 in which larvae fed on *Artemia* (160-214 μ) gave significantly higher growth in terms of total length than treatments 1 and 2 in which larvae fed on unsieved *Artemia* and nauplii $\leq 160\mu$ respectively ($F=6.07$, $Pr>F=0.0007$, Table 6, Figure 12). Maximum total length was found in treatment 4 (1.38cm), fed on the largest nauplii and minimum total length occurred in treatment 2 (1.30cm), fed on the smallest nauplii. No significant differences were found between treatments 4 and 3 as well as between treatments 1 and 2 (Table 6).

Differences in dry weight were not significant ($F=1.20$, $Pr>F=0.3687$). However, larvae fed on bigger sizes of *Artemia* (T3, 160-214 μ and T4, 214-315 μ) tended to be heavier than those receiving smaller sizes of *Artemia* (Table 6, Figure 13). Larvae fed on the smallest size of *Artemia* ($\leq 160\mu$) had a lower dry weight but significant differences were not detectable.

Survival was generally high and did not significantly differ among the four treatments ($F=1.06$, $Pr>F=0.4192$). Survival was highest in treatment 1 (84.3%) and lowest (77.3%) in treatment 2. There was no significant correlation between growth (total length) and survival among the 12 tanks. Significant differences were not detectable in specific growth

rates of total length ($F=2.02$, $Pr>F=0.1894$, Table 7) and specific growth rates of dry weight ($F=2.40$, $Pr>F=0.1430$). However, treatments 4 and 3 had a tendency to give slightly higher specific growth rates in both total length and dry weight than treatments 1 and 2 but not significantly different.

Table 6. A comparison of final total length, dry weight and survival of larvae fed unsieved and size graded *Artemia* nauplii for 21 days after hatching. (Experiment 1, 1992; Means¹ ±SE²)

Treatments	Total Length ³ (cm)	Dry Weight ⁴ (mg)	Survival(%)
T1,unsieved	1.319 ^b ± 0.018	3.253 ^a ± 0.373	84.33 ^a ± 1.17
T2, ≤160μ	1.297 ^b ± 0.011	2.983 ^a ± 0.133	77.33 ^a ± 5.13
T3,160-214μ	1.377 ^a ± 0.017	3.627 ^a ± 0.081	81.17 ^a ± 0.44
T4,214-315μ	1.379 ^a ± 0.019	3.360 ^a ± 0.269	78.17 ^a ± 2.35

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

³after preservation in 5% formalin for 24h.

⁴after drying at 80°C for 24h.

Figure 12. Experiment 1, 1992. The effect of food particle size on the total length (cm) of larvae. T1 unsieved nauplii, T2 nauplii $\leq 160\mu$, T3 nauplii $160-214\mu$ and T4 nauplii $214-315\mu$. The vertical bars are the standard error of the estimate.

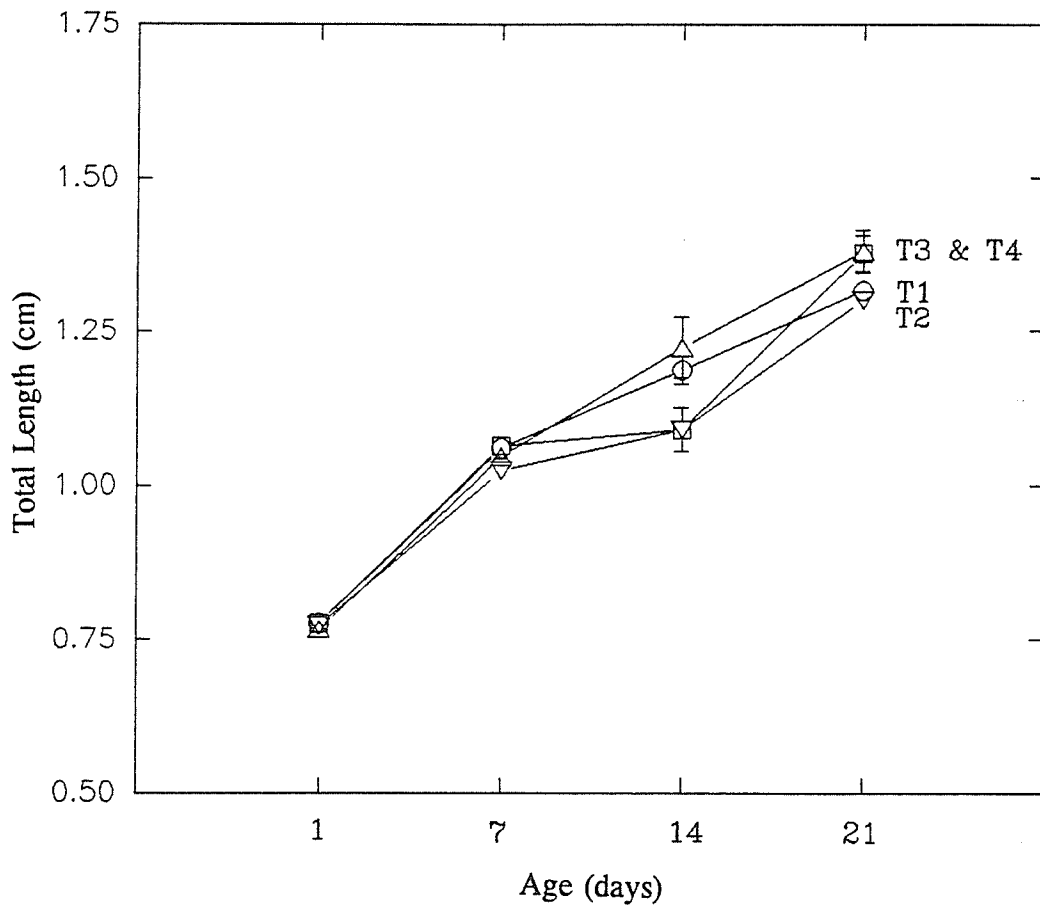


Figure 13. Experiment 1, 1992. The effect of food particle size on the dry weight (mg) of larvae. T1 unsieved nauplii, T2 nauplii $\leq 160\mu$, T3 nauplii $160-214\mu$ and T4 nauplii $214-315\mu$. The vertical bars are the standard error of the estimate.

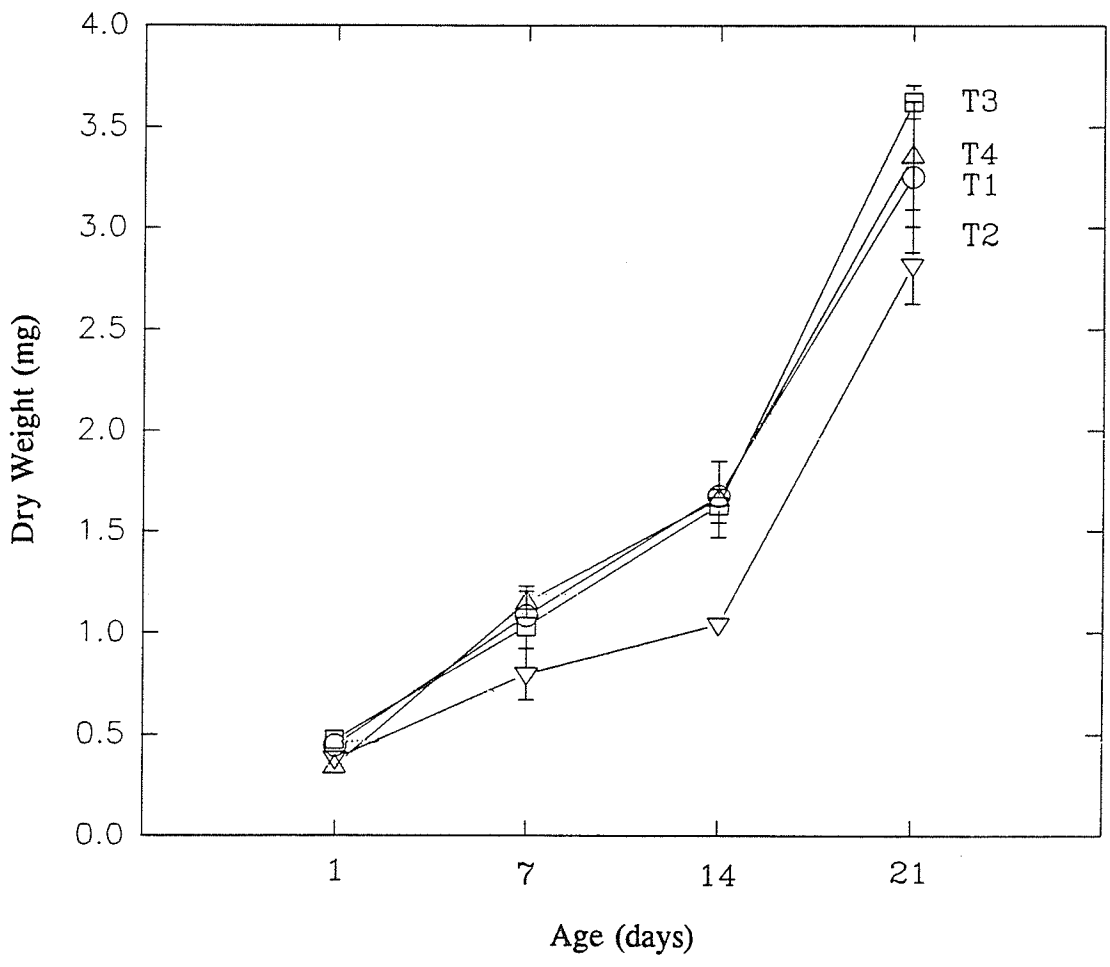


Table 7. A comparison of specific growth rates, relative growth rates and increments for total length and dry weight of larvae fed unsieved and size graded *Artemia* nauplii for 21 days after hatching. (Experiment 1, 1992; Means¹±SE²)

Treatments	Total Length		
	Specific Growth	Relative Growth	Increment
T1, unsieved	2.51 ^a ± 0.05	3.31 ^a ± 0.08	0.54 ^a ± 0.01
T2, ≤160μ	2.47 ^a ± 0.04	3.24 ^a ± 0.06	0.53 ^a ± 0.01
T3, 160-214μ	2.72 ^a ± 0.11	3.68 ^a ± 0.19	0.60 ^a ± 0.03
T4, 214-315μ	2.80 ^a ± 0.18	3.82 ^a ± 0.33	0.61 ^a ± 0.04

	Dry Weight		
	Specific Growth	Relative Growth	Increment
T1, Unsieved	9.40 ^a ± 0.35	29.71 ^a ± 2.43	2.81 ^a ± 0.35
T2, ≤160μ	9.56 ^a ± 0.75	31.58 ^a ± 5.82	2.44 ^a ± 0.22
T3, 160-214μ	10.04 ^a ± 0.05	34.49 ^a ± 0.38	3.19 ^a ± 0.07
T4, 214-315μ	10.85 ^a ± 0.13	41.75 ^a ± 1.26	3.02 ^a ± 0.25

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

Effect of Food Combination on Growth and Survival (1992).

In this experiment, larvae which fed on *Artemia* for the first 10 days followed by powdered prepared food for the remaining 11 days (T3) of the 21 day experimental period grew significantly larger in both total length (1.77cm, $F=30.41$, $Pr>F=0.0001$) and dry weight (6.62mg, $F=41.90$, $Pr>F=0.0001$) than larvae in the other treatments in which *Artemia* and prepared food were provided for different time periods (Table 8). The worst growth in terms of total length and dry weight occurred when larvae received powdered food throughout the 21 day experimental period (T5). In general, diets consisting entirely of *Artemia* or entirely of prepared food were inferior to those in which larvae began feeding on *Artemia* and were then switched to powdered prepared food. Of the two treatments in which larvae received only prepared food in either a powder (T5) or a paste-like form (T6), those (T6) receiving it in the paste-like form showed superior growth in terms of both total length and dry weight (Table 8, Figures 14 and 15). Specific growth rate, relative growth rate and increment of both total length and dry weight were significantly higher in treatment 3 than in the other treatments ($P<0.01$, Table 9).

Dissection of the stomachs of larvae indicated both powdered and paste-like prepared food were accepted at an early stage of exogenous feeding, however, young larvae of this catfish have a preference for live food (*Artemia*) insofar

as those receiving *Artemia* (treatments 1, 2, 3, and 4) had significantly more food in their stomachs than those receiving prepared food (Figure 16). Older larvae tended to feed more successfully on prepared food (Figure 17).

The best survival (87.9%) was attained in treatment 2, however survival levels of 85.9% and 84.3% in treatments 3 and 1 respectively, which also received both *Artemia* and prepared food, were not significantly different from that of treatment 2. Larvae which received only prepared food had significantly poorer survival ($F=57.73$, $Pr>F=0.0001$, Table 8). There was a significant positive correlation between growth (total length) and survival ($r^2=0.52$, $b=0.004$); differences in specific growth rates, relative growth rates and increments shown in Table 9 are therefore effects of treatments rather than differential survival. There were highly significant differences in specific growth rate of total length ($F=17.18$, $Pr>F=0.0001$) and dry weight ($F=14.76$, $Pr>F=0.0001$). The best specific growth rate of total length and dry weight over a 21 day period were found in treatment 3. In addition, a comparison of specific growth rate at different ages is shown in Table 10. There was a strong trend of higher specific growth rate after switching to prepared food in treatment 2, treatment 3 and treatment 4 in comparison to other treatments at each age.

Table 8. A comparison of final total length, dry weight and survival of larvae fed different diets for 21 days after hatching. (Experiment 2, 1992; Means¹±SE²)

Treatments	Total Length ³ (cm)	Dry Weight ⁴ (mg)	Survival(%)
T1 ⁵	1.489 ^{cd} ± 0.023	3.843 ^c ± 0.226	84.26 ^a ± 1.54
T2 ⁶	1.553 ^{bc} ± 0.021	4.487 ^c ± 0.121	87.87 ^a ± 1.33
T3 ⁷	1.771 ^a ± 0.045	6.617 ^a ± 0.156	85.92 ^a ± 0.81
T4 ⁸	1.633 ^b ± 0.029	5.830 ^b ± 0.394	57.78 ^b ± 8.93
T5 ⁹	1.259 ^e ± 0.023	2.170 ^d ± 0.321	12.78 ^c ± 3.43
T6 ¹⁰	1.417 ^d ± 0.037	4.157 ^c ± 0.054	17.22 ^c ± 3.43

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

³after preservation in 5% formalin for 24h.

⁴after drying at 80°C for 24h.

⁵*Artemia* throughout the entire 21 days.

⁶*Artemia* followed by powdered prepared food on day 16.

⁷*Artemia* followed by powdered prepared food on day 11.

⁸*Artemia* followed by powdered prepared food on day 6.

⁹powdered prepared food throughout the entire 21 days.

¹⁰paste-like prepared food throughout the entire 21 days.

Figure 14. Experiment 2, 1992. The effect of food combination on the total length (cm) of larvae. T1 nauplii, T2 nauplii and switch to powdered prepared food on day 16, T3 nauplii and switch to powdered prepared on day 11, T4 nauplii and switch to powdered prepared food on day 6, T5 powdered prepared food and T6 prepared food in paste-like form. The vertical bars are the standard error of the estimate.

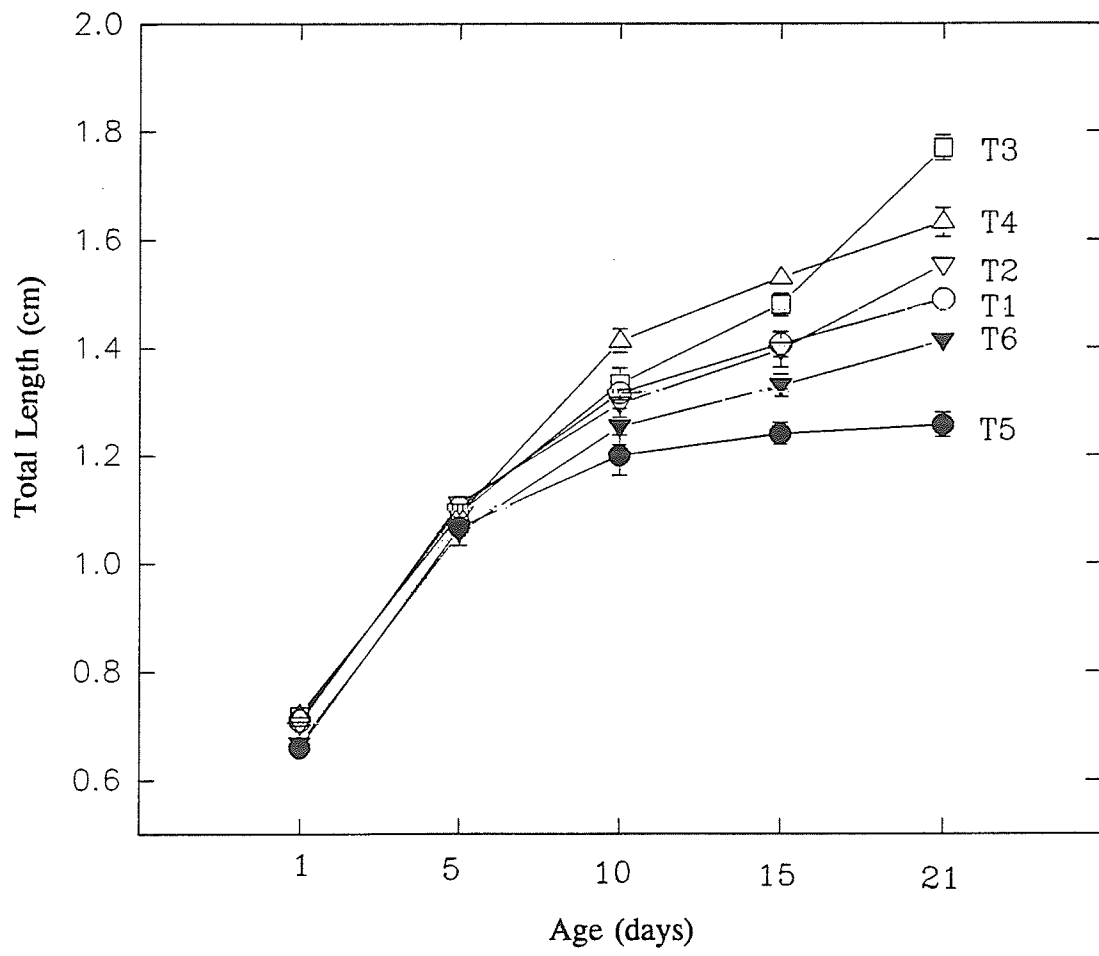


Figure 15. Experiment 2, 1992. The effect of food combination on the dry weight (mg) of larvae. T1 nauplii, T2 nauplii and switch to powdered prepared food on day 16, T3 nauplii and switch to powdered prepared on day 11, T4 nauplii and switch to powdered prepared food on day 6, T5 powdered prepared food and T6 prepared food in paste-like form. The vertical bars are the standard error of the estimate.

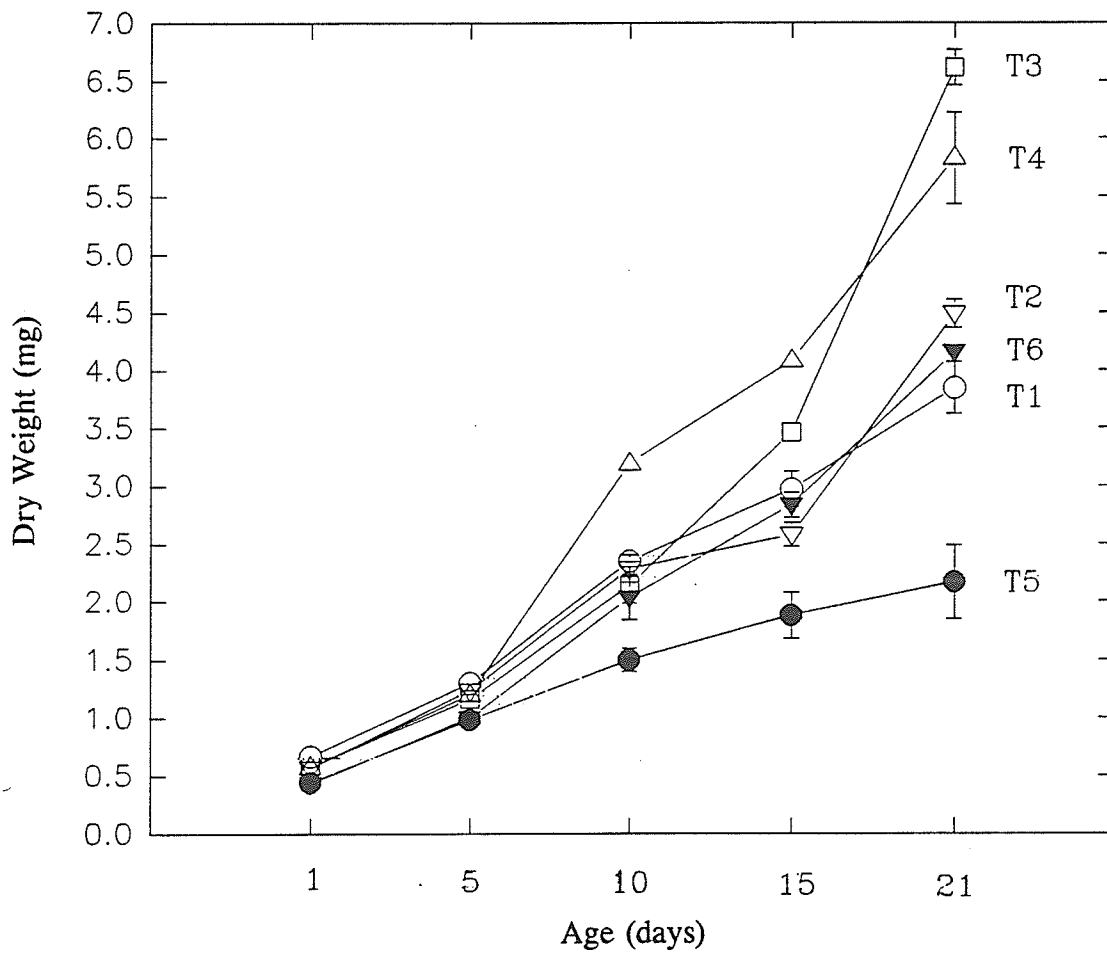


Table 9. A comparison of specific growth rates, relative growth rates and increments for total length and dry weight of larvae fed different diets for 21 days after hatching. (Experiment 2, 1992; Means¹±SE²)

Treatments	Total Length		
	Specific Growth	Relative Growth	Increment
T1	3.53 ^c ± 0.04	5.23 ^c ± 0.08	0.78 ^{cd} ± 0.01
T2	3.77 ^{bc} ± 0.09	5.76 ^{bc} ± 0.19	0.85 ^{bc} ± 0.02
T3	4.30 ^a ± 0.04	7.00 ^a ± 0.11	1.05 ^a ± 0.02
T4	3.90 ^b ± 0.15	6.05 ^b ± 0.34	0.91 ^b ± 0.04
T5	3.07 ^d ± 0.12	4.31 ^d ± 0.22	0.60 ^e ± 0.03
T6	3.58 ^{bc} ± 0.11	5.34 ^c ± 0.24	0.75 ^d ± 0.02
Dry Weight			
	Specific Growth	Relative Growth	Increment
T1	8.33 ^c ± 0.22	22.66 ^{cd} ± 1.28	3.18 ^d ± 0.22
T2	9.88 ^b ± 0.32	33.30 ^{bc} ± 2.46	3.92 ^c ± 0.11
T3	11.66 ^a ± 0.75	51.76 ^a ± 8.94	6.03 ^a ± 0.20
T4	11.02 ^{ab} ± 0.22	43.56 ^{ab} ± 2.22	5.25 ^b ± 0.36
T5	7.47 ^c ± 0.48	18.33 ^c ± 2.27	1.73 ^e ± 0.30
T6	10.80 ^{ab} ± 0.33	41.44 ^{ab} ± 3.29	3.72 ^{cd} ± 0.02

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

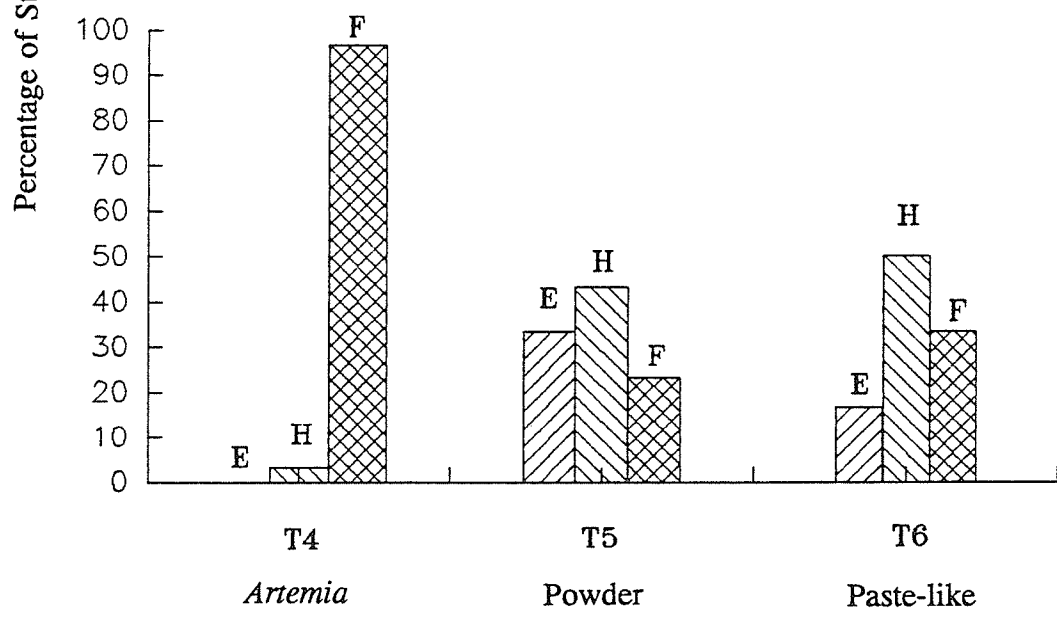
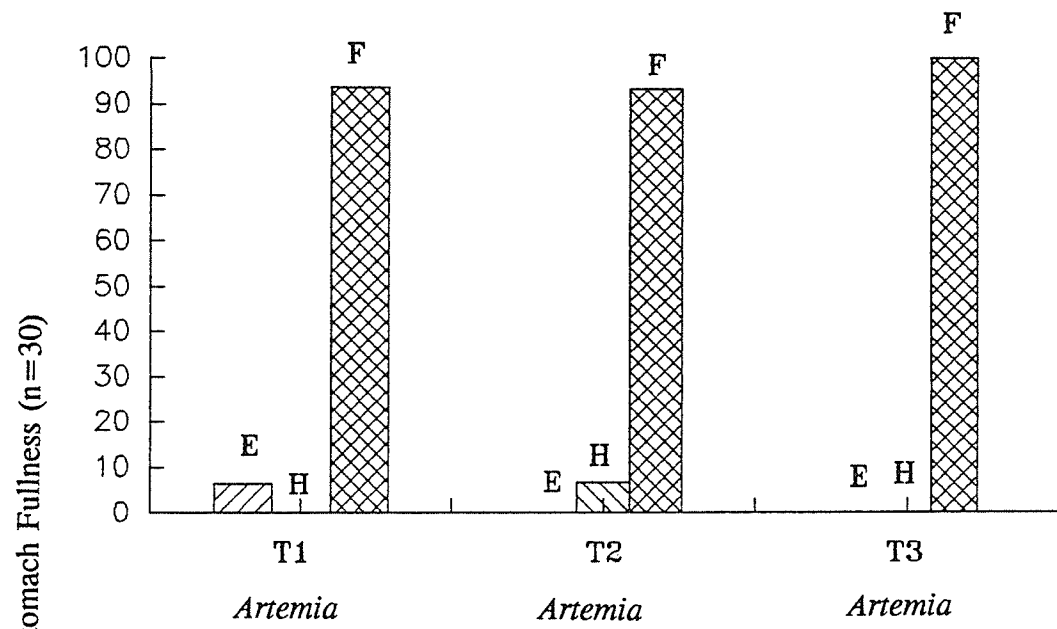


Figure 17, Experiment 2, 1992. The stomach fullness of larvae 1 day after the first presentation of prepared food in powdered form (E=empty, H=half-full and F=full). T2 larvae offered prepared food at 16 days of age. T3 larvae offered prepared food at 11 days of age. T4 larvae offered prepared food at 6 days of age. T5 larvae offered prepared food at 1 days of age.

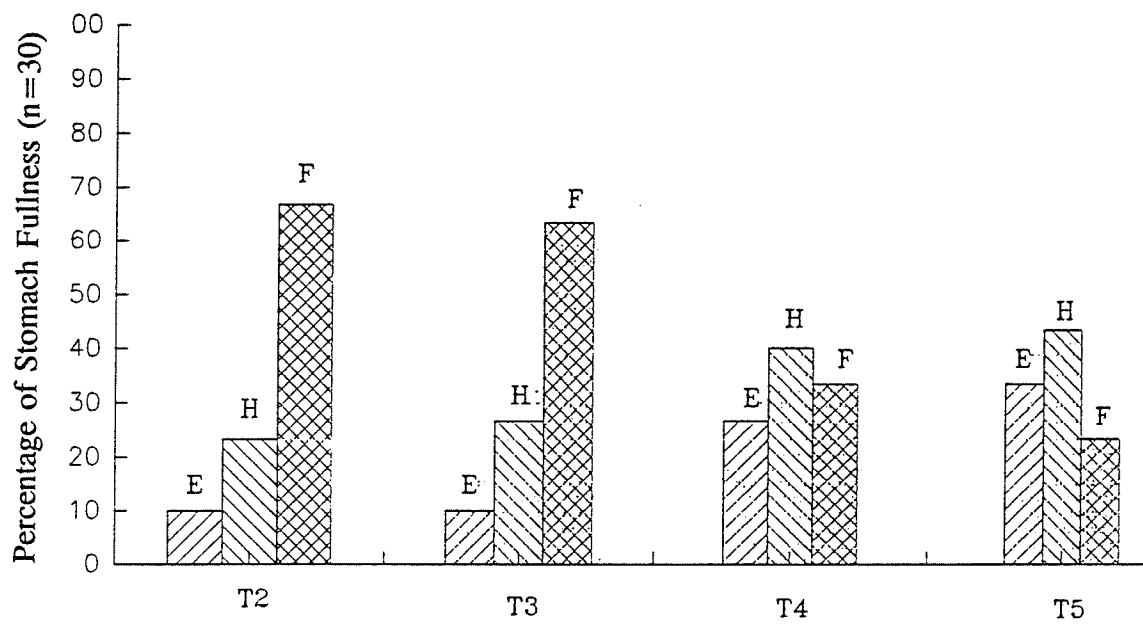


Table 10. A comparison of specific growth rate of total length and dry weight at different age in experiment 2, 1992.
(Means¹±SE²)

Age	Specific Growth Rate of Total Length					
	T1	T2	T3	T4	T5	T6
1-6	8.181 ^a ±0.217	9.128 ^a ±0.149	8.447 ^a ±0.109	8.298 ^a ±0.384	9.597 ^a ±0.463	9.208 ^a ±0.323
6-11	3.532 ^b ±0.268	3.101 ^b ±0.469	3.961 ^b ±0.327	5.191 ^a ±0.316	1.908 ^c ±0.217	3.409 ^b ±0.568
11-16	1.321 ^a ±0.066	1.483 ^a ±0.372	2.093 ^a ±0.261	1.588 ^a ±0.257	0.729 ^a ±0.395	1.197 ^a ±0.291
16-21	0.964 ^{bc} ±0.287	1.779 ^b ±0.578	2.983 ^a ±0.057	1.088 ^{bc} ±0.251	0.316 ^c ±0.226	1.016 ^{bc} ±0.357

Age	Specific Growth Rate of Dry Weight					
	T1	T2	T3	T4	T5	T6
1-6	13.335 ^a ±0.455	15.672 ^a ±2.458	14.215 ^a ±2.584	14.773 ^a ±1.817	15.928 ^a ±2.016	16.844 ^a ±1.155
6-11	11.864 ^{bc} ±1.346	12.296 ^{bc} ±2.057	12.133 ^{bc} ±0.865	19.594 ^a ±0.150	8.416 ^c ±1.490	14.062 ^b ±1.714
11-16	4.632 ^{bc} ±1.508	2.435 ^c ±0.252	9.699 ^a ±1.672	4.902 ^{bc} ±0.348	4.382 ^{bc} ±0.870	6.775 ^{ab} ±1.122
16-21	4.284 ^{cd} ±0.225	9.236 ^{ab} ±0.882	10.783 ^a ±0.209	5.861 ^{bc} ±1.148	2.205 ^d ±2.175	6.389 ^{bc} ±0.738

¹Means in the same row with the same superscript are not significantly different.

²The standard error of the estimate.

Effect of Feeding Frequency on Growth and Survival (1992).

Different feeding schedules during the 21 day experimental period following hatching resulted in significant differences in dry weight (based on two-factor ANOVA $F=5.48$, $Pr>F=0.0008$) but no significant difference was found in total length ($F=2.30$, $Pr>F=0.0690$, Table 11). There was no significant interaction between treatments and time. One day-time feeding (Treatment 1) gave the best dry weight (7.78mg) and one night-time feeding (Treatment 2) gave the poorest dry weight (6.05mg, Table 11, Figure 18 and 19).

Survival levels were not significantly different among the various treatment groups ($F=2.86$, $Pr>F=0.0807$). Treatment 2 yielded lower survival (68.4%) than other treatments. However, increasing feeding frequency probably tended to improve survival (Table 11).

Although total length and survival were not significantly different among the treatments means, there was a tendency for total length and survival of larvae fed at night-time to be lower than larvae in other treatments in which food was given in the day-time. In addition, *C. macrocephalus* fed during day-time achieved significantly greater dry weight than those fed during the night. Survival among those fed in the day-time was also higher than among those fed during the night-time but it was not significantly different. Although, significant differences were not detectable in total length, there was a

strong trend for one-time feeding (T1) to give better total length over a 21 day period. There was no significant correlation between growth (total length) and survival among the 15 tanks.

Significant differences in specific growth rates, relative growth rates and increments were not evident (Table 12).

Table 11. A comparison of final total length, dry weight and survival of larvae receiving food once in the day, once at night, twice, three and four times in the day, for 21 days after hatching. (Experiment 3, 1992; Means¹±SE²)

Treatments	Total Length ³ (cm)	Dry Weight ⁴ (mg)	Survival(%)
T1,once (D)	2.180 ^a ± 0.037	7.783 ^a ± 0.291	76.03 ^a ± 2.55
T2,once (N)	2.163 ^a ± 0.027	6.050 ^c ± 0.265	68.41 ^a ± 8.93
T3,twice	2.140 ^a ± 0.033	6.600 ^{bc} ± 0.340	87.38 ^a ± 3.70
T4,three	2.191 ^a ± 0.023	6.650 ^{bc} ± 0.401	91.74 ^a ± 1.79
T5,four	2.212 ^a ± 0.028	7.000 ^{ab} ± 0.465	79.44 ^a ± 4.58

¹Means in the same columns with the same superscript are not significantly different.

²The standard error of the estimate.

³after preservation in 5% formalin for 24h.

⁴after drying at 80°C for 24h.

Figure 18. Experiment 3, 1992. The effect of feeding schedule on the total length (cm) of larvae. T1 one time feeding at day-time, T2 one time feeding at night-time, T3 two times feeding at day-time, T4 three times feeding at day-time and T5 four times feeding at day-time. The vertical bars are the standard error of the estimate.

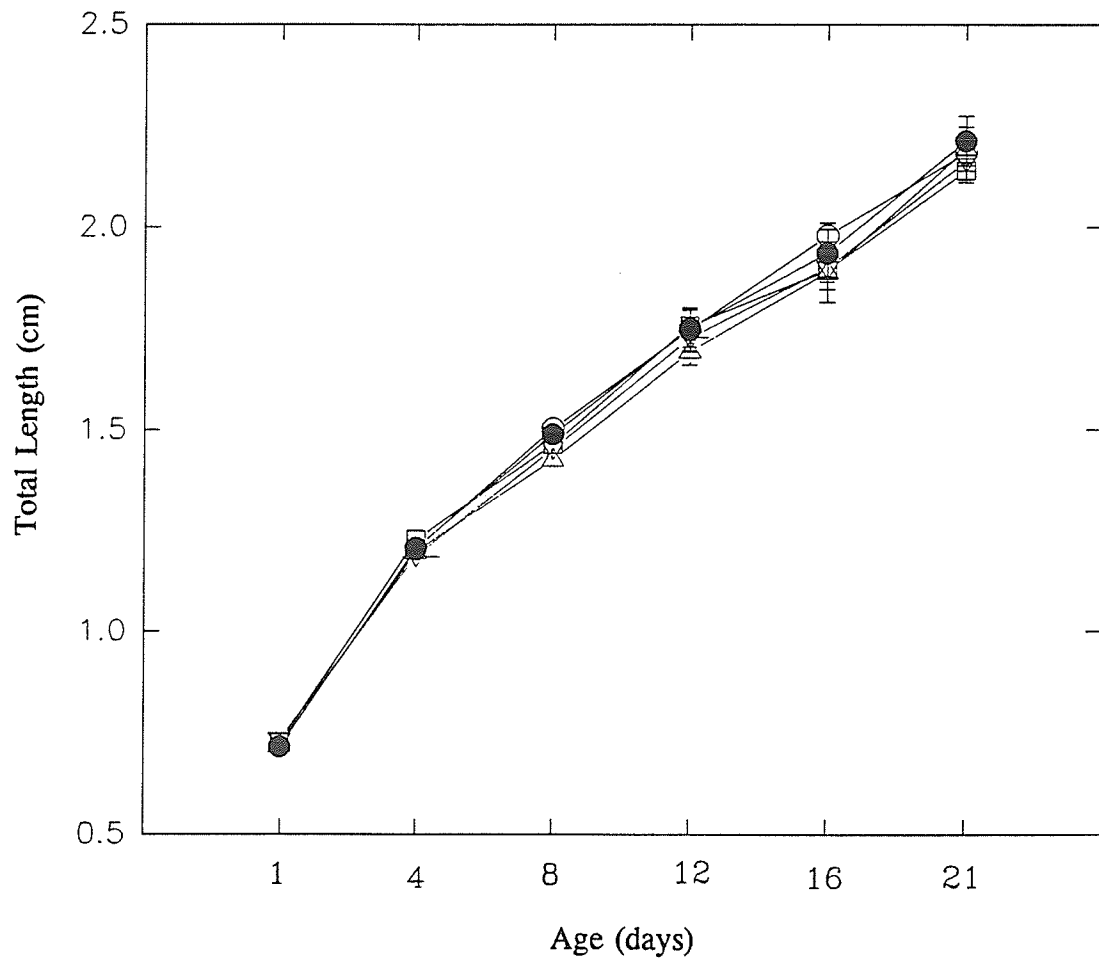


Figure 19. Experiment 3, 1992. The effect of feeding schedule on the dry weight(mg) of larvae. T1 one time feeding at day-time, T2 one time feeding at night-time, T3 two times feeding at day-time, T4 three times feeding at day-time and T5 four times feeding at day-time. The vertical bars are the standard error of the estimate.

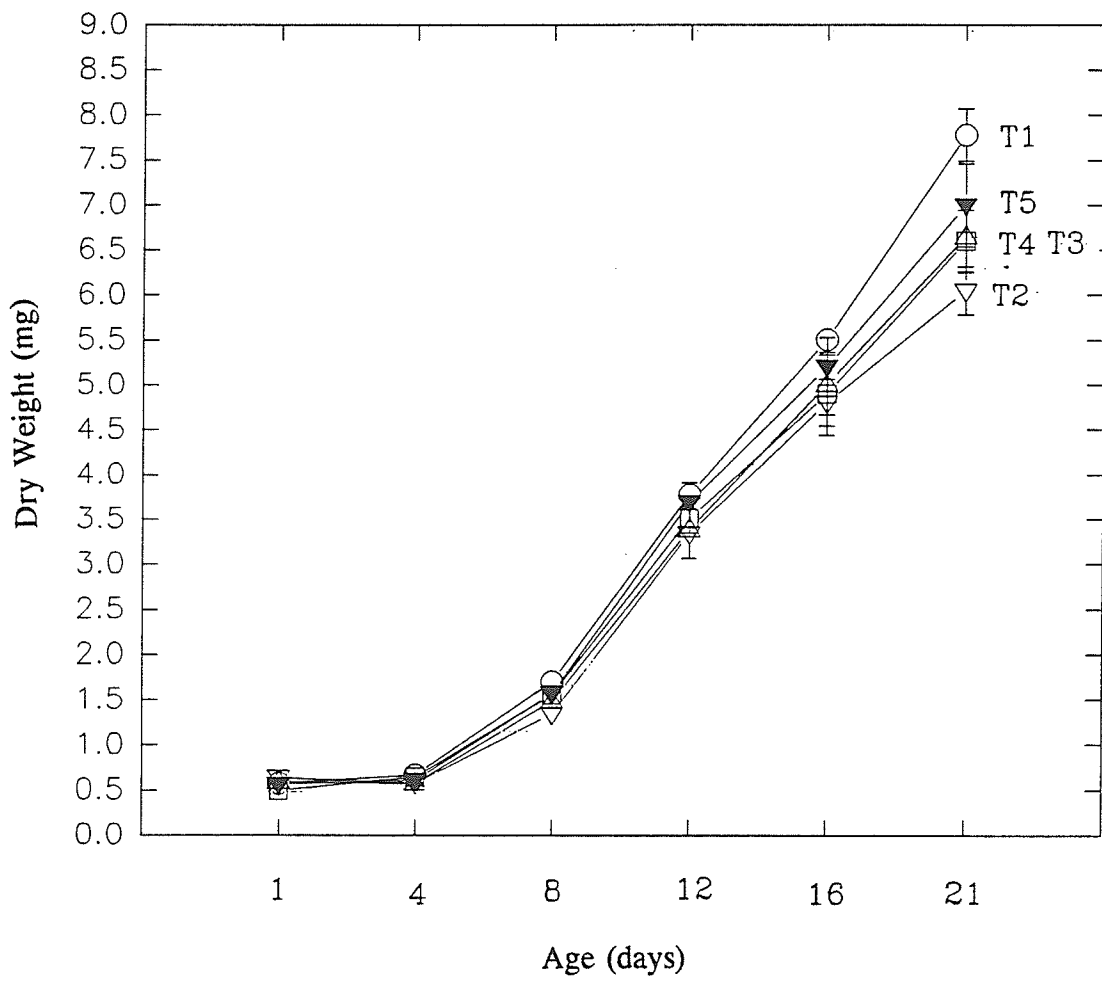


Table 12. A comparison of specific growth rates, relative growth rates and increments for total length and dry weight of larvae receiving food once in the day (D), once at night (N), twice, three and four times in the day, for 21 days after hatching. (Experiment 3, 1992; Means¹±SE²)

Treatments	Total Length		
	Specific Growth	Relative Growth	Increment
T1,once(D)	5.30 ^a ± 0.07	9.75 ^a ± 0.21	1.47 ^a ± 0.02
T2,once(N)	5.15 ^a ± 0.11	9.29 ^a ± 0.33	1.43 ^a ± 0.05
T3,twice	5.14 ^a ± 0.07	9.26 ^a ± 0.20	1.41 ^a ± 0.03
T4,three	5.09 ^a ± 0.10	9.13 ^a ± 0.30	1.38 ^a ± 0.05
T5,four	5.37 ^a ± 0.17	9.95 ^a ± 0.53	1.50 ^a ± 0.07

Treatments	Dry Weight		
	Specific Growth	Relative Growth	Increment
T1,once(D)	12.42 ^a ± 0.08	59.84 ^a ± 1.10	7.21 ^a ± 0.28
T2,once(N)	10.69 ^a ± 0.46	40.57 ^a ± 4.43	5.41 ^a ± 0.29
T3,twice	12.32 ^a ± 0.58	59.41 ^a ± 7.80	6.10 ^a ± 0.36
T4,three	11.52 ^a ± 0.37	49.07 ^a ± 4.26	6.06 ^a ± 0.41
T5,four	11.95 ^a ± 0.38	54.23 ^a ± 4.82	6.43 ^a ± 0.47

¹Means in the same column with the same superscript are not significantly different.

²The standard error of the estimate.

Discussion

Results of experiment 1 in 1991 indicated that *C. macrocephalus* larvae showed improved growth when offered bigger *Artemia*. The experiment was repeated in 1992 to avoid the confounding effect of unequal food density among treatments which existed in the 1991 experiment. The results of the 1992 experiment confirmed that *C. macrocephalus* larvae supplied a bigger size of *Artemia* and fed to excess, under laboratory conditions, achieved better growth. A particle size equivalent to 2.2% of total larval length is optimal for *C. gariepinus* according to Uys and Hecht (1985), however, it is difficult to maintain this size relationship during larval culture as the larvae increase in length while the size of unfed *Artemia* nauplii remains constant.

Larvae in all treatments could be observed to accept *Artemia* very well and generally had full stomachs within about 30 minutes of being offered food, after which they formed a school at the bottom of the tanks. This demonstrated the acceptability of live food (*Artemia*) and confirmed observations of Omorinkoba et al. (1990) in which *Heterobranchus* and *Clarias* larvae accepted *Artemia* and zooplankton fairly well and showed comparatively good growth.

The analysis of growth parameters indicate an *Artemia* size range from 160 to 315 μ is more suitable than *Artemia* $\leq 160\mu$ for *C. macrocephalus* larval reared under laboratory

conditions. The ability to successfully capture food depends on larval size and prey size. Halver (1989) stated that too small food particles are not consumed efficiently. A prepared food size of 125μ appeared to be too small for *C. macrocephalus* larvae (Unprasert and Sinthusen, 1989). A food particle size of $50-90\mu$ is considered suitable for silver carp larvae, $90-150\mu$ for grass carp larvae and $150-270\mu$ for bighead carp larvae (Dabrowski and Bardega, 1984). Hasan and Macintosh (1992) suggested that food particle sizes in the ranges of $125-300\mu$, $300-500\mu$, $300-790\mu$ and $500-1,000\mu$ in diameter were most appropriate for carp larvae weighing 15-23mg (TL 13-18mm), 46-97mg (TL 17-22mm), 105-209mg (TL 20-25mm) and 210-466mg (TL 24-31mm) respectively. Zagarese (1988) stated that food partitioning may occur when larger prey items are available for larvae of the South African catfish (*Rhamdia sapo*) larvae. In the case of *C. macrocephalus* the mouth gape is still unknown and Torrains (1986) has stated that as the larvae grow, they will usually select progressively larger prey items. Further investigations should therefore be undertaken using wider size ranges of larval food items and older larvae.

The differences in growth among larvae receiving different food sizes may result from a higher energy requirement when larvae feed on smaller sizes of food compared to those feeding on bigger food items (Pitcher and Hart, 1982). More energy may be required to capture small food

items. Survival in the 1992 experiment was higher than in the experiment in 1991 in which tank cleaning was difficult. Good sanitation is vital for successful larval rearing. A comparison of growth using *Artemia* (experiment 1, 1991) versus prepared food (experiment 2, 1991) confirmed that *C. macrocephalus* larvae prefer or are more successfully cultured on a live food. Larvae fed on prepared food showed poor growth and survival. This could result from physiological immaturity with little or no capacity to synthesize certain digestive enzymes (Lam, 1991; Hofer, 1985). Dabrowski and Glogowski (1977) stated that proteolytic enzymes present in natural live food contribute substantially to the digestion process in fish. Hepher (1988) similarly stated that exogenic enzymes may increase the digestibility of food and this was considered to be a major advantage of live food. The low enzymes production as well as the absence of a stomach in the first few weeks after hatching are two important reasons for difficulties in rearing coregonids (hybrids of *Coregonus wartmanni* and *Coregonus lavaretus*) according to Lauff and Hofer (1984). Limited digestibility of dry diets has been the assumed cause of poor growth and survival in *Polydon spathula* larvae (Webster et al., 1991). In addition, a short digestive tract might contribute to some difficulties resulting from absence of certain enzymes or lack of time due to the short tract (Hofer, 1985). Nevertheless, Uys and Hecht (1985) suggested that prepared food could be used to successfully rear *C.*

gariiepinus larvae. The quality of the prepared food is probably critical when larvae are involved. In the 1991 experiments, prepared food used in all treatments showed signs of poor palatability, nutrient leaching and breakdown. These results contradict the hypothesis that the risk of mortality can be reduced by using sterilized, prepared food. However, if prepared food of very high quality was available, the outcome might be better.

The results of experiment 2 in 1992 revealed that *C. macrocephalus* larvae fed on live food (*Artemia*) attained good growth and survive in high numbers. *Clarias gariiepinus* requires live food organisms such as *Artemia* or zooplankton for the first period of life (Msiska, 1981). Likewise, *C. batrachus* larvae fed on live food exhibited significantly superior growth relative to those fed artificial diets (Alam and Mollah, 1988). Live *Artemia* resulted in better growth than artificial feed in *C. batrachus* larvae according to Bairage et al. (1988). Species other than catfish such as grass carp and bighead carp larvae also grow better when given live food than when fed on commercial dry diets (Rottman et al., 1991). Although there was evidence in the present study that *C. macrocephalus* larvae accepted prepared food to some extent at the start of exogenous feeding, larvae fed solely on prepared food in powder form not only grew poorly but also had poor survival. Conversely *C. macrocephalus* fed on paste-like prepared food attained good growth but had poor survival.

Survival of larvae fed on a paste-like form was slightly higher than those fed on prepared food in a powdered form but not significantly different. This indicates that an appropriate physical form may be important for good results. Santiago et al. (1987) likewise reported that Nile tilapia (*Oreochromis niloticus*) larvae fed on pelleted crumbles had significantly higher survival than those fed on unpelleted diets. Other catfish species (*Ictalurus punctatus*) only start accepting artificial food 5 weeks after stocking (Zagarese, 1988). The duration of the transitional period from live food organisms to artificial diets in *C. macrocephalus* larval rearing is still uncertain. The length of the feeding period on live food may be crucial if larvae are to maximize growth and achieve a high survival rate. The food combination applied in this study, in which larvae fed on *Artemia* for the first 10 days and subsequently fed on powdered prepared food for the remaining 11 days of the 21 day experimental period resulted in the best growth and survival. The findings in this study support and even explain a study conducted by Fermin and Bolivar (1991) in which the best specific growth rate (12.38% per day) and highest survival rate (71-86%) of *C. macrocephalus* larvae were obtained when larvae fed on a mixture of *Artemia* and dry food (*Artemia* and dry diet at 50% of body weight) when compared to those supplied either zooplankton or a dry artificial diet alone. The findings of the present study imply that prepared food may not be consumed

efficiently at the start of exogenous feeding. Prepared food should therefore, be given after a period of live food feeding to avoid poor growth as well as water quality problems. Okoye et al. (1991) suggested that zooplankton alone cannot support the growth and survival of *C. gariepinus* larvae in outdoor tanks and that artificial diets containing at least 30% crude protein are necessary to supplement the natural food in the tanks at a particular period for good growth and survival. Dabrowski (1984) similarly suggested that growth rates of Cyprinid larvae improved when larvae were transferred to a dry diet at an individual wet weight of 5-6mg. Feeding on zooplankton and subsequently on an artificial diet revealed some improvement in both growth and survival of common carp larvae (Dabrowski, 1987). Growth of *C. gariepinus* larvae halted when food was changed from *Artemia* to a commercial diet between the age of 1.8 and 4.1 days according to Verreth and Tongeren (1989).

Conversely, Damme et al. (1990) found that the best growth rate of *C. gariepinus* larvae could be obtained when larvae fed on *Artemia* alone. Feeding on *Artemia* and a dry diet resulted in intermediate growth and poor growth was obtained when larvae fed only on a dry diet. Carp (*Cyprinus carpio*) larvae showed good growth and low mortality when fed on dry food under intensive conditions (Applbaum and Dor, 1978).

The rationale for the examination of the effect of feeding frequency on *C. macrocephalus* larval rearing success

was based on the fact the larvae have a small stomach but a high metabolic rate. Feed should therefore be given at an appropriate time. More frequent feeding may stimulate larvae to feed more and thus result in better growth. The frequency of larval feeding varies depending on species, age, feeding level, and other variables. Feeding three times per day was suggested to be optimal for *Cirrhinus mrigala* (Hamilton) larvae (Sampath and Ravindran, 1987). Feeding twice a day was sufficient to obtain maximal growth in 50g channel catfish (*Ictalurus punctatus*) (Andrews and Page, 1975).

In the present study increase in feeding frequency improved neither growth nor survival. A feeding frequency of three times per day appeared to be suitable for rearing *C. macrocephalus* larvae at ambient temperature (26-31°C) according to Mollah and Tan (1982). The different results may be a consequence of different feeding level applied. Satiation feeding was utilized by Mollah and Tan (1982) whereas feeding to excess was used in the present study. In the present study excess *Artemia* survived well and were available until the next feeding time. Given that *C. macrocephalus* larvae can be classified to some extent as scavengers, the effect of an increase in feeding frequency may thus not have been directly tested. However, the increase in feeding frequency from 2 to 4 times per day did not significantly increase growth and survival, therefore one daily feeding is acceptable at least under laboratory conditions and when excess live food is

supplied. A comparison of day and night-time feeding did not indicate a significant difference in growth and survival. However, *C. macrocephalus* fed on *Artemia* during the day-time had slightly better growth (but not significantly different) and survival than those fed at night. *Clarias batrachus* which has similar feeding behaviour to *C. macrocephalus* is also considered to be diurnally active (Leenanone, 1981). Larvae of other catfish species such as *C. gariepinus* are considered to be primarily nocturnally active, tactile feeders according to Britz and Piennaar (1992), however this may not be the case with *C. macrocephalus*.

In conclusion, the studies revealed that *Artemia* nauplii in the size range of 160 μ to 315 μ produced better growth than *Artemia* smaller than 160 μ . *Artemia* and paste-like prepared food produced better growth than powdered prepared food. Larvae fed on *Artemia* also attained higher survival, unlike those fed on paste-like prepared food in which poor survival resulted. However a combination diet beginning with *Artemia* and then switching to prepared food on day 11 resulted in better growth than combinations of other time periods, prepared food or *Artemia* alone. A feeding schedule of one daily feeding is acceptable when larvae are fed to excess using live food (*Artemia*). *C. macrocephalus* larvae appear to be diurnal feeders. The physical form of prepared food may be important to avoid nutrient leaching and water pollution.

These studies show simple changes in the culture

procedures can improve the production of *C. macrocephalus* larvae. Further study of the feeding of *C. macrocephalus* larvae may make the culture of this species more attractive to fish farmers in Northeastern Thailand.

The culture of *C. macrocephalus* larvae will be more efficient if the following issues can be clarified:

- Optimal food particle size over a wider size range, older fish, and other prepared foods.
- Ideal feeding frequency using prepared food.
- The best time to switch to prepared food within the period from 5 days to 10 days old in order to create base line data which would allow length or weight to be used to determine the best time to switch from nauplii to prepared food.
- A mouth (gape) size study should be undertaken since it is still unknown.
- The ontogeny of the digestive system and its enzyme production. This knowledge could result in a better understanding of the age at which different diets could be assimilated.

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Appendixes

Appendix 1. Randomized tank allocation for experiment in 1991 and 1992 (T=treatments and R=replications)

1.1 Experiment 1, 1991.

T5R1	T4R2	T1R3	T4R1	T3R3	T2R1	T3R2	
T1R2	T2R3	T5R2	T1R1	T5R3	T4R3	T3R1	T2R2

1.2 Experiment 2, 1991.

T3R3	T4R4	T3R1	T1R3	T1R1	T2R4	T4R2	T4R1
T1R2	T3R2	T2R1	T2R2	T3R4	T2R3	T4R3	T1R4

1.3 Experiment 1, 1992.

T1R1	T4R3	T2R3
T3R2	T4R1	T2R1

T1R2	T2R2	T3R3
T1R3	T3R1	T4R2

1.4 Experiment 2, 1992.

	T3R2	T6R3	T6R1	T2R2
T5R3	T1R1	T4R1	T3R1	T2R3

T3R3	T5R1	T4R2	T2R1	T5R2
	T1R3	T6R2	T4R3	T1R2

1.5 Experiment 3, 1992.

T5R2	T2R2	T2R1	T4R1
T5R1	T1R3	T3R2	T1R1

T3R1	T3R3	T5R3	T4R3
	T1R2	T2R3	T4R2

Appendix 2. Means of the total length of experiments in 1991 and 1992.

2.1 Experiment 1, 1991.

Trt. Rep.		Total Length ¹ (cm)								H ²
		Age 1	3	5	7	9	11	13	15	
T1	R1	0.65	0.92	0.94	1.04	1.18	1.32	1.36	1.14	
	R2	0.71	0.93	0.97	1.13	1.20	1.40	1.45	1.58	NS
	R3	0.72	0.90	0.95	1.05	1.13	1.22	1.30	1.40	
T2	R1	0.71	0.82	0.82	0.86	0.89	0.97	0.96	0.98	
	R2	0.65	0.84	0.86	0.87	0.85	0.91	0.95	0.99	NS
	R3	0.72	0.86	0.88	0.92	0.90	1.01	0.95	0.94	
T3	R1	0.65	0.85	0.87	0.88	0.91	1.08	1.16	1.38	
	R2	0.66	0.86	0.87	0.92	1.00	1.17	1.20	1.25	NS
	R3	0.65	0.87	0.90	0.91	0.96	1.08	1.00	1.09	
T4	R1	0.65	0.97	0.99	1.15	1.20	1.39	1.41	1.51	
	R2	0.62	0.93	1.08	1.27	1.31	1.42	1.56	1.75	NS
	R3	0.63	0.94	0.99	1.14	1.19	1.48	1.59	1.73	
T5	R1	0.63	0.90	0.97	1.20	1.25	1.48	1.51	1.65	
	R2	0.65	0.95	1.00	1.14	1.17	1.28	1.35	1.50	NS
	R3	0.65	0.90	0.95	1.16	1.21	1.32	1.42	1.49	

¹after preservation in 10% formalin for 24h.

²Hartley's Test for homogeneity of variance.

2.2 Experiment 2, 1991.

Trt. Rep.		Total Length ¹ (cm)							H ²
Age		1	3	5	7	9	11	13	
T1	R1	0.67	0.82	0.98	1.02	1.09	1.06	1.12	NS
	R2	0.66	0.83	0.98	1.04	1.10	1.09	0.95	
	R3	0.58	0.79	0.86	0.87	0.92	0.96	1.06	
	R4	0.60	0.80	0.96	1.05	1.08	1.08	1.08	
T2	R1	0.61	0.79	0.90	1.01	1.12	0.96	1.04	NS
	R2	0.60	0.77	0.86	0.88	0.98	0.96	0.99	
	R3	0.65	0.85	0.94	0.99	1.07	1.03	1.00	
	R4	0.66	0.80	0.89	0.99	0.98	1.02	1.09	
T3	R1	0.60	0.77	0.88	0.94	0.89	0.89	0.89	NS
	R2	0.65	0.83	0.97	1.03	1.03	1.03	1.02	
	R3	0.62	0.76	0.81	0.86	0.91	0.93	0.98	
	R4	0.65	0.80	0.82	0.90	0.94	1.06	1.08	
T4	R1	0.61	0.78	0.82	0.84	0.94	0.88	0.94	NS
	R2	0.63	0.81	0.91	0.97	1.02	1.10	1.05	
	R3	0.60	0.79	0.85	0.91	0.92	0.94	1.05	
	R4	0.67	0.82	0.94	1.04	1.06	1.01	1.10	

¹after preservation in 10% formalin for 24h.

²Hartley's Test for homogeneity of variance.

2.3 Experiment 1, 1992.

Trt.	Rep.	Total length ¹ (cm)				H ²	
		Age	1	7	14		21
T1	R1		0.78	1.03	1.17	1.32	NS
	R2		0.77	1.06	1.16	1.33	
	R3		0.78	1.09	1.23	1.30	
T2	R1		0.77	1.02	1.08	1.28	NS
	R2		0.77	1.03	1.10	1.29	
	R3		0.78	1.02	1.09	1.33	
T3	R1		0.78	1.08	1.16	1.42	NS
	R2		0.78	1.06	1.06	1.32	
	R3		0.77	1.05	1.05	1.39	
T4	R1		0.74	1.05	1.16	1.42	NS
	R2		0.78	1.03	1.32	1.31	
	R3		0.78	1.06	1.19	1.41	

¹after preservation in 5% formalin for 24h.

²Hartley's Test for homogeneity of variance.

2.4 Experiment 2, 1992.

Trt. Rep.		Total Length ¹ (cm)					H ²
Age		1	6	11	16	21	
T1	R1	0.72	1.10	1.28	1.36	1.49	NS
	R2	0.70	1.11	1.33	1.42	1.49	
	R3	0.71	1.10	1.34	1.44	1.49	
T2	R1	0.72	1.12	1.25	1.38	1.56	NS
	R2	0.70	1.11	1.34	1.46	1.52	
	R3	0.69	1.10	1.30	1.35	1.58	
T3	R1	0.71	1.08	1.32	1.45	1.73	NS
	R2	0.72	1.09	1.29	1.47	1.77	
	R3	0.72	1.11	1.39	1.52	1.81	
T4	R1	0.70	1.09	1.37	1.51	1.66	NS
	R2	0.74	1.08	1.43	1.51	1.58	
	R3	0.72	1.10	1.44	1.57	1.66	
T5	R1	0.67	1.06	1.19	1.21	1.25	NS
	R2	0.65	1.10	1.27	1.28	1.30	
	R3	0.66	1.04	1.14	1.23	1.22	
T6	R1	0.69	1.08	1.32	1.37	1.40	NS
	R2	0.65	1.01	1.23	1.30	1.43	
	R3	0.66	1.08	1.21	1.32	1.41	

¹after preservation in 5% formalin for 24h.

²Hartley's Test for homogeneity of variance.

2.5 Experiment 3, 1992.

Trt. Rep.		Total Length ¹ (cm)						H ²
	Age	1	5	9	13	17	21	
T1	R1	0.72	1.19	1.48	1.64	1.92	2.23	NS
	R2	0.73	1.22	1.52	1.82	2.02	2.16	
	R3	0.70	1.20	1.51	1.78	2.00	2.16	
T2	R1	0.74	1.17	1.50	1.78	1.84	2.24	NS
	R2	0.72	1.23	1.43	1.73	1.96	2.17	
	R3	0.74	1.16	1.43	1.67	1.90	2.08	
T3	R1	0.73	1.24	1.50	1.77	1.93	2.19	NS
	R2	0.72	1.24	1.45	1.73	1.95	2.14	
	R3	0.73	1.20	1.44	1.77	1.80	2.09	
T4	R1	0.71	1.23	1.41	1.63	1.74	2.10	NS
	R2	0.73	1.19	1.44	1.71	1.95	2.19	
	R3	0.72	1.17	1.43	1.74	1.98	2.01	
T5	R1	0.71	1.22	1.51	1.84	1.98	2.31	NS
	R2	0.73	1.21	1.46	1.69	1.82	2.10	
	R3	0.71	1.19	1.50	1.72	2.01	2.23	

¹after preservation in 5% formalin for 24h.

²Hartley's Test for homogeneity of variance.

Appendix 3. Means of the dry weight of experiments in 1991 and 1992.

3.1 Experiment 1, 1991.

Trt. Rep.		Dry Weight ¹ (mg)								H ²
Day		1	3	5	7	9	11	13	15	
T1	R1	0.40	0.59	0.84	1.15	1.81	2.51	2.22	5.38	NS
	R2	0.31	0.64	1.16	1.63	1.90	2.97	3.41	4.74	
	R3	0.34	0.58	0.89	1.10	1.64	2.47	2.33	3.30	
T2	R1	0.33	0.42	0.43	0.56	0.74	0.85	0.68	1.17	NS
	R2	0.31	0.34	0.42	0.48	0.54	0.59	0.73	0.87	
	R3	0.33	0.40	0.54	0.56	0.51	0.76	0.78	1.05	
T3	R1	0.40	0.42	0.58	0.65	0.80	1.17	1.03	2.48	NS
	R2	0.31	0.41	0.54	0.67	0.91	1.40	1.97	2.50	
	R3	0.30	0.42	0.45	0.55	0.83	1.03	0.94	1.30	
T4	R1	0.51	1.27	1.23	1.50	2.14	3.29	2.88	4.14	NS
	R2	0.34	0.66	1.38	2.21	2.70	3.36	4.22	4.69	
	R3	0.30	0.76	1.11	1.94	2.50	3.70	4.76	5.24	
T5	R1	0.41	0.56	1.57	1.88	1.51	4.10	3.14	5.56	NS
	R2	0.40	0.44	1.12	1.52	1.73	2.11	3.00	3.29	
	R3	0.32	0.61	1.11	1.45	2.33	3.63	3.99	5.31	

¹after drying at 100°C for 24h.

²Hartley's Test for homogeneity of variance.

3.2 Experiment 2, 1991.

Trt. Rep.		Dry Weight ¹ (mg)							H ²
Day		1	3	5	7	9	11	13	
T1	R1	0.40	0.50	0.64	0.68	0.80	1.09	1.23	NS
	R2	0.36	0.45	0.67	0.79	0.87	1.11	1.05	
	R3	0.39	0.44	0.60	0.70	0.90	1.06	0.98	
	R4	0.41	0.47	0.73	1.07	1.11	0.97	1.06	
T2	R1	0.39	0.46	0.60	0.87	0.89	0.83	0.83	NS
	R2	0.40	0.43	0.51	0.60	0.70	0.72	0.88	
	R3	0.35	0.41	0.64	0.78	0.77	0.85	0.88	
	R4	0.41	0.48	0.50	0.78	0.88	0.92	0.87	
T3	R1	0.43	0.46	0.48	0.70	0.56	0.57	0.69	NS
	R2	0.33	0.40	0.67	0.85	0.86	0.72	0.80	
	R3	0.35	0.38	0.45	0.63	0.68	0.86	0.78	
	R4	0.37	0.43	0.49	0.50	0.58	0.68	0.80	
T4	R1	0.32	0.39	0.46	0.70	0.75	0.60	0.87	NS
	R2	0.34	0.41	0.64	0.80	1.01	0.81	1.01	
	R3	0.35	0.43	0.63	0.89	0.74	0.78	0.89	
	R4	0.43	0.47	0.69	0.85	0.83	0.97	1.11	

¹after drying at 100°C for 24h.

²Hartley's Test for homogeneity of variance.

3.3 Experiment 1, 1992.

Trt.	Rep.	Dry Weight ¹ (mg)				H ²	
		Day	1	6	13		21
T1	R1		0.44	1.17	1.78	3.39	NS
	R2		0.49	1.01	1.62	3.82	
	R3		0.41	1.07	1.63	2.55	
T2	R1		0.44	1.03	1.13	2.54	NS
	R2		0.32	0.77	1.04	3.18	
	R3		0.38	0.59	0.95	2.73	
T3	R1		0.42	1.35	1.75	3.48	NS
	R2		0.45	0.66	1.66	3.64	
	R3		0.45	1.08	1.47	3.76	
T4	R1		0.38	1.25	1.99	3.89	NS
	R2		0.31	1.06	1.33	3.02	
	R3		0.34	1.14	1.66	3.17	

¹after drying at 80°C for 24 h.

²Hartley's Test for homogeneity of variance.

3.4 Experiment 2, 1992.

Trt. Rep.		Dry Weight ¹ (mg)					H ²
Day		1	6	11	16	21	
T1	R1	0.67	1.33	2.18	2.98	3.76	NS
	R2	0.65	1.21	2.49	2.70	3.50	
	R3	0.68	1.36	2.39	3.23	4.27	
T2	R1	0.57	1.31	2.11	2.44	4.70	NS
	R2	0.64	1.11	2.51	2.78	4.48	
	R3	0.49	1.29	2.24	2.52	4.28	
T3	R1	0.61	1.13	1.99	3.52	6.89	NS
	R2	0.43	1.13	1.98	3.49	6.61	
	R3	0.71	1.23	2.46	3.38	6.35	
T4	R1	0.61	1.17	3.13	4.10	5.63	NS
	R2	0.49	1.23	3.31	4.09	5.27	
	R3	0.63	1.20	3.15	4.06	6.59	
T5	R1	0.41	0.98	1.31	1.55	1.62	NS
	R2	0.43	1.08	1.65	2.24	2.16	
	R3	0.49	0.89	1.54	1.85	2.73	
T6	R1	0.44	1.02	2.43	3.05	4.13	NS
	R2	0.37	0.95	1.84	2.76	4.08	
	R3	0.49	1.03	1.84	2.70	4.26	

¹after drying at 80°C for 24h.

²Hartley's Test for homogeneity of variance.

3.5 Experiment 3, 1992.

Trt. Rep.		Dry Weight ¹ (mg)							
		Day	1	5	9	13	17	21	H ²
T1	R1		0.55	0.64	1.63	3.64	5.51	7.25	
	R2		0.58	0.66	1.68	4.04	5.39	7.85	NS
	R3		0.59	0.70	1.79	3.65	5.61	8.25	
T2	R1		0.58	0.65	1.35	3.84	5.30	6.55	
	R2		0.62	0.55	1.41	3.32	4.68	5.65	NS
	R3		0.73	0.53	1.27	2.87	4.43	5.95	
T3	R1		0.55	0.76	1.66	3.18	5.21	5.95	
	R2		0.54	0.57	1.43	3.71	5.50	7.10	NS
	R3		0.41	0.61	1.58	3.63	3.99	6.75	
T4	R1		0.61	0.60	1.37	3.32	4.39	6.20	
	R2		0.57	0.54	1.52	3.36	5.08	7.45	NS
	R3		0.59	0.59	1.56	3.47	5.54	6.30	
T5	R1		0.60	0.57	1.54	3.88	4.35	7.10	
	R2		0.56	0.59	1.56	3.50	5.51	6.15	NS
	R3		0.54	0.68	1.60	3.70	5.54	7.75	

¹after drying at 80°C for 24h.

²Hartley's Test for homogeneity of variance.

Appendix 4. Water quality of experiments in 1991 and 1992.

4.1 Experiment 1, 1991.

Parameters	Treatment Combinations ¹														
	11	12	13	21	22	23	31	32	33	41	42	43	51	52	53
T ²	28	28	28	28	28	28	29	28	28	28	29	28	28	28	28
p ³	7.7	7.6	8.0	7.8	7.6	7.7	7.9	7.6	7.7	7.7	8.2	7.7	7.7	7.8	7.7
D ⁴	5.1	5.0	3.7	5.2	4.9	4.8	5.0	5.0	5.2	5.0	4.8	5.2	5.0	5.1	5.5
T	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
p	7.5	7.6	7.6	8.2	7.6	7.6	7.7	8.1	7.6	7.8	7.8	7.9	7.6	7.7	7.8
D	5.0	5.2	5.0	5.0	5.0	5.2	4.9	5.1	5.0	5.0	4.8	5.2	4.9	5.0	5.3
T	29	28	28	28	28	28	28	28	28	28	28	28	28	28	28
p	7.9	8.0	7.9	8.2	8.0	8.0	8.0	8.0	7.9	8.0	8.2	8.2	7.8	8.0	8.0
D	5.1	4.9	4.8	5.0	4.9	4.9	5.2	5.1	5.0	4.8	5.0	5.0	5.1	5.2	5.1
T	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
p	8.0	8.0	8.2	7.9	8.2	8.0	8.1	8.2	8.0	8.0	8.2	8.1	8.0	8.0	8.0
D	5.0	5.0	4.6	5.1	5.0	5.0	5.5	4.9	4.8	5.1	5.0	5.1	5.0	4.9	4.9

¹No. of treatment and replication.²Temperature (°C).³pH.⁴Dissolved Oxygen (mg/L).

4.2 Experiment 2, 1991.

Parameters	Treatment Combinations							
	11	12	13	14	21	22	23	24
Temp. (°C)	28	28	28	28	28	28	28	28
pH	7.5	7.6	7.7	7.6	7.7	7.7	7.7	7.5
DO. (mg/L)	5.0	4.8	5.2	4.9	5.6	5.2	5.2	5.3
Temp.	28	28	28	29	28	28	28	28
pH	7.6	7.6	7.9	7.6	7.9	7.9	7.7	7.7
DO.	4.8	5.1	5.0	4.5	5.3	5.1	5.0	4.8
Temp.	28	29	28	28	28	29	29	28
pH	7.9	7.7	8.1	8.4	8.0	8.1	8.1	8.2
DO.	3.5	5.4	5.1	4.7	5.1	5.1	4.9	3.7

Parameters	Treatment Combinations							
	31	32	33	34	41	42	43	44
Temp.	28	28	28	28	28	28	28	28
pH	7.7	7.7	7.6	7.7	7.6	7.7	7.8	7.7
DO.	4.5	4.7	5.2	5.2	5.2	4.8	5.1	5.3
Temp.	28	29	28	28	28	28	29	28
pH	7.7	7.9	7.7	8.0	7.6	7.8	7.8	7.9
DO.	4.8	5.1	5.0	5.1	5.0	5.0	5.1	5.2
Temp.	28	28	28	28	28	28	28	28
pH	7.9	8.0	8.2	8.2	7.6	8.5	7.9	8.0
DO.	5.1	5.5	5.0	5.2	4.8	5.1	5.2	5.1

4.3 Experiment 1, 1992.

Parameters	Treatment Combinations ¹											
	11	12	13	21	22	23	31	32	33	41	42	43
T ²	25	25	25	25	25	25	25	25	25	25	25	25
p ³	7.9	7.8	7.9	8.0	8.0	8.0	8.0	8.0	7.9	7.8	7.9	7.8
D ⁴	6.8	6.9	6.1	6.2	6.4	6.5	6.6	6.2	6.7	6.1	6.5	6.4
A ⁵	0.9	0.9	1.0	0.9	0.8	0.9	0.9	0.9	1.0	1.0	1.0	0.9
T	25	25	25	25	25	25	25	25	25	25	25	25
p	7.8	7.8	7.9	8.0	8.0	8.0	8.1	8.1	8.1	7.7	7.8	7.8
D	5.9	6.5	6.4	6.1	6.5	6.6	6.4	6.4	6.2	6.7	6.2	6.8
A	0.9	1.1	1.2	0.9	1.2	1.2	1.1	1.3	1.0	0.9	1.2	1.1
T	25	25	25	25	25	25	25	25	25	26	26	26
p	7.8	7.9	7.8	7.8	8.0	7.9	8.0	8.1	8.0	8.0	7.9	7.8
D	6.8	6.8	6.5	6.2	6.3	6.4	6.5	6.8	6.4	6.4	6.4	6.7
A	0.9	1.2	1.2	1.1	1.3	1.1	0.9	0.9	1.3	1.0	1.2	1.0
T	28	28	28	28	28	28	28	28	28	28	28	28
p	7.9	8.1	8.2	8.1	8.1	8.0	8.2	8.1	8.0	8.0	8.0	8.0
D	6.5	6.8	6.4	6.3	6.3	6.3	6.5	6.5	6.7	6.5	6.4	6.4
A	1.2	1.3	1.2	1.4	1.2	1.3	1.1	1.2	1.3	1.1	1.1	1.4

¹No. of treatment and replication.

²Temperature (°C).

³pH.

⁴Dissolved Oxygen (mg/L).

⁵Total Ammonia (mg/L).

4.4 Experiment 2, 1992.

Parameters	Treatment Combinations								
	11	12	13	21	22	23	31	32	33
Temp. (°C)	26	26	26	26	26	26	26	26	26
pH	7.6	7.8	7.9	7.6	7.8	7.8	7.7	7.6	7.6
DO. (mg/L)	6.4	6.5	5.8	6.4	6.4	6.4	6.8	6.4	6.1
Ammonia (mg/L)	0.9	1.2	1.4	1.3	1.2	1.3	1.2	1.3	1.3
Temp.	27	27	27	27	27	27	27	27	27
pH	6.9	6.9	7.1	7.1	7.0	7.5	7.0	7.3	6.9
DO	6.5	6.8	6.4	6.3	5.9	6.4	6.0	6.8	6.1
Ammonia	1.0	1.0	1.1	1.3	1.1	1.1	1.1	1.1	1.1
Temp.	25	25	25	25	25	25	25	25	25
pH	7.7	7.7	7.8	8.0	7.7	7.9	7.7	7.7	7.6
DO	6.9	10	8.2	8.8	6.9	7.5	8.2	6.9	7.8
Ammonia	1.1	1.2	0.9	1.1	1.2	1.1	1.1	1.1	1.2
Temp.	27	27	27	27	27	27	27	27	27
pH	7.0	7.2	6.9	7.3	7.1	7.0	6.9	7.5	7.0
DO	5.4	4.9	4.7	4.8	4.9	4.7	4.4	3.9	4.6
Ammonia	1.4	1.3	0.8	0.9	0.8	0.8	0.8	0.8	1.1
Temp.	25	25	25	25	25	25	25	25	25
pH	6.9	7.2	7.3	7.6	7.3	7.7	7.4	7.6	7.4
DO	5.9	6.2	6.7	7.2	6.6	6.5	5.8	7.2	7.2
Ammonia	0.9	1.0	0.8	0.7	0.7	0.7	0.6	0.5	0.5

Experiment 2, 1992 (Cont'd)

Parameters	Treatment Combinations								
	41	42	43	51	52	53	61	62	63
Temp. (°C)	26	26	26	26	26	26	26	26	26
pH	7.5	7.3	7.6	7.9	7.9	7.9	8.0	8.0	7.8
DO. (mg/L)	5.9	6.3	6.4	6.7	6.2	6.8	5.9	6.5	6.4
Ammonia (mg/L)	1.3	1.3	1.2	0.8	0.9	0.7	1.1	0.7	0.9
Temp.	27	28	27	27	27	27	28	27	28
pH	6.9	8.0	7.0	7.7	7.2	7.6	8.2	6.9	7.7
DO	7.2	8.0	6.3	6.4	6.4	6.8	7.2	7.0	6.1
Ammonia	1.1	1.2	1.1	1.6	1.2	1.5	1.6	1.5	1.6
Temp.	25	25	25	25	25	25	25	25	25
pH.	8.0	8.1	7.5	7.8	7.6	7.8	8.0	7.7	7.9
DO	7.8	7.5	7.5	9.4	7.8	7.2	6.9	6.5	8.0
Ammonia	0.9	0.8	1.2	0.8	0.9	1.0	1.0	1.0	0.8
Temp.	27	27	27	27	27	27	27	27	27
pH	6.9	6.9	7.1	6.9	7.0	7.1	7.3	6.9	6.9
DO	4.7	4.4	4.5	4.8	4.4	4.5	4.4	6.0	5.0
Ammonia	0.7	0.7	0.6	0.5	0.4	0.5	0.4	0.4	0.6
Temp.	25	25	25	25	25	25	25	25	25
pH	7.4	7.8	7.5	7.5	7.0	6.9	7.2	6.9	7.8
DO	8.0	9.0	6.0	6.2	5.7	6.6	6.8	6.7	7.2
Ammonia	1.1	1.6	1.6	1.0	1.0	1.0	1.1	1.0	1.0

4.5 Experiment 3, 1992.

Parameters.	Treatment Combinations ¹														
	11	12	13	21	22	23	31	32	33	41	42	43	51	52	53
T ²	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
p ³	7.8	7.8	7.9	8.0	7.6	7.6	7.7	7.6	7.9	8.0	8.0	7.9	7.9	7.8	7.8
D ⁴	8.5	8.0	8.2	9.0	8.9	8.0	10	8.6	8.9	9.0	8.3	8.5	10	8.5	7.9
A ⁵	0.8	0.9	1.3	0.9	0.8	1.1	0.9	1.1	1.2	1.1	1.1	1.2	1.1	1.3	0.9
T	26	25	25	25	26	25	25	25	25	25	25	25	25	26	25
p	7.9	8.1	7.6	8.0	7.9	7.9	7.7	7.7	7.6	7.9	8.1	7.6	8.0	8.0	7.8
D	8.5	8.2	8.0	8.5	8.3	8.3	8.9	8.4	9.0	10	8.6	8.5	8.5	8.9	8.6
A	0.9	0.9	0.9	0.8	0.9	1.0	0.9	1.0	0.9	0.8	0.9	0.9	0.9	0.9	1.0
T	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
p	8.0	7.6	7.6	8.1	7.9	7.9	7.6	7.6	7.9	7.6	7.4	7.7	7.9	8.0	8.0
D	10	8.5	8.0	6.8	8.0	8.2	7.6	8.0	8.5	8.4	7.2	8.5	6.8	7.9	8.9
A	0.8	0.7	1.0	0.8	0.9	0.9	0.8	0.8	0.9	1.1	0.9	0.9	1.0	0.8	0.8
T	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
p	7.7	7.7	8.0	7.6	8.0	7.5	8.1	8.0	7.7	7.9	8.0	8.0	7.7	7.7	7.6
D	8.0	10	8.5	8.7	8.8	8.8	9.5	8.9	7.9	8.1	9.0	8.8	9.4	10	7.9
A	0.9	1.1	1.2	0.9	0.9	1.2	1.0	0.9	0.9	1.1	1.2	0.8	1.1	1.0	1.2
T	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
p	7.4	7.7	8.0	7.9	7.5	7.5	7.8	7.7	7.7	8.0	8.1	7.6	8.0	8.0	7.5
D	5.9	6.7	6.1	6.5	6.2	5.7	6.5	6.7	6.8	6.7	7.2	6.4	6.6	5.8	7.2
A	1.3	1.4	1.4	1.2	1.1	1.4	1.3	1.2	1.1	1.2	1.4	1.2	1.3	1.3	1.2

¹No. of treatment and replication.

²Temperature (°C).

³pH.

⁴Dissolved Oxygen (mg/L).

⁵Total Ammonia (mg/L).

Appendix 5. The analysis of variance of experiments in 1991 and 1992.

5.1 Analysis of Variance Procedure of Total Length(TL), Dry Weight(DW) and Survival(Y) of Experiment 1, 1991.

Dependent Variable: TL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	8.86189600	2.21547400	73.64	0.0001
Error	145	4.36212000	0.03008359		
Corrected	149	13.22401600			
	R-Square	C.V.	Root MSE		TL Mean
	0.670137	12.76842	0.173446		1.35840000
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	4	8.86189600	2.21547400	73.64	0.0001

Dependent Variable: DW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	35.64844000	8.91211000	12.80	0.0006
Error	10	6.96333333	0.69633333		
Corrected	14	42.61177333			
	R-Square	C.V.	Root MSE		DW Mean
	0.836587	24.53349	0.834466		3.40133333

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Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	4	35.64844000	8.91211000	12.80	0.0006

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.34051932	0.58512983	2.62	0.0987
Error	10	2.23242430	0.22324243		
Corrected	14	4.57294361			
	R-Square	C.V.	Root MSE		Y Mean
	0.511819	36.74690	0.472485		1.28578307
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	4	2.34051932	0.58512983	2.62	0.0987

5.2 Analysis of Variance Procedure of Total Length(TL), Dry Weight(DW) and Survival(Y) of Experiment 2, 1991.

Dependent Variable: TL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.08065688	0.02688563	1.78	0.1531
Error	156	2.35554250	0.01509963		
Corrected	159	2.43619938			
	R-Square	C.V.	Root MSE		TL Mean
	0.033108	11.96573	0.122881		1.02693750

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	3	0.08065688	0.02688563	1.78	0.1531

Dependent Variable: DW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.21751875	0.07250625	10.69	0.0010
Error	12	0.08137500	0.00678125		
Corrected	15	0.29889375			
	R-Square	C.V.	Root MSE		DW Mean
	0.727746	8.944830	0.082348		0.92062500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	3	0.21751875	0.07250625	10.69	0.0010

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.71485011	0.23828337	1.96	0.1740
Error	12	1.45942374	0.12161864		
Corrected	15	2.17427384			
	R-Square	C.V.	Root MSE		Y Mean
	0.328776	51.92297	0.348739		0.67164621

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	3	0.71485011	0.23828337	1.96	0.1740

5.3 Analysis of Variance Procedure of Total Length(TL), Dry Weight(DW) and Survival(Y) of Experiment 1, 1992.

Dependent Variable: TL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.15268667	0.05089556	6.07	0.0007
Error	116	0.97203333	0.00837960		
Corrected	119	1.12472000			
	R-Square	C.V.	Root MSE		TL Mean
	0.135755	6.816094	0.091540		1.34300000
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	3	0.15268667	0.05089556	6.07	0.0007

Dependent Variable: DW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.63789167	0.21263056	1.20	0.3687
Error	8	1.41260000	0.17657500		
Corrected	11	2.05049167			
	R-Square	C.V.	Root MSE		DW Mean
	0.311092	12.71112	0.420208		3.30583333
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	3	0.63789167	0.21263056	1.20	0.3687

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.04899332	0.01633111	1.06	0.4192
Error	8	0.12356372	0.01544547		
Corrected	11	0.17255705			
	R-Square	C.V.	Root MSE		Y Mean
	0.283925	5.588677	0.124280		2.22377850
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	3	0.04899332	0.01633111	1.06	0.4192

5.4 Analysis of Variance Procedure of Total Length(TL), Dry Weight(DW) and Survival(Y) of Experiment 2, 1992.

Dependent Variable: TL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4.73637778	0.94727556	30.41	0.0001
Error	174	5.41992000	0.03114897		
Corrected	179	10.15629778			
	R-Square	C.V.	Root MSE		TL Mean
	0.466349	11.64870	0.176491		1.51511111
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4.73637778	0.94727556	30.41	0.0001

Dependent Variable: DW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	36.67669444	7.33533889	41.90	0.0001
Error	12	2.10066667	0.17505556		
Corrected	17	38.77736111			
		R-Square	C.V.	Root MSE	DW Mean
		0.945827	9.262250	0.418396	4.51722222
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	36.67669444	7.33533889	41.90	0.0001

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	9.21855707	1.84371141	57.73	0.0001
Error	12	0.38327091	0.03193924		
Corrected	17	9.60182798			
		R-Square	C.V.	Root MSE	Y Mean
		0.960084	10.28697	0.178716	1.73730058
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	9.21855707	1.84371141	57.73	0.0001

5.5 Analysis of Variance Procedure of Total Length(TL), Dry Weight(DW) and Survival(Y) of Experiment 3, 1992.

Dependent Variable: TL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	29	20.35317889	0.70183375	209.43	0.0001
Error	60	0.20106667	0.00335111		
Corrected	89	20.55424556			
	R-Square	C.V.	Root MSE		TL Mean
	0.990218	3.771802	0.057889		1.53477778

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIME	5	20.29112556	4.05822511	1211.01	0.0001
TRT	4	0.03084000	0.00771000	2.30	0.0690
TIME*TRT	20	0.03121333	0.00156067	0.47	0.9698

Dependent Variable: WT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	29	499.3923556	17.2204261	126.86	0.0001
Error	60	8.1446667	0.1357444		
Corrected	89	507.5370222			
	R-Square	C.V.	Root MSE		WT Mean
	0.983953	12.18191	0.368435		3.02444444

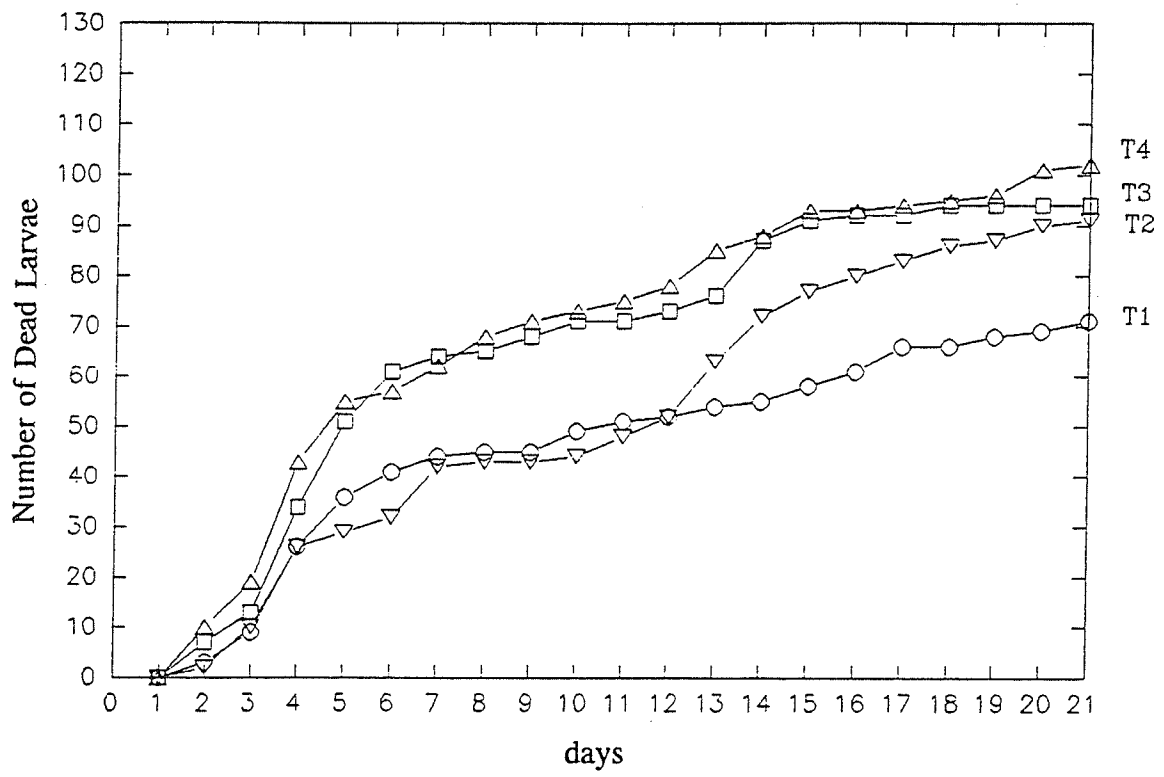
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIME	5	492.9248889	98.5849778	726.25	0.0001
TRT	4	2.9771889	0.7442972	5.48	0.0008
TIME*TRT	20	3.4902778	0.1745139	1.29	0.2242

Dependent Variable: Y

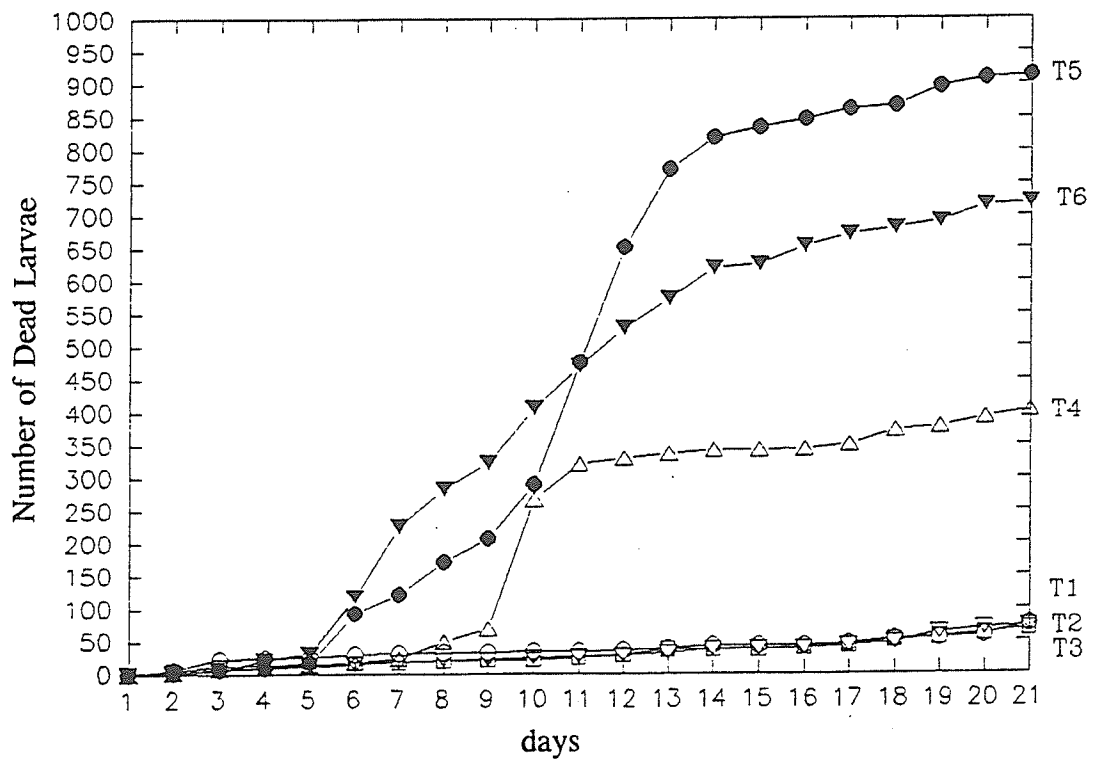
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.50888981	0.12722245	2.86	0.0807
Error	10	0.44429327	0.04442933		
Corrected	14	0.95318308			
	R-Square	C.V.	Root MSE		Y Mean
	0.533885	9.428744	0.210783		2.23553279
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	4	0.50888981	0.12722245	2.86	0.0807

Appendix 6. The observed accumulated mortality of experiments in 1992.

6.1 The observed accumulated mortality of experiment 1, 1992. Dead larvae were pooled among three replications (initial larvae = 960 ,sampled for total length and dry weight = 360, and missing larvae between 22-45).



6.2 The observed accumulated mortality of experiment 2, 1992. Dead larvae were pooled among three replications (initial = 1800, sampled for total length and dry weight = 720, and missing larvae = 29-175).



6.3 The observed accumulated mortality of experiment 3, 1992. Dead larvae were pooled among three replications (initial = 1800, sampled for total length and dry weight = 540, and missing larvae between 5-48).

