FOOD AND FEEDING OF THE BRINE SHRIMP Artemia franciscana (BRAZILIAN "MACAU" POPULATION) IN SEMI-INTENSIVE CULTURE

ALBERTO ALVES CAMPOS

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FOOD AND FEEDING OF THE BRINE SHRIMP *Artemia franciscana* (Brazilian "Macau" population) IN SEMI-INTENSIVE CULTURE

By

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT

The brine shrimp *Artemia* spp. is a widely used food item in aquaculture. Almost the entire worldwide production of brine shrimp eggs and biomass comes from extensive harvesting of inland saline lakes or coastal solar saltworks. Declining salt prices and growing aquaculture demand has switched the focus to *Artemia* production in hypersaline environments. NE Brazil has a salt-producing area of 35,000 ha, half of which is abandoned or under strictly artisanal operation. The present research evaluated the effect of supplemental feeding on *Artemia franciscana* (Brazilian "Macau" population) production and developed semi-intensive culture techniques for this region.

Preliminary laboratory trials with dried microalgae (*Spirulina maxima*) were performed to test feeding rations and a feeding table was produced. Low cost feedstuffs available in NE Brazil were evaluated for their digestibility and processing yields, and three brewery by-products were selected (i.e., malt bran, brewer's yeast and spent grains). These were then tested individually and combined in laboratory feeding trials, and their performance was recorded as growth and survival of the *Artemia*. Malt bran showed significantly better results for both parameters and was selected to be tested in the field.

Malt bran was efficiently converted into biomass by the *Artemia* (FCE = 39.16 %) and supplemental feeding was very effective in increasing biomass production. Monthly yields in fertilized control ponds averaged 306.17 kg· ha^{-1}, while feeding ponds produced up to 977.78 kg· ha^{-1}. Increasing feeding levels (rations), although not showing marked differences in size and survival, exhibited gains in individual weight and, consequently, in biomass output. The fatty acid profile of the cultured *Artemia* was greatly influenced by the diet, while protein content and amino acids did not show significant variations among treatments.

High salinities were the most efficient predator control method in semi-intensive field systems, and a minimum of 90 ppt was identified as a safe limit to

avoid the major predators in the area (i.e., cyclopoid copepods and the fish, *Poecilia vivipara*). The high mortalities experienced (above 70%) were believed to be related to high temperatures and stocking densities. Measures should be taken to reduce pond water temperature (deeper ponds, shading) and stocking densities below 100 ind· L^{-1} are recommended.

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1.0 INTRODUCTION

1.1 *Artemia* production and its uses in aquaculture

Since the 1930's, when the importance of using newly hatched brine shrimp nauplii was first related to the successful development of marine fish larvae (Seale 1933; Rollefsen 1939), *Artemia* has been the most widely used live food animal in fish and shrimp hatcheries throughout the world (Dhert and Sorgeloos 1995). Besides its nutritional value and very high acceptability by larval predator species, one of the major advantages of utilizing *Artemia* in hatcheries is related to the animal's capability of producing resistant eggs (cysts) that is conveniently available as an off-the-shelf product to be hatched and offered to the larvae according to a hatcheries' daily needs.

Almost all the adult *Artemia* biomass and cysts consumed worldwide originates from natural populations extensively harvested in saline lakes (e.g., Great Salt Lake, Utah) or large-scale coastal saltworks (e.g., San Francisco Bay, California). Every year, over 2,000 metric tonnes of dry *Artemia* cysts are marketed worldwide for on-site hatching (van Stappen 1996). Cysts are usually processed (washed and dried) and vacuum packed in cans, preserving their hatching characteristics for several months. In this way, they can be conveniently stored and shipped to hatcheries all over the world without compromising quality. High cyst prices (around US$ 150.00 per kilogram for a good quality vacuum canned product) and fluctuating harvests and quality (i.e., hatching

characteristics, nutritional quality), however, have created a need to reduce cyst consumption in hatcheries. While formulated feeds have not been shown to be as efficient for many marine cultured species as live food organisms, feeding strategies have to be devised to reduce hatchery operational costs without compromising larval quality or quantity. Nash and Shehadeh (1980) when feeding bigger *Artemia* nauplii and juveniles to older mullet (*Mugil cephalus*) larvae found that a reduction of 50-60% in total hatchery operational costs could be achieved by the decreased cyst consumption.

The development of aquaculture, with a rapid increase in the number of species cultured, and in hatchery and nursery techniques, has also created a demand for live feed animals of different sizes. Together with rotifers and *Artemia* nauplii, many hatcheries and growers have been utilizing *Artemia* juveniles and adults for late larval stages or as nursery diets to reduce the stress of acclimation to a new pond/tank environment, eventually reducing larval mortalities and, especially, cannibalism (Léger and Sorgeloos 1992; Dhert and Sorgeloos 1995).

Initially sold as a frozen product to the ornamental fish market, adult *Artemia* biomass is now marketed live, and in several forms of frozen or dehydrated (dried, freeze-dried) products. Energetic advantages (e.g., better growth, improved physiological condition) when feeding bigger nauplii and juveniles to older marine shrimp larvae (Abelin 1992), and better growth and survival of shrimp postlarvae (*Penaeus monodon*, *P. japonicus*, *P. vannamei*, *P. kerathurus* and *Macrobrachium rosembergii*) in nurseries when fed ongrown

Artemia (Dhont *et al.* 1993) were also reported. First feeding coho salmon fed with adult *Artemia* show increased growth when compared to dry pelleted commercial diets (Kim *et al.* 1996). Broodstock diets rich in *Artemia* have been found to improve maturation performance in penaeid shrimps (Rocha and Camara 1986; Browdy *et al.* 1987; Bueno 1990).

Despite the high nutritional quality of adult *Artemia* as prey animals (Léger *et al.* 1986; Lavens and Sorgeloos 1991), inert products (e.g., freeze-dried flakes) do not posses important qualities of the live biomass (attractiveness, stability in the water) and probably lack some essential nutrients related to growth and survival (Fluechter 1982). Although annual harvests of *Artemia* biomass (wet weight) are estimated to be more than 5.000 metric tonnes (Lai 1991, Abelin, 1992), only a very small portion of this catch is marketed as a live product due to high transportation costs. In that sense, several culture systems have been proposed to provide a reliable source of live brine shrimp in various life stages according to the predator's needs, and placed close to the market or integrated with the aquaculture operation.

1.2 *Artemia* taxonomy and general characteristics

The brine shrimp *Artemia* are crustaceans belonging to the class

Branchiopoda ("gill feet") - since their thoracic appendages are flattened, leaflike structures that performs gas exchange -, and to the Order Anostraca ("shell-less") since they lack a rigid external carapace, or exoskeleton.

The original species first described by Schlosser from the salterns of Lymington, England (1755, *in* Kuenen and Baas-Becking 1938) and named by Linnaeus (1758) as *Artemia salina* is now considered extinct, and several sibling species and superspecies are recognized today (Browne and Bowen 1991). These authors, following the recommendations from a consensus of scientists attending the first two International *Artemia* Symposia, suggested that further work should refer to brine shrimp by its generic name *Artemia* (Leach 1819) followed by the superspecies group - when known - and its geographical origin.

Two main groups are usually distinguished, in terms of their reproductive mode: a bisexual and a parthenogenetic one (consisting of all-female populations). The *Artemia* dealt in this research belong to the neotropical bisexual group most commonly found in the Americas, the *A. franciscana* superspecies. Figure 1.1 displays some of the life stages of this strain, belonging to the Brazilian "Macau" population.

Artemia are the largest of the Branchiopods, measuring up to 19 mm total length (TL) in parthenogenetic populations, and up to 12 mm in bisexual strains. They have a life span of about six months, becoming sexually mature two to four weeks after hatching. Reproductive mode can be oviparous

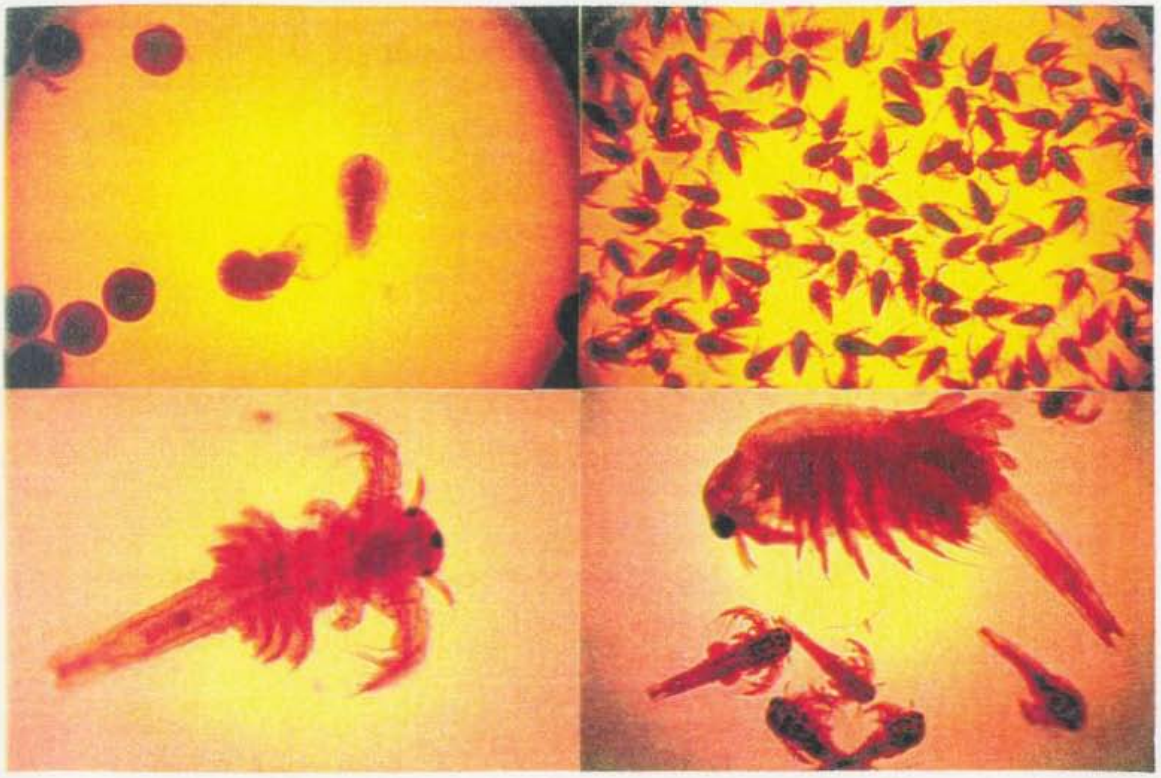


Figure 1.1 - Life stages of *Artemia franciscana* belonging to the Brazilian "Macau" population. Clockwise, from upper left: decapsulated cysts (resistant eggs) and hatching embryos still attached to embryonic cuticle; newly hatched instar I nauplii (400 μm); full-grown adult about 10 mm long, with metanauplii (2-3 mm); approximately one week old juveniles (5 mm), thoracopods (thoracic appendages) still not fully developed.

(producing "wintering eggs" or diapause cysts) or ovoviviparous (females incubating shell-free eggs in their broodsacs and then releasing free-swimming nauplii), depending on genetic tendencies and seasonal fluctuations in the environment (Sorgeloos *et al.* 1986). *Artemia*'s physiological adaptations to high salinities provide a very efficient ecological defense against predation; they possess a unique osmoregulatory system that allows them to tolerate the broadest salinity range in the animal kingdom, withstanding salt concentrations of 5 to 300 ppt; and the capacity to synthesize very efficient respiratory pigments to cope with the low oxygen levels found at high salinities (van Stappen 1996). *Artemia* can be found in hypersaline bodies of water (saline lakes, coastal lagoons, saltworks) in more than 500 natural and man-made habitats throughout the tropical, subtropical and temperate zones of the five continents, in habitats characterized by communities with low species diversity and simple trophic structures (Lenz and Browne 1991). *Artemia franciscana* superspecies are found in the Americas, the Caribbean, Australia, New Zealand and some Pacific Islands, but have also been introduced in several other countries, particularly in SE Asia, because of their desirable culture (tolerates higher temperatures), nutritional (better fatty acid profile) and physical (smaller naupliar size) characteristics.

1.3 Feeding behavior

The brine shrimp *Artemia* is a continuous, nonselective, particle-filtering organism. It feeds on live microorganisms (microalgae, protozoa, bacteria, yeasts) or on any organic or inorganic particles that might be available in the water column. The combined activity of propulsion, gas exchange, and filtration by the eleven pairs of thoracopods (thoracic appendices) in adult brine shrimp results in a practically continuous filter feeding activity (Barlow and Sleigh 1980). It is an obligate particle feeder with regard to fulfilling its protein and carbohydrate needs (D'Agostino 1980), but can utilize some micronutrients dissolved in the medium if the concentrations are sufficiently high (Hernandorena, 1983). No selection in size or sorting of particles has been observed in the brine shrimp (Reeve 1963a; Dobbeleir *et al.* 1980) and although maximal particle sizes that can be ingested by the naupliar and postnaupliar stages have not been determined exactly , particle diameter should not exceed 50 to 70 µm (Lavens and Sorgeloos 1991).

1.4 Nutritional requirements

It has been shown that various *Artemia* strains and superspecies have different nutritional requirements (D'Agostino and Provasoli 1968, D'Agostino 1980). An example of this variation is the inability of a tetraploid strain of Camacchio (Italy) to grow on artificial media which supports good growth of a diploid stain (Utah, USA), as demonstrated by Provasoli and Pintner (1980).

The requirements referred in this section are related to the bisexual, diploid neotropical superspecies group *Artemia franciscana*, found in the major cyst and biomass production areas of the world, i.e., San Francisco and San Pablo Bay (California), and Great Salt Lake (Utah). It is by far the most commonly available strain of *Artemia* for aquaculturists and researchers and, therefore, the best known from a culturing and nutritional point of view.

Difficulties in estimating nutritional requirements of *Artemia* are related to the bacteria and protozoans that easily develop in the culture medium, and that are able to biosynthesize essential nutrients as they use the supplied brine shrimp food as a substrate (Dhont *et al.* 1993). Together with the possibility of taking up and digesting exogenous microflora as part of the diet, *Artemia* can also utilize dissolved nutrients, when these are present at a minimum concentration.

As stated by D'Agostino (1980), *Artemia* is one of a very select few species of Crustacea that have been reared bacteria-free on chemically defined media under aseptic conditions. The little that is known of the nutrient requirements of *Artemia* under such conditions can be summarized as follows (Lavens and Sorgeloos 1991):

(a) the optimal protein:carbohydrate ratio is between 1:3 to 1:5;

(b) the essential amino acids are probably the same as for Crustacea in general;

(c) exogenous nucleotides and sterols are essential;

(d) the essential vitamins are thiamin, nicotinamide, calcium pantothenate, pyridoxin, riboflavin, folic acid, and putrescin. D'Agostino (1980) comments that the requirement for putrescin is unique for *Artemia* and one species of beetles. He speculates some reasons for this essentiality but concludes that "its function is yet to be determined";

(e) highly unsaturated fatty acids are not essential for growth but stimulate reproduction.

1.5 *Artemia* diets

The nonselective and continuous filtration behaviour of *Artemia* allows the utilization of a great diversity of living and non-living foods for its culture, provided the feed particles are kept in suspension and are sufficiently small to be ingested (<50 µm).

The range of suitable diets can be conveniently broadened to include waste products of poor nutritional quality, since the nutritional composition of the diet does not play the most critical role in the selection of foods for *Artemia* culture (Lavens and Sorgeloos 1991). This is probably due to the interaction between the brine shrimp and the bacterial flora that develops in the culture medium and/or around the surface of feed particles, as verified by Douillet (1987). He suggested that *Artemia* cultures could be enhanced by the exogenous microflora present in the media and/or their metabolic products, and concluded that the best diets were those which "enhanced colonization by adventitious

microflora". Consequently, from a cost-effectiveness point of view, other criteria such as particle size, solubility, buoyancy, digestibility, availability and cost can play a more definitive role in feedstuff selection, especially for semi-intensive culture purposes where deficiencies in the diet can be compensated by naturally available foods.

Since *Artemia* is a highly efficient feed converter, containing as much as six times more protein than its culture feed (Ronsivalli and Simpson 1987), and it has been demonstrated that, apart from the lipid content, the nutritional quality of *Artemia* biomass produced in semi-intensive or intensive systems is similar to wild caught biomass (Dhont and Lavens 1996), it can be expected that this high quality animal protein can be produced with cheap agricultural wastes and/or fertilizers in a low technology culture system.

1.6 Brine shrimp culture systems

1.6.1 Extensive culture

Since the early 1980's, attempts have been made to integrate extensive *Artemia* culture with salt production, leading to several inoculations of brine shrimp in salt-producing countries, particularly in SE Asia and Latin America (Davis 1980; Camara and Rocha 1987; Castro *et al.* 1987; Jumalon *et al.* 1987; Tackaert and Sorgeloos 1991a).

Extensive culture methods are usually performed in the large evaporation ponds of coastal saltworks. The production of marine salt involves the flowing of

seawater, or high salinity estuarine water, though a series of shallow evaporation ponds, until it reaches a certain concentration (260-300 ppt) when sodium chloride (NaCl) begins to precipitate. Intermediate ponds with salinities high enough to ensure a predator-free environment (100-200 ppt) are inoculated with *Artemia* cysts or newly hatched nauplii. No feed is supplied and fertilization to increase algal populations is performed in very few cases, since excessive particulate matter in the water could decrease salt quality and/or delay sodium chloride precipitation. The large size of the storage and evaporation ponds in these enterprises (up to several hundred hectares) also makes it impractical to perform effective fertilization procedures. Culture densities are very low due to a low food availability for the brine shrimp, resulting in low productivity. This type of system is oriented for salt production, and *Artemia* are treated as a byproduct.

1.6.2 Intensive and Super-intensive culture

The need for controlled production of high quality, nutritionally balanced, fixed age populations of *Artemia*, has generated an increasing interest and research efforts to produce biomass under intensive or super-intensive systems (up to 20,000 individuals per liter). Several systems have been designed (Bossuyt and Sorgeloos 1980, 1981; Lavens *et al.* 1985; Dhert *et al.* 1992, 1993) and specific diets formulated. Although these systems can be much better controlled, mainly in terms of producing disease-free *Artemia* of specific sizes, their production costs can be prohibitive and are obviously confined to regions

where outdoor production is impracticable. Super-intensive farms have been established in the last decade in some temperate countries (e.g., USA, France, UK), growing brine shrimp in tanks and raceways to supply local demand for live *Artemia* at specific life stages (Dhont and Lavens 1996).

1.6.3 Semi-intensive culture

Several attempts have been made to establish semi-intensive farming practices for the production of *Artemia*, mainly in developing countries like Costa Rica (Naegel 1987), Thailand (Tarnchalanukit and Wongrat 1987), Mexico (Tom 1987), Vietnam (Quynh and Lam 1987) and the Philippines (Jumalon *et al.* 1987). Most of these were established either in abandoned saltpans, or in existing saltfarms, by trying to increase *Artemia* productivity though fertilization in order to harvest brine shrimp as the main product. Recently, however, attempts have been made to supplement natural food organisms with locally available inert feeds (Thanh, pers. comm.), mainly residues or byproducts from local industries, in order to take advantage of the highly efficient feed conversion rate of *Artemia* (Ronsivalli and Simpson, 1987). Semi-intensive culture methods were mainly developed through the efforts of the Artemia Reference Center (Gent, Belgium) in Asian countries (Tackaert and Sorgeloos 1991b, Baert *et al.* 1996).

Recently, attention has been directed to the establishment of brine shrimp production units to support existing aquaculture enterprises, especially marine shrimp farming, since this activity is perhaps the major consumer of live and

frozen *Artemia* biomass, and also because the most suitable ecosystems for *Artemia* outdoor production share very similar characteristics to shrimp producing areas (tropical, estuarine tidal plains).

1.7 *Artemia* cyst and biomass production in NE Brazil

Since it was first inoculated in the saltworks around Macau (Rio Grande do Norte State, NE Brazil), in 1977, from a San Francisco Bay (California) population (Persoone and Sorgeloos 1980), *Artemia* has dispersed - through human, bird and wind dissemination - to a large salt-producing region along the states of Ceará, Piauí and Rio Grande do Norte (Camara and Rocha 1987).

Artemia were tremendously successful in adapting to these rich estuarine areas and, in a short period of time, were producing a modest, though significant, amount of very high-quality cysts (Guimaraes and do Rego 1987). Peak harvests of cysts were reached in 1979, with a production of more than 30 metric tonnes (Camara and Rocha 1987). Since then, cyst production has decreased steadily to reach an annual average around 1,000 kg.

It seems that *Artemia*'s reproduction in the evaporation ponds has been gradually switching from oviparous to ovoviviparous (Guimaraes and do Rego 1987). The cause of this marked depletion is still unclear to this day, since the factors governing reproductive mode and, consequently, cyst production are not yet completely understood (van Stappen 1996). Cysts are considered to be an environmentally triggered adaptation for survival in seasonally changing habitats.

Thus, females would switch to oviparity when some environmental conditions were limiting for the existing population (e.g., high salinity, low oxygen levels, low food availability). *Artemia* is supposed to have adapted to the stable environmental conditions of Brazilian saltworks (Machado 1984). Recent speculations, however, argue that a combined effect of environmental and genetic conditions might have caused this depletion in cyst production (Camara and Tackaert 1994). Newman (pers. comm.), based on observations from the Great Salt Lake (Utah, USA) argued that there seems to be two *Artemia* genotypes, one more inclined to produce cysts and another prone to ovoviviparity. Thus, the continuous harvesting of cysts would put much more pressure on the population exhibiting oviparous reproduction.

Artemia adult biomass is extensively harvested from large evaporation ponds, mainly processed as a frozen product for the ornamental fish market and for marine shrimp nurseries and broodstock maturation programs. No management procedures seem to be applied in the *salinas*, apart from some reinoculations with cysts in the early ponds of the brine circuit.

1.8 Potential for *Artemia* culture in NE Brazil

According to Sorgeloos (1983), millions of hectares of non-arable land exist in the tropical belt, much of which would be favorable to *Artemia* production. Camara and Rocha (1987), when evaluating the potential for *Artemia* culture in Brazil, quantified the saltwork areas in northeastern Brazil and found that only in

the two neighboring states of Ceará and Rio Grande do Norte, there are 16,517 hectares of abandoned saltworks that could be used to improve the income of local people through *Artemia* farming and encourage the development of other industries (e.g., solar salt production, fish and shrimp farming).

This large area of neglected ponds mainly consists of small, artisanally operated *salinas* (local name for solar salt operations) that were abandoned in the late sixties because they couldn't afford the competition with larger enterprises, which were recently mechanized with aids from the federal government. Owners of these small properties have either managed to find a job in the larger enterprises, have switched to other activities (fishing, harvesting *Artemia*, small business, temporary work), or have left these areas and moved to larger urban centres. These small units are much more manageable and require only few modifications (i.e., deepening the ponds, and/or raising dikes, screening the water intake to avoid predators) to be suitable for *Artemia* culture.

The northeastern Brazilian coast has also been experiencing a rapid development in marine shrimp aquaculture (Nunes 1995). New enterprises, together with the intensification of culture methods and the switch from a local species (*Penaeus subtilis*) - whose postlarvae could be collected in the adjacent estuarine waters to stock ponds - to the exotic *P. vannamei* successfully cultured in Ecuador, have created an enormous demand for postlarvae and, consequently, resulted in an increasing number of backyard and large-scale hatcheries. Farms and hatcheries are usually located not far from the salt

producing areas, since these two activities share a similar ecosystem, and represent a growing market for *Artemia* products (cysts, live animals in various stages, frozen biomass).

Since the semi-intensive culturing of *Artemia* has a fairly recent history, many questions regarding the optimal culture methods remain unsettled. As in any partially controlled system, the influence of environmental conditions plays a key role in determining appropriate culture methods, and regional techniques have to be developed for the particular prevailing conditions. Most of the previous work done with *Artemia* pond production have focused on enhancing the natural productivity of saltpans by fertilization, while feeding has been restricted to intensive systems. Thus the objectives of this research were to (a) investigate the potential of locally available feedstuffs , (b) conduct feeding trials to evaluate their performance on *Artemia* biomass production, and (c) to determine the optimum supplemental feeding regime (ration) for a particular feedstuff under the standard fertilization procedures. In addition, this study also aimed to provide some background on the cost-effectiveness of using supplemental feeding on *Artemia* semi-intensive pond production, and to test the feasibility of adapting the methods developed by the *Artemia* Reference Center in Asia to the Brazilian climatic and socio-economic characteristics.

Since one kilogram of adult brine shrimp biomass is sold at the equivalent price of one tonne of salt, this seems to be a viable opportunity for local communities to improve their income and make a better use of their land by

utilizing aquaculture as an alternative to reduce the rural exodus that afflicts the region.

2.0 MATERIALS AND METHODS

2.1 Preliminary laboratory trials

Eighteen 500-mL containers filled with filtered seawater (S‰=35 ppt) and gently aerated by aquarium pumps were set up for the 14-day feeding trials. The containers were inoculated with newly hatched *instar* 1 *Artemia* nauplii from the Brazilian "Macau" population (*Artemia franciscana* strain) at a density of 250 nauplii per liter. Cysts were obtained from wild sources, collected in the saltworks around the municipality of Grossos (Rio Grande do Norte State, NE Brazil). These were washed, dried, measured, decapsulated and hatched according to the standard techniques described by Sorgeloos *et al.* (1986) and updated by Lavens and Sorgeloos (1996).

The spray-dried blue-green microalga *Spirulina maxima* (Microfine Spirulina, Argent Chemical Laboratories, Richmond, BC) was chosen as a test diet as it is known as an effective *Artemia* diet (Person-Le Ruyet 1976, Douillet 1987) and its suitable size (8-10 µm diameter, 20 µm length). A feeding schedule (Table 2.1) was created based on values presented in the literature for similar culture conditions (Reeve 1963b,c; Vanhaecke *et al.* 1984; Coutteau *et al.* 1992) and the diet was offered in five different rations. All experiments were replicated three times and unfed samples were used as controls. The dried algae were suspended in a solution with the same filtered seawater used for the cultures, counted in an improved Neubauer counting

Table 2.1 - Feeding schedule for preliminary laboratory trials. Values are given as number of algae cells (x 10^4) per individual per day.

	Ration[a]				
Day	1	2	3	4	5
1	15	30	60	120	240
2	30	60	120	240	480
3	30	60	120	240	480
4	30	60	120	240	480
5	45	90	180	360	720
6	45	90	180	360	720
7	60	120	240	480	960
8	75	150	300	600	1,200
9	125	250	500	1,000	2,000
10	145	290	580	1,160	2,320
11	150	300	600	1,200	2,400
12	180	360	720	1,440	2,880
13	200	400	800	1,600	3,200
14	220	440	880	1,760	3,520

[a] Rations were arbitrarily chosen based on values presented in the literature for several species of microalgae and inert foods.

chamber (La Fontaine-Dynatech, Germany), diluted according to the ration prescribed for each treatment, and then offered daily to the *Artemia*.

To estimate growth, 10 *Artemia* from each container were collected every other day with plastic disposable pipettes (their ends cut according to the size of the animals) fixated in a lugol solution, and measured from the anterior portion of the head to the tip of the caudal furca (not including the setae) using a microscope or a dissecting microscope (depending on the size of the animal) equipped with a micrometer scale previously calibrated with a stage micrometer. During sampling, aeration was discontinued for some time, allowing settling of excess feeds, molts, dead animals and fecal pellets. These were siphoned daily and a water exchange of about 10% was performed to minimize water quality related problems (nitrification, bacterial blooms). At the end of the feeding trial, remaining animals were counted and survival was calculated taking into account the ones that were removed during sampling.

Salinity and pH were recorded daily with a hand refractometer (Atago, Japan) and a portable pH meter (pHep 1), respectively. Temperature was recorded three times each day using a bulb thermometer.

2.2 Feedstuff selection and evaluation

Since the nutritional composition of the diet is not a critical factor in the selection of *Artemia* diets (see Section 1.5), agricultural and food processing residues and byproducts locally available in the northeastern region of Brazil (wheat bran, rice polish, coconut cake meal, sugarcane molasses, brewery

residues) and other supposedly abundant natural resources, such as macroalgae (*Gracilaria* sp) were considered as possible feeds for *Artemia*.

Based on the feeding characteristics of *Artemia* (non-selective, continuous, suspension filter-feeder), the nature of the semi-intensive culture system where the feeds are a supplement to natural food items, and on the cost-effective orientation of the aquaculture operation, the following criteria were considered most critical for the selection of feedstuffs.

2.2.1 Availability and cost

Monthly production and seasonality of potential feedstuffs were investigated in their sources (industries, breweries, grain wholesalers, algae collectors in coastal communities). Only materials that were available in reasonably large quantities all year round, and at a very low or practically no cost, were considered as potential feedstuffs.

2.2.2 Digestibility

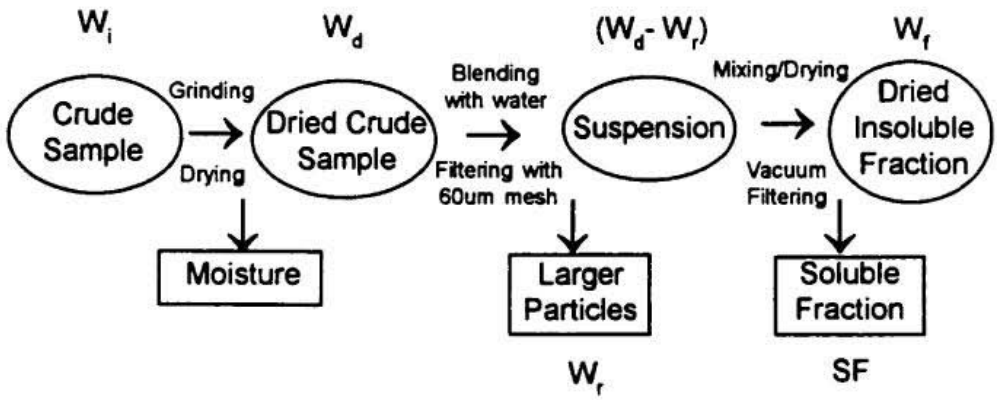
Selected feedstuffs were offered to an established culture of *Artemia* consisting of individuals of various life stages. Digestibility of a particular feed was observed visually under a compound microscope. Feeds were considered digestible when they were not present as whole particles in fecal pellets collected from the bottom of the culture vessel.

2.2.3 Particle size and solubility

A subsample with a known dry weight was blended in distilled water and

pressed through a 60-µm mesh plankton net to obtain the particle size suitable for the brine shrimp (Dobbeleir et al. 1980, Lavens and Sorgeloos 1991). The material retained on the net was then dried and weighed (for yield and soluble fraction calculations). The filtered solution containing the particles smaller than 60 µm was then thoroughly mixed using strong aeration. Aeration was discontinued after two hours and the particles in the solution were then left to settle for another hour. The supernatant was discharged and the sediment remaining in the bottom of the container was dried and weighed. The difference between initial and final weight should indicate the soluble fraction of the material and the yield of the process (Fig. 2.1). Besides its importance in the calculation of the overall yield of the feedstuff. the solubility can give some indication of the impact of a material in the quality of the culture medium. Materials that were extremely soluble in water were rejected for not being available to the *Artemia*.

Considering the above criteria. three feedstuffs were then selected to be tested in the laboratory feeding trials for their performance in growth and survival.



Calculations:

$$SF = (W_d - W_r) - W_f$$

$$Yield = 100 \times W_f / W_i$$

where: W_i = wt of crude sample
W_d = dry wt of crude sample
W_r = dry wt of particles retained in 60mm mesh
W_f = dry wt of insoluble particles smaller than 60 um
SF = soluble fraction

Figure 2.1 - Sample processing and evaluation. The Dried Insoluble Fraction obtained is the portion of the sample which is available for *Artemia* consumption. See 'Calculations' for abbreviations used in the processing diagram.

2.3 Laboratory trials

Laboratory trials were conducted in June-July 1997 in the Laboratory of Planctology of the Fisheries Engineering Department of the Federal University of Ceará, Fortaleza, Brazil.

2.3.1 Experimental set up

An experimental set of thirty 2-L containers filled with 1.5 L filtered hypersaline water (S‰ = 100 ppt) were gently aerated by aquarium pumps and set up for the 15-day feeding trials. Saline water was brought to Fortaleza from the nearby saltworks where the field trials would be conducted, kept in the dark for two months and filtered through a 5-µm mechanical filter. The containers were inoculated with newly hatched instar I *Artemia* nauplii from the Brazilian "Macau" strain, at a density of 250 nauplii· L^{-1}. Intensive systems use densities above 2,000 nauplii· L^{-1} (Dhert *et al.* 1992) and inoculations in salinas for extensive cultures are made with densities of 50 nauplii· L^{-1} (Jumalon *et al.* 1987). The density used here was arbitrarily chosen in order to achieve a reasonable biomass production without compromising the water quality in the semi-stagnant (low water exchange) field conditions. Cysts were collected from wild sources, washed, dried, decapsulated and hatched following the standard technique described by Sorgeloos et al. (1986) and updated by Lavens and Sorgeloos (1996).

2.3.2 Feeding

Each of the three selected feedstuffs (F_1, F_2 and F_3) were tested individually, under three different rations, and in three different combinations (F_1:F_2, F_1:F_3, F_2:F_3) of equal proportions. Feeding rations were scheduled as shown in Table 2.1 and based on the results obtained in the preliminary laboratory trials. The three most effective rations in terms of growth and survival (rations 3-5) were selected for these feeding trials.

The feeding trials were conducted under three different rations for each feedstuff, or combination of feedstuffs, and divided in two 15-day periods. All treatments were conducted in triplicate, and three unfed controls were always kept under the same density and culture conditions for each trial. Dried feedstuff samples were treated daily, as described for the feedstuff evaluation (weighed, blended, suspended in seawater, sieved in 60-µm mesh plankton net) and distributed in the experimental vessels according to their pre-established daily ration.

2.3.3 Diet evaluation

Performance of each diet was evaluated through the growth and survival of the animals in the culture vessels. To estimate growth, ten individuals from each container were collected every other day, fixed in a lugol solution, and measured from the anterior portion of the head to the tip of the caudal furca (not including the setae) using a compound microscope or a dissecting microscope

(depending on the size of the animal) equipped with a micrometer scale. During sampling, aeration was discontinued for some time, allowing settling of excess feeds, molts, dead animals and fecal pellets. These solids were siphoned daily and mortalities recorded. At the end of the feeding trial, the remaining animals were counted and their survival rate (%) calculated taking into account the ones that were removed during sampling.

2.3.4 Water quality

Besides siphoning of excess feeds, molts, dead animals and fecal pellets, a daily water exchange of about 10% was performed to minimize water quality related problems (nitrification, bacterial blooms). Temperature, salinity and pH were recorded with a min./max. thermometer, a hand refractometer (Atago, Japan) and a portable pHmeter (pHep 1), respectively.

2.4 Field trials

Field trials were conducted during the peak of the dry season, from October to December of 1996, in the municipality of Grossos (Rio Grande do Norte do Norte State, NE Brazil). One feedstuff (malt bran) was selected from the laboratory trials and then tested under field conditions, in a semi-intensive culture system supplied with fertilized water. The experimental setup was built in one of the evaporation ponds of an abandoned salina, which had to be adapted for *Artemia* culture.

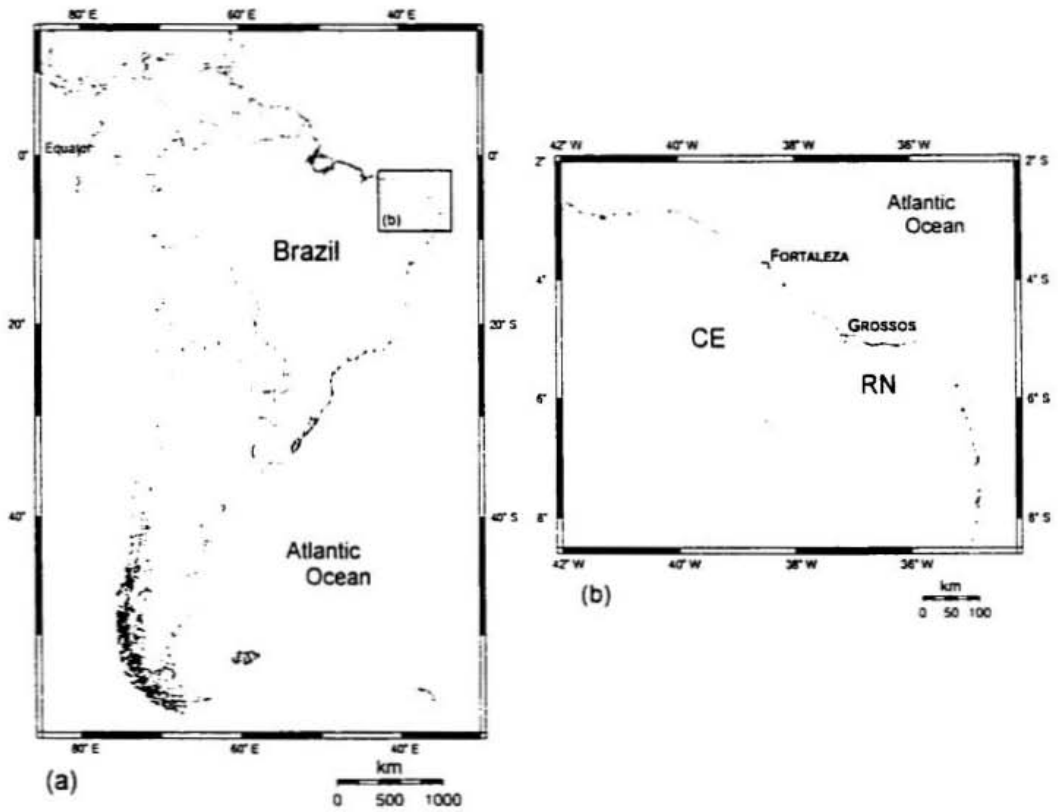


Figure 2.2 - The study area. Map (a) shows the location of Brazil in South America and outlines the section of its northeastern portion that is enlarged in (b). Laboratory trials were performed at the Federal University of Ceará (CE), situated in Fortaleza; fieldwork was conducted in the *salinas* around Grossos, Rio Grande do Norte State (RN).

A marked rainy season usually occurs in the first semester of the year, with the highest precipitation in March, April and May. From August until November, rainfall is close to zero. Total annual precipitation ranges from 800 to 1,000 mm. Humidity is around 70% during the first semester and decreases to an average of 60% during the dry season. Winds are predominantly northeasterly, averaging 2.0 m/s during the rainy season and increasing to an average of 5.0 m/s during the rest of the year. Total annual sunlight is usually around 3,000 h. Average evaporation is high, ranging from 100 mm in the peak of the rainy season to 300 mm in the second semester (ESAM 1996).

Tidal fluctuations range from 2 to almost 4 m. The highest spring tide recorded during the experiment being 3.6 m above mean sea level (DHN 1996).

2.4.2 Experimental set up

Nine *Artemia* experimental culture ponds ranging in depth from 40 to 50 cm were built in the evaporation pond mentioned above. Each pond had an approximate area of 27 m^2 (3.6 x 7.5 m) with an average volume of 10-11 m^3. The remaining area of the pond was kept as a reservoir to supply fertilized water to the culture ponds (Fig. 2.3). Water was pumped from the supply canal to the reservoir pond with a portable gasoline pump. When reaching the desired salinity and transparency, the water from the reservoir pond was then transferred by gravity to the culture ponds through 100-mm

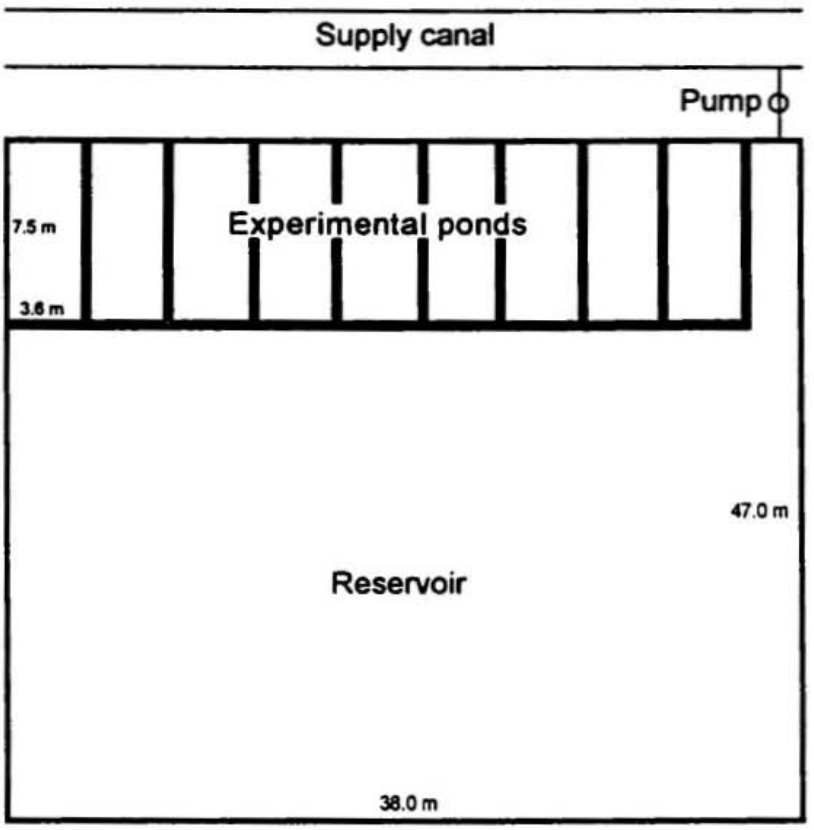


Figure 2.3 - Overhead view of a transformed evaporation pond and supply canal, showing experimental ponds, pumping station and pond dimensions. Thicker lines represent the walls that were built. Average depth was 45 cm in experimental ponds and 20 cm in the reservoir. Not drawn to scale.

(4") PVC pipes stopped by conical glass bottles and covered with 1000-µm mesh nylon screens. Salinity in the culture ponds was kept close to 100 ppt to avoid predation by fish and copepods.

2.4.2.1 Pond preparation

Pond preparation procedures included the building of experimental ponds, deepening the supply canal, measuring and adjusting soil pH, and the application of fertilizers to boost algal development.

2.4.2.1.1 Canal and pond modifications

The basic structural modification required to adapt evaporation ponds to *Artemia* production is to increase the water depth to prevent lethal high temperature conditions for *Artemia* and to promote the development of phytoplankton (Tackaert and Sorgeloos, 1991b). Deep ponds can also sustain a larger production per unit area, since *Artemia* is a planktonic organism. Depending on the size of the pond and the water dynamics, this is usually done by deepening the ponds and/or raising its walls and dikes. In this particular case, besides these pond modifications, the supply canal also had to be unobstructed and deepened, since the *salina* had been abandoned for more than ten years and the sediment-rich estuarine waters had deposited almost a meter-high layer of mud. During intertidal periods, when the canal would be holding little water, a stretch of about 40 m was manually excavated to reach a depth of one meter, and a slightly deeper well was dug around the spot where the inlet pipe of the pump was to be placed.

As soon as water could reach the pumping station, the evaporation pond was flooded with a thin layer of water to soften the hard bottom (Fig. 2.4a). Using the clay-rich bottom mud, a dike was erected to separate the experimental ponds and the reservoir (Fig. 2.4b). This section was then subdivided into nine experimental ponds and deepened to the desired depth (40-50 cm). Newly built pond walls were then covered with (coconut) palm tree leaves, "nailed" to the walls with V-shaped wooden pieces, to prevent erosion by water and wind action (Fig. 2.5a). The finished pond system, completely filled up and ready to receive the *Artemia* is shown in Fig 2.5b.

2.4.2.1.2 Pond fertilization

Calcium nitrate ($Ca(NO_3)_2$) was the fertilizer used during the experiment (Hydro brand, 15-16% N). Although organic fertilizers (e.g., manures) are cheaper, they are usually very bulky, their composition is variable and their action is much slower than inorganic compounds. Fertilizer amounts and fertilizing procedures were done according to Baert *et al.* (1996), until a turbidity of 30-40 cm was obtained. Fertilization was performed only in the reservoir pond, where a lower salinity would permit a faster response in terms of algal development. The addition of 1 mg nitrogen per liter of water was enough to promote an algal bloom and reach the desired turbidity in five to seven days.

(a)

(b)

Figure 2.4 - Evaporation pond modifications and experimental pond building: (a) perimeter walls of the former salt pond were rebuilt and it was flooded to soften its hard bottom mud; (b) a wall was raised to separate the *Artemia* culture ponds from the fertilized water reservoir.

(a)

(b)

Figure 2.5 - Pond finishing: (a) *Artemia* culture ponds were covered with palmtree leaves to prevent erosion, (b) ponds were filled and ready to receive the *Artemia* nauplii.

The calculated amount of fertilizer was dissolved in freshwater and uniformly distributed over the reservoir pond area. No further addition of fertilizer was necessary during the 15-day culture cycle, since the water dynamics of the system (i.e., pumping estuarine water from the supply canal to the reservoir pond and water flow to the culture ponds) seemed to be sufficient to keep turbidity in the desired range.

2.4.2.1.3. Soil pH and liming

Soil pH was measured with a Soil pH meter (Argent Laboratories, Richmond, BC). Liming was not necessary since both the soil and water presented satisfactory pH values (above 7.5) for *Artemia* culture (Baert *et al.* 1996).

2.4.2.2 Water intake

The ponds were supplied by a long canal that was flooded during high spring tides with the waters from the River Mossoró estuarine complex. The water was trapped in this narrowing canal - about 6 m wide where it meets the river (and where the sluice gates were placed) narrowing to a width of 1.5 m where the experimental ponds were located, six km from the gates - which is utilized by several small artisanal saltworks along the way to supply their evaporation ponds. During the high tide cycles, when the supply canal was flooded, which lasted for approximately three to five days depending on tide height, water was pumped continuously to the reservoir pond, with a portable 1.5 HP gasoline pump (OleoMac, Italy), with a capacity of 10 m^3 per hour.

Water from the reservoir pond could be delivered by gravity to the culture ponds. Each of the nine experimental culture ponds had a bottom PVC inlet pipe, stoppered by a conical brandy bottle (Dreher brand) commonly used by local *salina* operators and that efficiently fits tightly to the 100-mm (4") PVC pipes.

2.4.2.3 Predator control

Screening of intake waters, and lethal high salinities were the methods used to avoid the presence of predators in the culture ponds. A 1000-µm mesh stainless steel screen was used in the intake pipe of the pump and a nylon screen of the same mesh size fitted to the PVC pipes that supplied water from the reservoir pond to the culture ponds. Lethal salinities were obtained by filling up the *Artemia* ponds to a level of 10 to 15 cm, in order to insure higher temperatures and, thus maximum evaporation. Then, gradually filling up the ponds to the desired depth and never letting the salinity drop below 80 ppt. This method was much more effective than the screening as it will be discussed later.

2.4.3 Hatching and inoculation

Hatching procedures followed the recommendations by Baert *et al.* (1996) for field conditions. Cysts from the Brazilian "Macau" strain were obtained locally and tested for their hatching characteristics (hatching percentage, hatching efficiency and hatching rate). The number of cysts needed to inoculate the ponds in the desired density was then calculated and a 30% mortality at the time of stocking was taken into account. Cysts were hatched in the field laboratory under

constant light and strong aeration provided by six aquarium pumps, in a 100-L plastic container. Seawater (S‰ = 35 ppt) filtered with a 50 µm filter bag was used and less than 1 g of cysts per liter of water was added to avoid water quality related problems in the hatching container.

The nauplii were harvested in the first instar stage (see Fig. 1.1), since older stages would not survive the salinity shock from the hatching container (S‰ = 35 ppt) to the culture ponds (S‰ = 80 ppt), with a 125-µm mesh sieve and transferred to another 100-L container filled with pondwater. Naupliar density was again estimated and the container was transferred with a wheelbarrow to the culture ponds located approximately 300 m from the field laboratory. Initial stocking density was reduced from 250 to 200 individuals per liter, as suggested by Sorgeloos (pers. comm.). Inoculation of *Artemia* nauplii took place in the late afternoon, when water temperature was low and continued to drop until the early morning. The amount of nauplii required for each pond was calculated taking into account depth readings, and the required number of individuals was then uniformly distributed over the nine experimental *Artemia* ponds.

2.4.4 Food processing and feeding

Dried crude samples of the feedstuff selected for the field trials (malt bran) were processed at the field laboratory. Processing followed the same steps described for the laboratory trials, except for the last drying step, since the losses during the processing were now known and the amount of crude sample needed to obtain the desired quantity of sieved and washed feeds to be offered to the *Artemia* could be calculated. This greatly reduced the labor and equipment (i.e., drying oven) required to process the samples in the field and avoided possible nutritional losses during the drying step.

Crude samples were sieved through a set of four stainless steel sieves (500, 250, 125, and 63 µm mesh), mixed with filtered seawater in a large (100-L) plastic container with strong aeration for two hours and left to settle for approximately one hour. The supernatant was then discharged and the remaining particles were again mixed with filtered seawater and kept in suspension with aeration until the feeding time, following the recommendations of Thanh and Sorgeloos (pers. comm.). This suspension was prepared daily, usually 20-24 h before being offered to the *Artemia*, and the pre-determined daily rations were distributed evenly in the ponds during the first hours in the morning.

Daily rations for each feeding treatment were calculated based on the same feeding tables used for the laboratory trials. The tables, however, were adapted to a supplemental feeding regime and transformed to a 'mg of dry food per liter' basis instead of the initial 'number of particles per *Artemia*' basis used in the laboratory (Table 2.2). This was introduced for practical reasons in order to

adapt the procedure for larger volumes and for the work under field conditions. Treatments were randomly assigned to each pond, and these were labeled according to the feeding ration: Ration A (ponds A1, A2, and A3), Ration B (ponds B1, B2, and B3) and Control (no feeding) ponds (C1, C2, and C3).

2.4.5 Monitoring water quality parameters

2.4.5.1 Temperature

Air temperature was measured at least two times daily with a bulb thermometer, and minimum and maximum values were recorded daily with a min./max. thermometer placed near the ponds, almost at the ground level. Water temperatures were taken with a portable meter (Oxyguard Handy Mk III Portable meter, Point Four Systems, Inc., ON) three times every day.

2.4.5.2 Salinity

Salinity was recorded once a day with a temperature compensated hand refractometer (Atago, Japan). When the desired salinity was reached in the reservoir pond (S‰ = 50-80 ppt), culture ponds were slowly filled up. Evaporation losses could be compensated by regular pumping to the reservoir pond and by controlling the stoppers in the PVC pipes that fed the culture ponds.

Table 2.2 - Feeding table for *Artemia* cultured in fertilized ponds.

Day	Ration (dry weight)			
	A		B	
	mg· L^{-1}	g· $pond^{-1}$ [a]	mg· L^{-1}	g· $pond^{-1}$ [a]
1	1.5	15.80	3.0	31.32
2	3.0	31.59	6.0	62.64
3	3.0	31.59	6.0	62.64
4	3.0	31.59	6.0	62.64
5	4.5	47.38	9.0	93.96
6	4.5	47.38	9.0	93.96
7	6.0	63.18	12.0	125.28
8	7.5	78.97	15.0	156.60
9	9.0	94.77	18.0	187.92
10	15.0	157.95	30.0	313.20
11	15.0	157.95	30.0	313.20
12	18.0	189.54	36.0	375.84
13	22.5	236.92	45.0	469.80
14	22.5	236.92	45.0	469.80
Total		1,421.55		2,818.8

[a] calculations based on average pond volumes of 10.53 and 10.44 m^3 for rations A and B, respectively.

2.4.5.3 Dissolved oxygen

Dissolved oxygen and the percentage of oxygen saturation were measured with a portable DO meter (Oxyguard Handy Mk III Portable DO meter, Point Four Systems, Inc., ON). Since great variations in DO levels were expected, measurements were made three times daily at dawn (05:30) - to register the lowest concentrations caused by algal respiration during the night -, shortly before noon (11:30), and at dusk (17:30).

2.4.5.4 pH

The pH was recorded once a day with a portable pH meter (pHep 1).

2.4.5.5 Depth

Calibrated sticks were placed in each of the ponds to record water depth. Fluctuations in pond water level provided some information on evaporation rates, leakage, precipitation and pumping rates.

2.4.5.6 Turbidity

Turbidity was used as an indirect method to estimate algal development and abundance, and was measured daily with a Secchi disk.

2.4.6 Monitoring growth and survival

Samples of individuals were collected every other day from the culture ponds to estimate growth. After homogenizing the ponds with a paddle-like apparatus, samples were collected with a 125-µm mesh sieve attached to a 100-

mm PVC pipe. The animals were immediately fixed in a Lugol solution; samples were labeled and transferred to the field laboratory where 30 individuals from each sample were taken for measurements. As in the previous laboratory trials, the animals were measured from the anterior portion of the head to the tip of the caudal furca (not including the setae) using a compound microscope or a dissecting microscope (depending on the size of the animal) equipped with a micrometer scale.

In the last day of the trial, before the ponds were completely harvested, final *Artemia* density was calculated to estimate survival in each pond. After homogenizing the ponds, three 5-L samples of pond water were collected with a plastic bucket and filtered over a 125-µm mesh sieve. Samples were fixed in a Lugol solution, labeled and transferred to the field laboratory for counting.

2.4.7 Biomass harvesting and processing

Early in the morning on the 15th day of culture, when the animals were concentrated in the upper layers of the ponds, performing surface respiration due the low oxygen concentrations caused by algal respiration during the night, *Artemia* biomass was collected with a 500-µm mesh dip net and concentrated in 100-L plastic containers. At the same time, water was being pumped out from the pond being harvested and collected in a 10-L bucket with a 250-µm mesh sieve attached to its bottom, half-submerged to avoid damage to the animals. These were frequently transferred to the larger 100-L containers until the pond was completely empty.

The animals were then briefly washed in seawater to remove excessive brine, drained, weighed and placed in 250 g plastic heat-sealed bags (being very careful to completely remove the air from the bags), and immediately placed in a horizontal freezer. When completely frozen, the samples were transferred to the laboratory of the Food Technology Department, Federal University of Ceará, in Fortaleza, freeze-dried, and vacuum packed for analysis at a later time. Total biomass harvested in each pond was recorded for calculations on diet and ration performance (biomass output, yield, food conversion ratio).

Two samples of wild *Artemia* were also collected from unfertilized evaporation ponds in nearby saltworks, processed and packed in the same manner in order to evaluate and compare their nutritional value with the more intensively produced *Artemia*.

2.5 Sample analysis

2.5.1 Proximate composition

Samples were analyzed for their content of moisture, crude protein, total lipids, ash and carbohydrates.

2.5.1.1 Moisture

Moisture was determined by placing a thin layer of *Artemia* of known weight in an aluminum pan which was then placed in a drying oven at 90 °C. Samples were removed after 24 h, placed in a dessecator for 30 min to cool down and weighed. This procedure was repeated every 2 h afterwards until a constant weight was reached.

2.5.1.2 Protein content

Total organic nitrogen was determined by the Kjeldhal method. Approximately 400 mg of the freeze-dried sample was weighed on a nitrogen-free paper and transferred individually to a digestion tube together with two Kjeltabs (mercuric oxide type) and 20 mL of concentrated H_2SO_4. Tubes were then placed in a Buchi digestor, covered with an aspirator and digested for a minimum period of 45 min, until the solution was clear yellow color. Cooled digestion tubes were inserted in the Buchi distillation unit, and 100 mL of distilled water and 150 ml 25% (w/w) NaOH were added. Samples were distilled until 150 mL of condensate were collected in Erlenmeyer flasks containing 50 mL 4% (w/v) boric acid solution and 12 drops of a methyl red/methylene blue indicator (total volume of 200 mL). The condensate was then titrated with a standardized 0.1 N H_2SO_4 to a red point. Percentage of nitrogen in the sample was calculated using the formula shown in Appendix A.

2.5.1.3 Total lipids

Lipid extraction and analysis were performed using the Soxtec system, which is a modification of the Soxhlet principle. Crude freeze-dried samples were ground to a fine powder, weighed (approximately 3.0 g), placed in extraction thimbles, covered with cotton wool, and inserted in the Soxtec machine. Preweighed extraction cups with boiling chips and approximately 25-50 mL of hexane were also placed in the Soxtec system. Extraction was performed for 60 min in the "Boiling" position and, subsequently, for 30-45 min in the "Rinsing" position. After evaporation of the solvent, extraction cups were placed in the drying oven (at 100 °C) for 30 min. Cups were cooled in a dessecator and weighed. Crude fat could then be calculated (Appendix B).

2.5.1.4 Ash

Crucibles were first washed, dried (250 °C for 2 h), incinerated (550 °C for 2 h), cooled in dessecator and weighed. Samples were added to crucibles, weighed and placed in a muffle furnace (250 °C for 2 h). Temperature was then raised to 550 °C and samples were left overnight. After cooling in a dessecator, crucibles with samples were again weighed and ash content calculated.

2.5.1.5 Carbohydrates

This group of nutrients - e.g., sugars, starches and fiber - was estimated as the difference between the sum of the other constituents and the original dry weight of the sample.

2.5.2 Amino acid analysis

2.5.2.1 Total amino acids

Total amino acids were determined as described by Shahidi *et al.* (1990). Freeze-dried samples were hydrolyzed for 24 h at 110 °C with 6 N HCl (Blackburn 1978). The HCl was removed under vacuum, and dried samples were reconstituted using a lithium citrate buffer at pH 2.2. The hydrolyzed amino acids were then determined using a Beckman 121 MB amino acid analyzer (Beckman Instruments, Inc., Palo Alto, CA) interfaced with a HP integrator (Hewlett-Packard, Idaho) to enable accurate peak area analysis at the nanomole range. Tryptophan was determined separately by hydrolysis of the sample under vacuum in 3N mercaptoethanesulphonic acid at 110 °C, as described by Penke *et al.* (1974). Sulphur containing amino acids, cysteine and methionine were determined after performic acid oxidation prior to hydrolysis in 6 N HCl, and were measured as cysteic acid and methionine sulphone, respectively (Blackburn 1978).

2.5.2.2 Free amino acids

For determination of free amino acids, freeze-dried samples were homogenized using a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON) homogenizer in a small 50 mL centrifuge tube with 20 mL of ice-cold 6% perchloric acid for 2 min in an ice bath (Yamanaka 1989). The homogenized samples were then incubated in ice for 30 min before centrifugation (IEC Centra MP4 centrifuge, International Equipment Co., Needham Heights, MA) at 2000 x g

for 15 min. The residue was then re-extracted with 20 mL perchloric acid and centrifuged, as described above. The supernatants from the first and second extraction were combined and filtered through a Whatman No.4 filter paper. The pH of the filtrate was adjusted (Accumet pH meter, model 810, Fisher Scientific Co., Lawn, NJ) to 7.0 using a 33% (w/v) KOH solution and then centrifuged at 2000 x g for 10 min to remove precipitates of potassium perchlorate. The supernatant was then acidified to pH 2.2 using a 10 N HCl solution, and diluted to 50 mL with distilled water. Two milliliters of the extract were taken into a clean tube and 1.0 mL of lithium citrate buffer (pH 2.2; Beckman Instruments, Inc., Palo Alto, CA) was added. Samples were then analyzed on a Beckman 121 MB amino acid analyzer using Benson D-X 8.25 resin and a single column employing three-buffer lithium method as per Beckman 121 MB-TB-017 application notes.

2.5.3 Fatty acid analysis

Fatty acid composition of lipids was determined using gas chromatography (GC). Lipids were extracted with chloroform and methanol, based on the method described by Bligh and Dyer (1959). A known dry weight of finely ground freeze dried sample was added to a prerinsed vial with chloroform and, prior to extraction, 200 µL of a C17 internal standard (triheptadecanoin solution 99% pure, NU-CHEK-PREP, Inc.) was also introduced. Approximately 1.0 mL of methanol and 1.0 mL of chloroform-extracted water were added, and the tubes were topped with nitrogen and sonicated in crushed ice three times for 2 min,

allowing some cooling time between sonications. Samples were left in the freezer overnight for separation and then the chloroform bottom layer was double pipetted into a prerinsed vial. For the preparation of methyl esters of fatty acids, 500 µL of this lipid extract were transferred to prevoiusly well-cleaned Reacti-vials, and a drop of methanol was added to the samples to ensure complete water removal. Samples were then blow dried with nitrogen gas, after the addition of the transmethylation reagent (94.0 mL methanol; 6.0 mL concentrated sulphuric acid; 10.0 mg hydroquinone, as an antioxidant), and heated for 8 h at 70 °C. Vials were tightly stoppered and covered with aluminum foil, to keep them in complete darkness. After cooling, 1.0 mL of distilled water and 1.5 mL of hexane were added to the vials, which were vigorously shaken and then the phases were allowed to separate. Upper layers were then carefully pipetted, placed into prewashed vials, and blow dried with nitrogen gas. Dried lipid samples were dissolved in 25.0 µL of CS_2 (99%) and, with a syringe prerinsed 6 times in CS_2, 1.0 µL of the mixture was injected onto a Perkin-Elmer 8500 gas chromatograph (Perkin-Elmer Instrument Division, Norwalk, CT) equipped with a fused silica (30 m x 0.249 mm) capillary column coated with polyimide (film thickness: 0.25 µm), using helium as a carrier gas and a thermal gradient from 40 to 240 °C (injection at 225 °C; column temperature: 205 °C), and a split ratio of 35:1 (J & W Scientific, Folsom, CA). Peaks were recorded using a Perkin-Elmer Omega 2 computer package (Perkin-Elmer, Norwalk, CT) and identified by comparison with known standards. Relative areas (% of total fatty acid methyl

esters) were quantified as mg of fatty acid per gram of sample and in comparison with known amounts of the internal standard used.

2.6 Statistical treatment of data

Diets and rations were evaluated in terms of length and survival, in the laboratory trials, and also in terms of biomass output and pond yield, in the field trials. The effects of environmental and water quality parameters on diet performance was evaluated. Analysis of variance (ANOVA) was performed using the Statistical Package for the Social Sciences software, windows version 7.5 (SPSS 1996; SPSS Inc., Chicago, IL) with $\alpha = 0.05$. Scheffe's multiple range test was used as *a posteriori* comparisons to determine the nature of significant treatment differences and to group all homogeneous subsets. Percent data (e.g., survival) were transformed into the arcsine of their square roots to reduce deviations from normality (Zar 1996).

3.0 RESULTS

3.1 Preliminary laboratory trials

3.1.1 Growth

During the 14-day trial a total of 1,252 individuals were measured (eight less than the expected 1,260 due to complete mortalities in two of the control replicates) in the six treatments. Initial mean length (mm) for all treatments was 0.84±0.08 (s.d., n=180) and displayed no significant differences between all 18 containers (One-way ANOVA: $F=1.124$, $df=17,162$, $p>0.05$). The increasing mean length difference in size for each treatment can be seen in Table 3.1. Triplicates were pooled together since no significant differences among containers were detected in any of the seven sampling days for all six treatments ($p>0.05$). By the end of the trial, mean individual size was significantly different among all rations (Fig. 3.1) at the 0.05 level (One-way ANOVA: $F=423.4$, $df=5,166$, $p<0.001$, Appendix C).

3.1.2 Survival

Percent survival ranged from zero (in two of the control containers) to 57.45% in one of the containers in treatment 5. Except for the control, triplicates of all treatments displayed no significant statistical differences at the 0.05 level. Survival was significantly different among treatments and these could be grouped in four distinct homogeneous subsets (Fig. 3.1; One-

Table 3.1 - Length (mm) measurements of *Artemia* for the six different treatments (mean ± standard deviation), presented as the mean of n individuals (in parenthesis). Samplings were made every other day over a 14-day feeding trial. Treatments 1-5 represent increasing food rations.

Sampling day	Ration[a]					control[b]
	1	2	3	4	5	
2	0.86 ± 0.08 (30)	0.85 ± 0.08 (30)	0.85 ± 0.90 (30)	0.81 ± 0.06 (30)	0.84 ± 0.10 (30)	0.81 ± 0.07 (30)
4	1.10 ± 0.11 (30)	1.17 ± 0.11 (30)	1.23 ± 0.12 (30)	1.28 ± 0.14 (30)	1.31 ± 0.13 (30)	1.13 ± 0.09 (30)
6	1.46 ± 0.17 (30)	1.71 ± 0.16 (30)	1.80 ± 0.11 (30)	1.96 ± 0.18 (30)	2.20 ± 0.21 (30)	1.24 ± 0.13 (30)
8	1.88 ± 0.21 (30)	2.48 ± 0.22 (30)	2.94 ± 0.18 (30)	3.24 ± 0.33 (30)	3.34 ± 0.54 (30)	1.29 ± 0.14 (30)
10	2.29 ± 0.29 (30)	3.37 ± 0.28 (30)	3.94 ± 0.34 (30)	4.58 ± 0.61 (30)	5.11 ± 0.66 (30)	1.43 ± 0.14 (30)
12	2.74 ± 0.35 (30)	4.11 ± 0.34 (30)	4.98 ± 0.67 (30)	6.10 ± 0.69 (30)	7.35 ± 0.58 (30)	1.67 ± 0.24 (30)
14	3.20 ± 0.25 (30)	4.82 ± 0.50 (30)	5.82 ± 0.59 (30)	7.14 ± 0.90 (30)	8.28 ± 0.75 (30)	1.84 ± 0.30 (22)

[a] The feeding schedule for each ration can be seen in Table 2.1.

[b] No feeding.

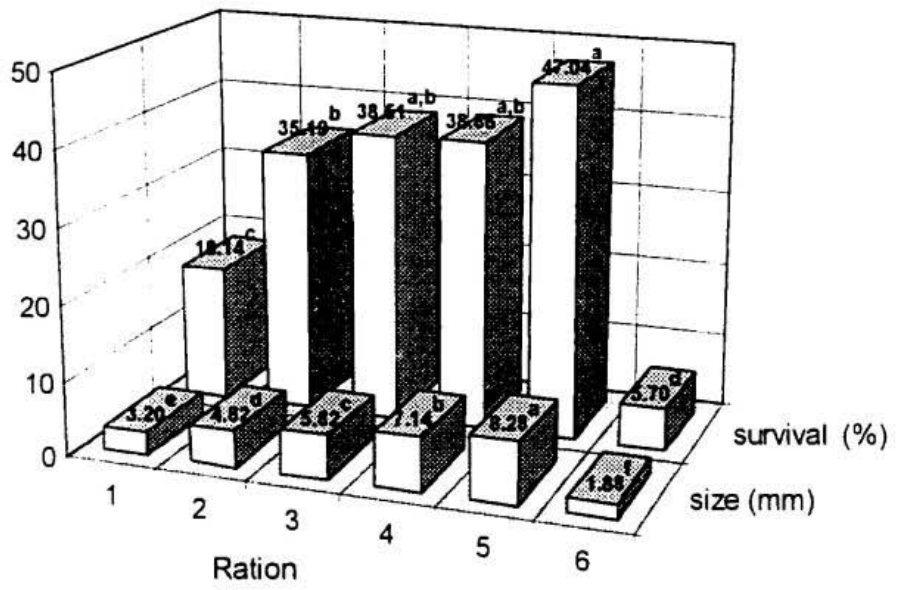


Figure 3.1 - Mean percentage survival and final size for the six treatments utilized in the preliminary laboratory trials (n=30). Distinct letters indicate significant differences at the α=0.05 level. Treatments 1-5 represent increasing rations (see Table 2.1); treatment 6 was the control (=no feeding).

way ANOVA; F=128.8, df=5,174, p<0.001, Scheffe's multiple comparison, Appendix D).

3.2 Feedstuff selection and evaluation

3.2.1 Availability and digestibility

Brans resulting from the polishing of grains (rice, wheat) were cost-prohibitive, as it will be discussed later. The use of macroalgae, although an abundant resource, was not considered because these are poorly digested and due to the prohibitive labor costs for collection and transportation of this bulky material. Sugarcane molasses are available from August until November, after the harvesting season. The dried product was considered digestible by the *Artemia*, but the amount of impurities and the high water content of this liquid residue made it difficult to process into a quantifiable feedstuff for research purposes.

Three residues from a local brewery could also be digested by the brine shrimp. The malt bran, resulting from the fine sieving of the kiln-dried sprouted barley culms; the spent grain, which are the solid wastes filtered off the 'wort' - or the liquid that will be fermented into beer - after the starches in the grain have been converted by enzymes into soluble fermentable sugars; and the brewer's yeast (*Saccharomyces uvarum*), microorganisms responsible for the fermentation process of this sugary substrate. The brewery is the largest in the region and produces close to a tonne a day of wet yeast residue and dried malt culms, and

more than 12 tonnes daily of spent grain, throughout the year.

3.2.2 Feedstuff processing and proximate composition

Dry weights, soluble fractions, weight of sieved particles (available to the *Artemia*) and the yield of processing are assembled in Table 3.2 for the malt bran, spent grain, brewer's yeast and sugarcane molasses. The three brewery residues were selected as potential *Artemia* feeds and analyzed for proximate composition (Table 3.3).

3.3 Laboratory trials

3.3.1 Diet evaluation

Feed types and rations were compared in terms of growth and survival. Table 3.4 summarizes the mean lengths for each of the six feed types offered in three rations together with an unfed control during the 14-day feeding trials. Means for all triplicates were pooled together since no significant differences were detected between containers receiving the same treatment (ANOVA, $p>0.05$).

Final shell length and survival were both significantly different among feed types and ration (Two-way ANOVA, Appendices E and G). Mean final length and survival for the six feed types and controls are shown in Fig. 3.2. In both cases, *Artemia* fed malt bran were significantly longer and had a greater survival than the other treatments (Scheffe's multiple comparison,

Table 3.2 - Evaluation of feedstuffs during processing. The dried insoluble fraction (W_f) obtained was the portion of the sample available for *Artemia* consumption and used to estimate the yield in the processing.

Sample	Wi (g)	Moisture (%)	Wd (g)	Wr (g)	SF (g)	Wf (g)	Yield (%)
Malt bran	25.06	13.44	21.69	13.13	1.97	6.59	26.28
Spent grain	50.14	87.68	6.18	4.76	0.18	1.24	2.46
Brewer's yeast	125.03	92.21	9.74	0.49	0.65	8.61	6.88
Sugarcane molasses	100.07	91.24	8.77	1.75	3.02	4.00	3.99

where: W_i = weight of crude sample

W_d = dry weight of crude sample (W_r+SF+W_f)

W_r = dry weight of particles retained in 60 µm mesh

SF = dry weight of soluble fraction

W_f = dry weight of insoluble particles smaller than 60 µm

Yield = 100 x W_f / W_i

Table 3.3 - Proximate composition of the three feedstuffs selected for testing in the laboratory feeding trials.

Feed type	Moisture (%)	% dry matter			
		CP[a]	EE[b]	Ash	CF+NFE[c]
Malt bran	13.44	20.1	2.6	8.8	68.5
Spent grain	87.68	25.2	3.8	5.3	65.7
Yeast	92.21	42.7	0.8	11.2	45.3

[a] Crude protein.
[b] Ether extract (total lipids).
[c] Crude fiber and Nitrogen-free extract (carbohydrates).

Table 3.4 - Length (mm) of *Artemia* for the six feed types offered in the four treatments (three rations and control). Values are the mean ± standard deviation of n=30 individuals. For each ration seven mean values are displayed which represent the samplings made every other day during the 14-day feeding trial.

Ration	Feed types					
	malt	grain	yeast	malt+grain	malt+yeast	grain+yeast
	0.76±0.06	0.80±0.08	0.80±0.08	0.81±0.07	0.79±0.07	0.80±0.07
	1.05±0.08	0.97±0.08	1.01±0.10	1.08±0.12	1.11±0.14	1.00±0.09
	1.10±0.08	1.13±0.16	1.18±0.13	1.11±0.11	1.20±0.16	1.12±0.09
1	1.34±0.07	1.20±0.09	1.27±0.15	1.31±0.11	1.30±0.14	1.30±0.10
	1.48±0.09	1.40±0.10	1.58±0.14	1.55±0.15	1.61±0.15	1.63±0.09
	1.69±0.18	1.62±0.20	1.69±0.17	1.73±0.16	1.90±0.15	1.72±0.15
	3.09±0.56	2.10±0.32	2.11±0.19	3.07±0.22	2.66±0.16	2.32±0.22
	0.81±0.05	0.82±0.09	0.79±0.07	0.79±0.06	0.80±0.07	0.79±0.06
	1.03±0.05	0.99±0.07	1.02±0.11	1.07±0.10	1.17±0.13	1.01±0.07
	1.12±0.08	1.18±0.10	1.12±0.13	1.15±0.14	1.17±0.14	1.17±0.09
2	1.31±0.15	1.28±0.13	1.34±0.13	1.30±0.10	1.36±0.18	1.33±0.10
	1.90±0.12	1.48±0.12	1.72±0.12	1.72±0.20	1.82±0.14	1.75±0.11
	3.13±0.34	1.81±0.17	2.31±0.19	2.94±0.15	2.80±0.13	2.20±0.20
	4.75±0.88	2.45±0.32	2.81±0.23	4.15±0.22	3.93±0.24	2.71±0.19
	0.85±0.09	0.80±0.07	0.79±0.07	0.78±0.06	0.80±0.6	0.82±0.06
	1.02±0.04	1.01±0.07	1.07±0.12	1.15±0.10	1.21±0.14	1.12±0.10
	1.20±0.15	1.21±0.14	1.25±0.15	1.24±0.16	1.31±0.16	1.25±0.11
3	1.90±0.19	1.51±0.14	1.51±0.14	1.42±0.12	1.68±0.16	1.49±0.09
	2.69±0.10	2.20±0.23	2.23±0.25	2.24±0.20	2.32±0.17	2.25±0.14
	5.81±0.71	3.15±0.34	3.28±0.37	3.83±0.31	3.72±0.19	3.24±0.23
	7.21±0.64	4.00±0.28	4.43±0.44	5.10±0.24	4.86±0.19	4.46±0.38
	0.79±0.08			0.79±0.07		
	0.97±0.07			1.02±0.09		
	1.01±0.11			1.04±0.11		
control[a]	1.15±0.15			1.26±0.24		
	1.33±0.22			1.44±0.26		
	1.69±0.21			1.62±0.15		
	1.88±0.25			1.99±0.25		

[a] The experiment was divided in two trials(individual feed types and combinations); one control was performed for each trial.

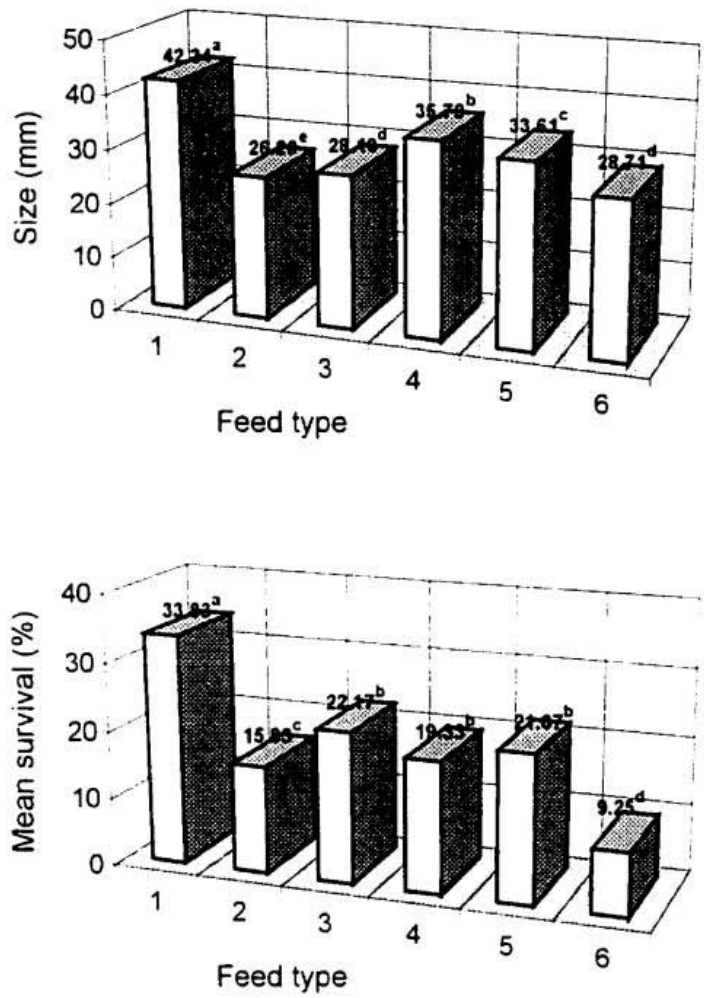


Figure 3.2 - Mean length and percentage survival for the six feed types utilized in the laboratory feeding trials (n=120). Distinct letters indicate significant differences at the α=0.05 level. Feed types are: 1, malt bran; 2. spent grain; 3. brewer's yeast; 4. malt+grain; 5. malt+yeast; and 6. grain+yeast.

Appendices F and H). Figure 3.3 shows the comparison of feed type performance for every ration utilized, plotted against mean survival and mean final length. *Artemia* fed ration 3 had the longest size (Scheffe's multiple comparison, Appendix F). However, *Artemia* fed ration 2 had a higher survival (Scheffe's multiple comparison, Appendix H). Differences among feed types, and in ration performance within a particular feed type, can be more clearly distinguished in the growth curves estimated for each treatment (Fig. 3.4).

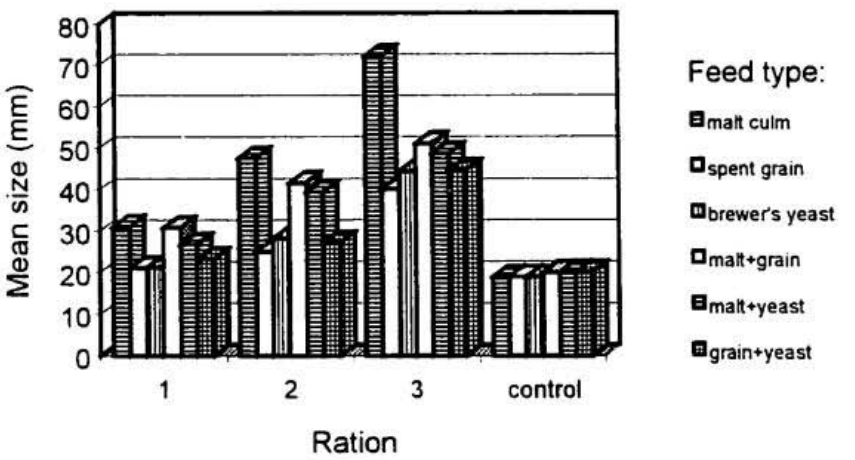
3.3.2 Water quality

Salinities stayed between 35 and 37 ppt in all containers during the 14-day experiment. Temperatures ranged from 24.6 to 27.3 °C and pH fluctuated between 7.7 to 8.1.

3.4 Field trials

3.4.1 Growth

Table 3.5 summarizes the mean values for the length of individual brine shrimp in each of the field treatments (two rations together with an unfed control) during the 14-day feeding trials. Figure 3.5 shows the growth curves plotted for each treatment. Ration 1,2 and the controls were found to be significantly different in terms of size (One-way ANOVA; $F=83.5$, $df=2,276$, $p<0.001$, Appendix I).



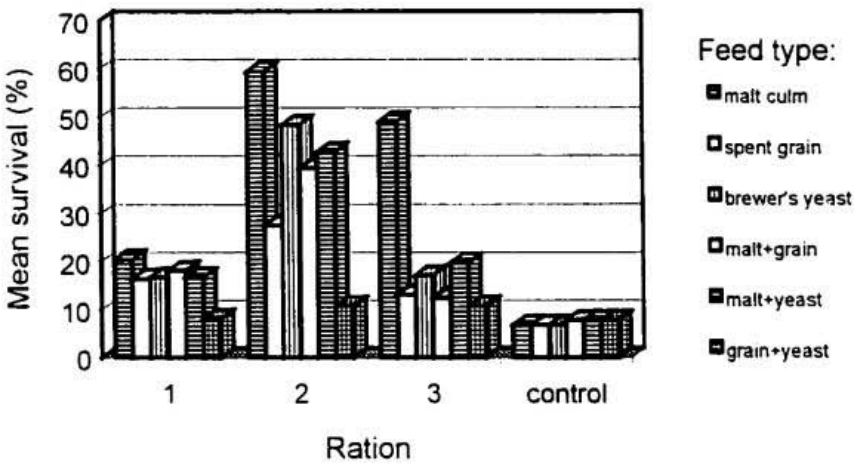


Figure 3.3 - Feed type performance (final length and percentage survival) for every ration utilized in the laboratory feeding trials. Rations were significantly different at the α=0.05 level for all feed types in terms of length and survival.

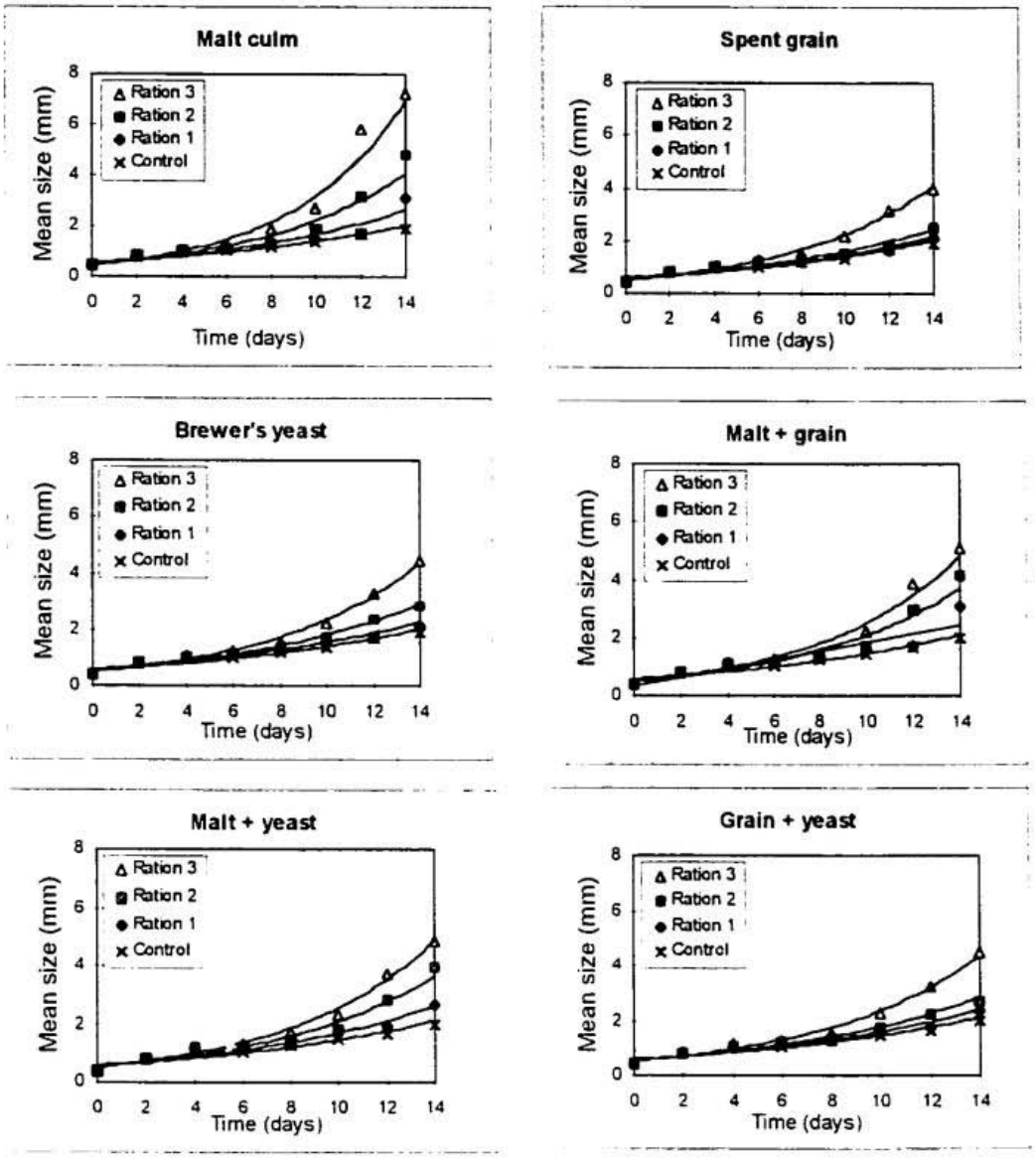


Figure 3.4 - Growth curves for *Artemia* reared under laboratory conditions (14-day feeding trials. six types of feeds offered in three rations and one control = no feeding). Exponential curves were fitted to observations and graphics adjusted to the same scale for comparison. Each point is the result of 30 observations (n=30).

Table 3.5 - Length (mm) measurements of *Artemia* for the three treatments performed in the field (two rations and a control). Values are presented as the mean ± standard deviation of n=90 individuals. Samplings were made every other day during the 14-day feeding trial.

Sampling day	Ration		control[a]
	1	2	
2	1.05 ± 0.14	1.15 ± 0.14	1.09 ± 0.15
4	1.42 ± 0.21	1.50 ± 0.26	1.51 ± 0.25
6	1.94 ± 0.26	2.11 ± 0.25	2.01 ± 0.30
8	2.86 ± 0.34	3.56 ± 0.46	2.43 ± 0.25
10	5.30 ± 0.77	6.16 ± 0.85	3.91 ± 0.62
12	6.34 ± 0.94	7.31 ± 0.75	4.72 ± 0.97
14	7.29 ± 1.01	7.91 ± 0.98	6.06 ± 1.05

[a] No supplemental feeding.

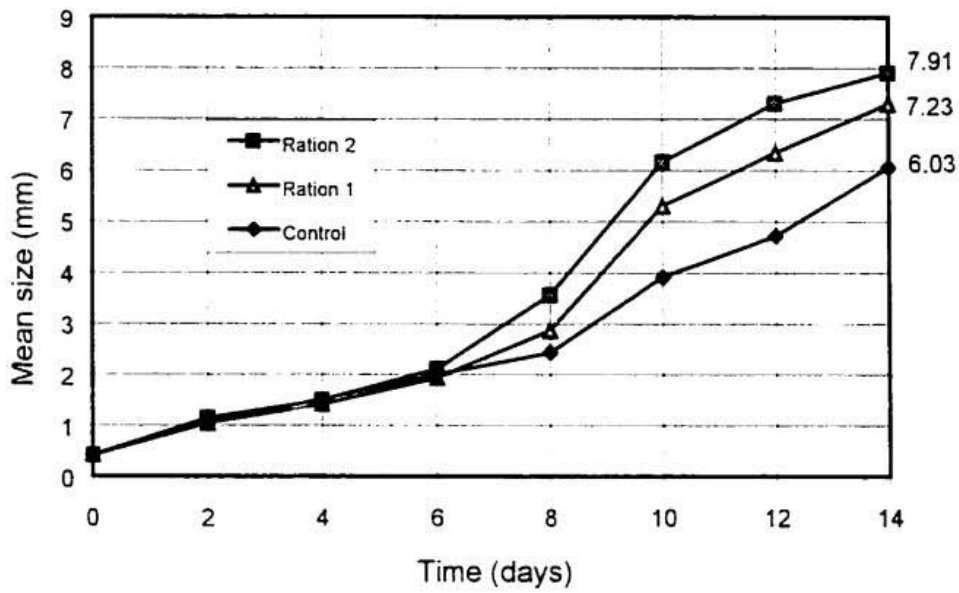


Figure 3.5 - Growth curves for *Artemia* reared under field conditions (14-day feeding trials). Malt bran offered in two rations with a control group; control ponds received no supplemental feeding, all ponds were supplied with fertilized water from the reservoir pond). Mean final length for each treatment is shown. Treatments were all significantly different at the α=0.05 level. Each point is the result of 93 observations (n=93).

3.4.2 Survival

Survival ranged from 13.63 to 29.74% in the nine experimental ponds, and was significantly different among treatments at the 0.05 level (ANOVA: F=52.7, df=2,276, p<0.001, Appendix J). *A posteriori* analysis revealed that all treatments (rations 1, 2 and control ponds) were significantly different in terms of survival (Fig. 3.6; Scheffe's multiple comparison, Appendix J).

3.4.3 Biomass output and overall yield

Total biomass harvested at the end of the feeding trial (g) and the yield for each pond ($g \cdot L^{-1}$) are presented in Table 3.6. Mean final length (mm), *Artemia* density ($ind \cdot L^{-1}$), individual wet weight (mg), and percentage survival are also included for an overall comparison of ration performance. Individual weights were calculated based on the values obtained for total biomass and Artemia density at harvest. There was a significant difference among treatments for both biomass output and yields (ANOVA's p<0.001). Ration 2 had a significant higher biomass yield compared to ration 1, which was greater than the control (Scheffe's multiple comparison).

3.4.4 Food conversion ratios and efficiencies

Food conversion ratios were calculated as the average amount of feeds used to produce the biomass (dry weight) divided by the average biomass harvested in treatments 1 and 2 (wet weight) less the average biomass produced in the control ponds. Conversion efficiencies in

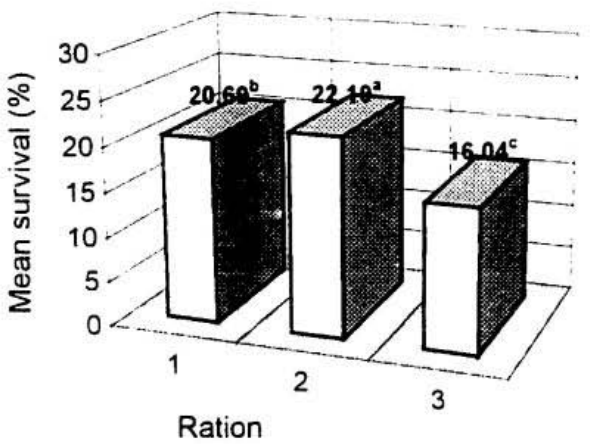


Figure 3.6 - Mean survival of *Artemia* for the three rations utilized in the field feeding trial. Distinct letters indicate significant differences at the α=0.05 level. Ration 3 represents the control (=no feeding).

Table 3.6 - Overall ration comparison in terms of growth (mean final length and individual wet weight), *Artemia* density at harvest, percent survival, total *Artemia* biomass harvested at the end of the feeding trial and the resulting yields for each pond.

Ration[a]	Pond	Length (mm)	Density (ind· L^{-1})	Survival[b] (%)	ind. wt[c] (mg)	Biomass (g)	Yield (g· m^{3-1})
	A1	7.96	30.02	15.01	2.92	970	87.62
1	A2	6.77	59.48	29.74	1.88	1,180	112.06
	A3	7.14	34.62	17.31	2.20	760	76.08
	B1	7.95	46.57	23.29	2.96	1,490	137.96
2	B2	8.67	35.21	17.61	3.63	1,380	127.78
	B3	7.12	51.34	25.67	2.18	1,090	112.14
	C1	7.11	32.52	16.26	1.74	640	56.44
control	C2	5.77	36.47	18.24	1.02	380	37.04
	C3	5.21	27.25	13.63	0.83	220	22.63
	Total					8,110	

[a] Feeding schedule for rations 1 and 2 can be seen in Table 2.2. Control ponds received no supplemental feeding, but all ponds received fertilized water from the reservoir pond.

[b] Calculated based on a 200 individual· L^{-1} initial stocking density.

[c] Individual wet weight at harvest.

percentages were calculated as the inverse of the ratios described above times one hundred. Table 3.7 shows the calculations and resulting FCR's and FCE's for the two rations tested in the semi-intensive culture system.

3.4.5 Predator control

Screening with a 1000-µm stainless steel mesh was only partially efficient in avoiding large fish predators - mainly the livebearer *Poecilia vivipara* - to enter the experimental ponds. Copepods were also found in large quantities in some deeper areas of the reservoir pond. Dense "clouds" of cyclopoid copepods, usually spread over an area of 4 to 10 m^2, and at an estimated density of 0.5 to 2 individuals· mL^{-1}, were consistently reported in the reservoir pond until the salinity reached 80-90 ppt. No potential predators were reported at salinities above 90 ppt. Aquatic insects (*Corixa* sp.) were collected in the experimental ponds even at high salinities (>100 ppt), but were only present in insignificant amounts.

3.4.6 Monitoring water quality parameters

Values for dissolved oxygen, temperature, salinity, pH, depth and turbidity are summarized in Table 3.8 for each treatment. No significant differences were found in these parameters (Figs. 3.7 and 3.8: One-way ANOVA, $p>0.05$, Appendix K), except for turbidity ($p=0.004$) where ration 2 was different from the control group (Fig. 3.9; One-way ANOVA, $F=6.12$, $df=2.60$, $p<0.05$, Scheffe's Multiple comparison, Appendix L).

Table 3.7 - Feed conversion ratios and efficiencies for the two rations tested in the semi-intensive culture system.

Ration	Food offered[a] (g)	Biomass harvested[b] (g)	Biomass balance[c] (g)	FCR[d]	FCE[e] (%)
1	1,421.55	970.00	556.67	2.55	39.16
2	2,818.80	1,320.00	906.67	3.11	32.17
control	-	413.33	-	-	-

[a] Average amount of food (dry wt) used in each treatment during the 14-day trial.

[b] Average biomass (wet weight) harvested at the end of the trial.

[c] Biomass harvested minus the average biomass output obtained in the control.

[d] Food Conversion Ration = $\frac{\text{dry wt food offered (g)}}{\text{wet wt biomass balance harvested (g)}}$

[e] Food Conversion Efficiency = $\frac{\text{wet wt biomass balance harvested (g)}}{\text{dry wt food offered (g)}} \times 100$

Table 3.8 - Fluctuation in water quality parameters monitored during the 14-day field feeding trial.

Parameter	Treatment	Mean	Std. Dev.	Min.	Max.	n
	Ration 1	31.43	2.64	26.10	34.80	63
Temperature[a]	Ration 2	31.44	2.68	25.70	35.00	63
(°C)	Control	31.46	2.58	26.00	35.00	63
	Ration 1	92.86	6.26	83.00	105.00	21
Salinity	Ration 2	89.71	6.42	79.00	103.00	21
(ppt)	Control	91.14	5.95	81.00	105.00	21
	Ration 1	8.38	0.12	8.20	8.64	21
pH	Ration 2	8.36	0.15	8.10	8.60	21
	Control	8.38	0.15	8.20	8.64	21
	Ration 1	4.38	0.65	3.50	5.60	63
DO[a]	Ration 2	4.38	0.60	3.60	5.60	63
($mg.l^{-1}$)	Control	4.53	0.60	3.60	5.70	63
	Ration 1	68.84	13.19	50.00	93.00	63
DO[a]	Ration 2	69.13	12.18	51.00	91.00	63
(% sat.)	Control	72.01	12.04	52.00	93.00	63
	Ration 1	39.86	2.06	36.00	44.00	21
Depth	Ration 2	39.62	2.18	35.00	43.00	21
(cm)	Control	39.48	2.82	35.00	45.00	21
	Ration 1	30.19	4.12	24.00	39.00	21
Turbidity	Ration 2	31.52	3.61	27.00	40.00	21
(cm)	Control	34.38	4.14	26.00	42.00	21

[a] Parameters recorded three times daily due to marked diel cycles.

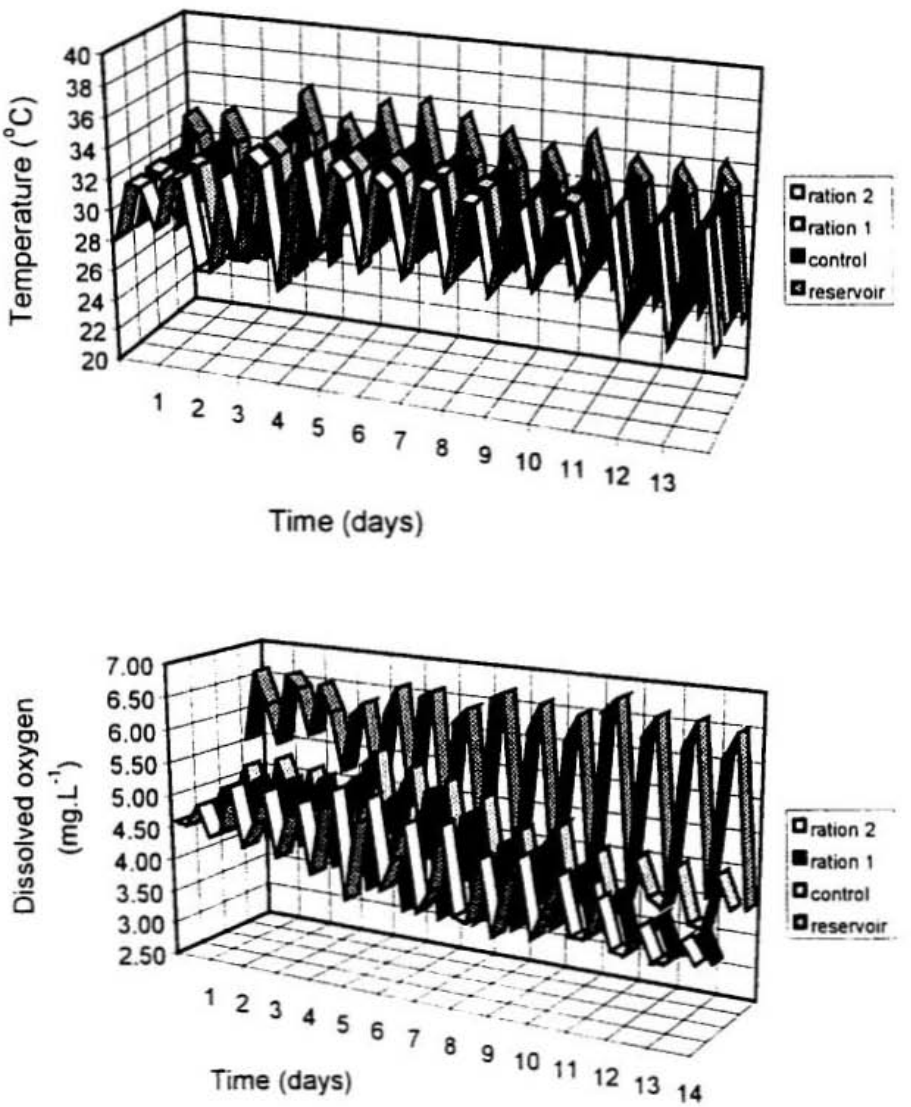


Figure 3.7 - Temperature and dissolved oxygen fluctuations during the 14-day field feeding trials in the reservoir and experimental ponds. No significant differences were found among the three treatments for DO and temperature. Observations were made three times daily due to pronounced diel cycles. Three dimensional presentation due to consistently overlapping values.

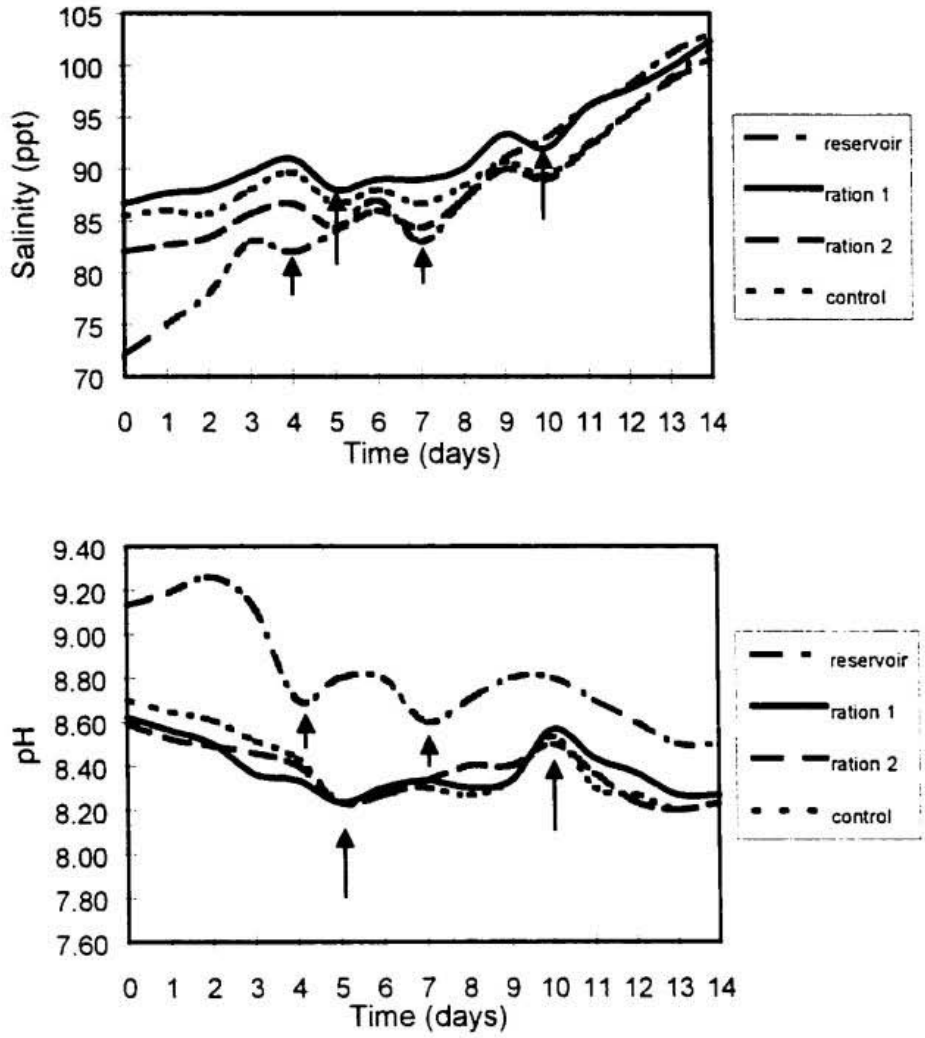


Figure 3.8 - Salinity and pH fluctuations in the reservoir and experimental ponds during the 14-day field feeding trials. No significant differences found among the three treatments (rations 1, 2, and control) for salinity and pH. Short arrows indicate pumping from the supply canal to the reservoir pond; long arrows represent water flowing from the reservoir to the experimental ponds.

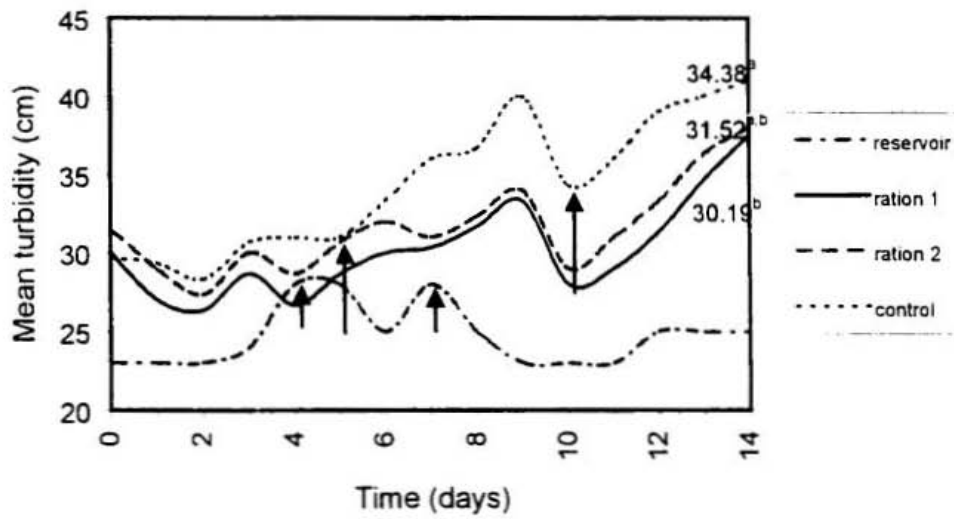


Figure 3.9 - Turbidity fluctuations during the 14-day field feeding trials. Overall means for each treatment are displayed. Distinct letters represent significant differences at the α=0.05 level (Scheffe's multiple comparison). Short arrows indicate pumping from the supply canal to the reservoir pond; long arrows represent water flowing from the reservoir to the experimental ponds.

3.5 Sample analysis

3.5.1 Proximate composition

Table 3.9 summarizes the proximate composition (moisture, crude protein, total lipids, ash and carbohydrates) of *Artemia* biomass samples representing each ration used in the field trials as well as naturally occurring individuals collected in nearby *salinas*.

3.5.2 Amino acid analysis

Results for total and free amino acids for cultured and wild *Artemia* are presented in Table 3.10. Results are reported as g of amino acid per 100 g of protein in the sample, and as mg per 100 g of sample for the free amino acids.

3.5.3 Fatty acid analysis

Fatty acid methyl esters are shown in Table 3.11 for the cultured and wild *Artemia*, as well as the feed used (i.e., malt bran). Results are presented in percentage area of total fatty acids and as mg per g of dry weight of sample.

Table 3.9 - Proximate composition of cultured and wild *Artemia*.

Artemia Sample	Ration	Moisture (%)	% dry matter			
			CP[a]	EE[b]	Ash	CF+NFE[c]
wild	-	90.40	57.32	4.39	26.88	11.41
cultured	1	88.7	54.04	3.79	22.36	19.81
cultured	2	87.9	52.67	3.44	27.12	16.77
cultured	control	88.5	56.28	3.92	25.61	14.19

[a] Crude protein.

[b] Ether extract (total lipids).

[c] Crude fiber and Nitrogen-free extract (carbohydrates).

Table 3.10 - Total and free amino acids from wild and cultured *Artemia* biomass.

Amino acid	Total AA (g· 100 g protein^{-1})		Free AA (mg· 100 g sample^{-1})	
	cultured	wild	cultured	wild
Alanine	6.61	6.96	636.92	564.03
Arginine	7.32	7.15	572.30	344.53
Aspartic acid[a]	10.42	11.15	332.09	57.90
Cysteine[b]	1.51	1.71	121.58	83.07
Glutamic acid[c]	9.99	10.86	367.83	222.00
Glycine	5.45	6.36	260.21	263.54
Histidine	2.17	2.32	95.98	92.42
Isoleucine	5.18	5.56	212.36	174.71
Leucine	8.26	8.67	355.64	291.19
Lysine	7.39	7.88	428.93	230.56
Methionine[d]	1.81	2.17	132.50	173.54
Phenylalanine	5.04	5.28	213.44	191.88
Proline	5.32	5.30	559.70	280.94
Serine	5.48	6.33	227.59	137.41
Threonine	4.28	4.69	139.25	127.32
Tryptophan	1.27	1.32	46.82	43.25
Tyrosine	4.21	5.18	221.75	243.06
Valine	6.11	6.52	284.35	207.47
Total	97.83	105.39	5,209.23	3,728.82

[a] Aspartic acid + asparagine

[b] Cysteic acid + cystine

[c] Glutamic acid + glutamine

[d] Methionine + methionine sulfone

Table 3.11 - Fatty acid methyl esters in the total lipids extracted from *Artemia* (cultured and wild) and from the feed used in the field trials.

Fatty acid	*Artemia* source					
	cultured		wild		malt bran	
	%[a]	mg· g^{-1}[b]	%[a]	mg· g^{-1}[b]	%[a]	mg· g^{-1}[b]
14:0	2.98	1.19	2.77	1.05	8.69	2.11
16:0	23.78	9.51	9.21	3.48	28.08	6.82
16:1	5.87	2.35	8.69	3.29	ND	ND
18:0	6.72	2.69	8.55	3.23	0.57	0.14
18:1	21.89	8.76	27.47	10.39	4.95	1.20
18:2n-6	15.95	6.38	7.73	2.92	30.40	7.38
18:3n-6	0.46	0.18	2.92	1.10	ND	ND
18:3n-3	6.42	2.57	9.88	3.74	10.05	2.44
18:4n-3	0.46	0.18	0.53	0.20	ND	ND
20:0	0.15	0.06	0.18	0.07	0.86	0.21
20:1	0.59	0.23	0.60	0.23	0.70	0.17
20:4n-6	1.20	0.48	4.24	1.60	ND	ND
20:3n-3	0.67	0.27	0.34	0.13	ND	ND
20:5n-3	2.51	1.01	2.35	0.89	ND	ND
22:6n-3	0.42	0.17	ND[e]	ND	ND	ND
ID Peaks (%)[c]	90.07	-	85.76	-	85.37	-
n-3 HUFA[d]	3.60	1.45	2.69	1.02	0	0

[a] Percentage of total fatty acids.
[b] Amount of fatty acid per gram of dried sample.
[c] Percentage of identified fatty acids (peaks) in chromatograph.
[d] Total n-3 Highly Unsaturated Fatty Acids.
[e] Not detected

4.0 DISCUSSION

4.1 Feeding tables and rations

The preliminary laboratory trials were mainly designed to evaluate the proposed feeding schedules and the rations that would be used later in the feedstuff evaluation for both laboratory and field trials. Feeding tables were designed so that they would produce significantly different results for the rations tested, mainly in terms of growth, allowing a comparison between rations and feed types used. Final lengths of cultured individuals were also desired to be below the maximum length obtained when fed to satiation, since the rations were expected to be supplemental. The influence of naturally available foods in the semi-intensive culture system could result in overfeeding, thus masking the comparison of rations in the field.

The results obtained in the preliminary laboratory trials and in the feedstuff evaluation trials supported the utilization of the proposed feeding tables, since the six rations tested in the former, and the four rations used in the latter for each feed type, presented significantly different values for growth. Final lengths obtained in the preliminary trials for the best ration (8.28 mm) and in the feedstuff evaluation trial (7.21 mm) for the best performing container fed malt bran under the highest ration, were satisfactorily below the maximum length expected for 2-week old *Artemia* fed to satiation under similar conditions (Sorgeloos *et al.* 1986).

Some culture conditions (mainly density, salinity and temperature) and the quality and quantity of the food offered, are reported to be the major factors

influencing the feeding behavior of *Artemia*, affecting its filtration rate, ingestion rate and food assimilation (Dhont and Lavens 1996). Although the culture conditions experienced in the field trials were fairly different from the ones produced in the laboratory, the feeding tables could be successfully extrapolated. Coutteau and Sorgeloos (1989) have demonstrated that light intensity had no effect on *Artemia* ingestion rates, and other parameters such as mechanical disturbance, and ammonia and nitrite concentrations, only had an influence in feeding when measured at extreme values. Temperature and salinity were the parameters that showed the highest variations between laboratory and field trials (22.1-24.6 °C to 25.7-35.0 °C, and 34-36 ppt to 79-105 ppt, respectively), but these ranges were not far from the optimal (90% survival) combinations of temperature and salinity for *A. franciscana* from the Brazilian "Macau" population (i.e., 18-28 °C, and 25-120 ppt, respectively) established by Vanhaecke *et al.* (1984).

Feed type had a marked impact on growth and survival as observed in the feedstuff evaluation laboratory trials. The quantity of food offered to the *Artemia* showed a pronounced effect in the final biomass produced in the field trials.

Control treatments, which received no feeding during the 14-day trials, still showed some growth and survival. This could be explained by the presence of bacteria which can be taken up by *Artemia* as observed by Douillet (1987). We did not attempt to measure bacterial levels in the culture vessels, since they were considered naturally available foods for all vessels and thus not affecting the comparison between feed types.

4.2 Feedstuff selection and evaluation

Brans and other agricultural wastes have been emplyed as cheap alternatives for *Artemia* culture, but mainly tested in laboratory scale trials (Sorgeloos *et al.* 1980; Basil *et al.* 1987; Lavens *et al.* 1987; Vieira 1987). Rice bran is widely available in SE Asian countries and has recently been used effectively as supplemental feeds for *Artemia* ponds in Vietnam (Sorgeloos, pers. comm.). The northern portion of northeastern Brazil (Fig. 2.2 b), where research for suitable feedstuffs was conducted, is an arid region, plagued by periodic droughts and consequently with a limited production of agricultural crops. Rice, corn, wheat and soybean are brought from other regions of the country and are generally processed (polished, milled) where they originate. Thus, the amount of agricultural wastes and by-products from grains produced locally is insignificant, and those available in wholesalers are cost-prohibitive for *Artemia* culture.

Macroalgae (*Ulva* sp., *Gracilaria* sp.) have been successfully tested as feeds for *Artemia* under laboratory conditions (Klein 1989). The brown alga *Gracilaria* sp. is abundant in NE Brazil. Recently, pharmaceutical companies have been purchasing sun-dried algae from the local harvesters to extract agar, thus raising the price of this resource and causing some environmental problems in reef communities due to overharvesting (Monteiro-Neto, pers. comm.). Vieira (1987) reported better growth, survival and food conversion efficiency for *Artemia*, from a Portuguese strain, when fed the green macroalga *Ulva* sp. as compared with wheat bran. In the observations made during the feedstuff

evaluations, *Gracilaria* sp. was very poorly digested by the *Artemia*. Differences between European and American strains of *Artemia* might also account for large variations in digestibility and nutritional requirements (D'Agostino and Provasoli 1968; D'Agostino 1980).

The three brewery residues (i.e., malt bran, yeast, spent grain), have a year-round availability in large quantities at a very low cost and good apparent digestibility.

Yeasts have also been used as a substitute to microalgal diets in *Artemia* culture. Torula yeast (*Candida utilis*) is considered a promising alternative (Blanco-Rubio 1987), but some trials with marine yeasts (Johnson 1980) and dried baker's or brewer's yeast (*Saccharomyces cerevisiae*) (Coutteau *et al.* 1990) have led to poor results on growth and survival of *Artemia*. Coutteau *et al.* (1992) have argued that enzymatic and chemical treatment could significantly improve *Artemia* cultures fed with yeasts, removing or permeabilizing yeast cell walls and, thus increasing their digestibility to the *Artemia*. This treatment was not performed with the yeasts used in this study and might explain why it did not perform as well as the malt bran did in the feeding trials, despite its higher protein content.

When processing the feedstuffs, the malt bran presented by far the highest yield (26.28% against 2.46% for the spent grain and 6.88% for the yeast) which was mainly a consequence of its lower moisture content and small particle size. During processing, drying was the most energy-consuming operation, and reducing samples to the desired particle size (<50-60 µm) was definitely the most

laborious step. Yeast cells were within the desired size range for *Artemia* ingestion. Malt bran, as a very fine powder, presented a high percentage of small particles, and reduced losses at sieving. Spent grain, on the other hand, had to be thoroughly crushed and homogenized in order to achieve the desired size ranges.

Results of the feedstuff evaluation feeding trials indicated that the best performing feed type was the malt bran, in terms of both growth and survival, followed by combinations of malt+grain, and malt+yeast. These results support the suitability of brans for *Artemia* culture as discussed by Sorgeloos *et al.* (1980) and Lavens *et al.* (1987). Yeasts might have shown a better performance if they were chemically treated, as described by Coutteau *et al.* (1992). However, the cost involved would certainly be prohibitive for a semi-intensive culture system, and thus not fitting the scope of this work.

The rations utilized in the feeding trials showed an increasing performance, in terms of brine shrimp size, as the ration increased. Mean final size was about half of that obtained for the *Spirulina* in the preliminary trials, which clearly demonstrates the differences in nutritional quality between microalgae and other foods. Survival was higher in ration 2 (intermediate ration) as compared to ration 3 (highest ration), probably due to water quality related problems caused by the excess of feeds in the small containers.

4.3 Field trials

4.3.1 Pond building and preparation

Little information about *Artemia* pond building and preparation is available apart from the two works produced by the *Artemia* Reference Center, Belgium (Tackaert and Sorgeloos 1991b; Baert *et al.* 1996). We basically followed their recommendations concerning pond design (depth, shape) and fertilization procedures. Some modifications were made as suggested by the local saltfarmers, and considering the low-technology orientation of this system. Covering newly built pond walls with palm tree leaves was one such modifications. It is a common practice in that area, where strong winds can cause serious damage to the dikes by wave erosion, and has proven to be essential to ensure wall integrity. When using coconut leaves, however, the sun-dried leaves have to be soaked in saltwater for a few days in order to release a reddish pigment they contain, otherwise ponds would have to be flushed several times.

Pond design seemed to be efficient enough to reduce temperature variations, as compared with the high variations experienced in the shallow reservoir pond (see Fig. 3.7). Maximum temperatures in the *Artemia* ponds, however, were higher than expected and perhaps pond depth in this region should be higher than those recommended by Baert *et al.* (1996).

Fertilization with calcium nitrate produced a fast response and a steady algal bloom. However, this procedure increased pH values in the reservoir (fertilized) pond up to 9.3 (see Fig. 3.8) and water management had to be more carefully done to avoid high variations in the culture ponds. This observations agrees with Baert *et al.* (1996) who noted that despite the fast action of nitrate, because it is directly available for the algae, this type of fertilizer can cause

increases in pH. It also supports their view that fertilization should not be performed directly in the culture ponds.

4.3.2 Predator control

Salinity was the best barrier against predators. Although screening helped to keep the larger predators away (e.g., *Mugil* sp., *Elops saurus*), small fish and copepods were still found in the reservoir pond, and had to be eradicated before the experiment could start. Adult copepods averaging 860 µm in length, were considered a potential threat to the newly hatched *Artemia* nauplii (about 400 µm) (Tackaert and Sorgeloos 1991b), and could easily pass through the 1000-µm mesh. Copepods could be found in dense 'clouds' in some areas of the fertilized pond until a salinity of approximately 85 ppt was achieved. Two smaller stainless steel meshes (500 and 250 µm) were tested at the inlet hose of the pump, but constant clogging made it impracticable to work even with the 500-µm mesh. A filter box with a series of meshes might alleviate the clogging problem, but since salinity was found to be so efficient in killing the copepods, this option was not explored.

Small livebearer fish species are reported to be a problem in *Artemia* producing saltfarms (e.g., *Cyprinodon variegatus* in Cuba, *Aphanius fasciatus* in SE Asia; Sorgeloos, pers, comm.). *Poecilia vivipara*, the species that was found preying on *Artemia* in the salinas around Grossos, and was abundantly present in the supply canal, is very similar in appearance to those mentioned above. It is a freshwater species with a very high resistance to salinity. They were probably

carried to the reservoir by the waterfowl that abound in the region, since even the fry would have difficulty in passing through the 1000-µm mesh and the pump rotor without some damage. They were observed, though very stressed, in salinities up to 80 ppt. Baert *et al.* (1996) recommend a lower limit of 70 ppt to exclude predators. We found that for the northeastern Brazilian region, 90 ppt was a safer lower limit to inoculate *Artemia* ponds.

4.3.3 Feed performance

Feed performance was evaluated as growth, survival, feed conversion ratio, and biomass output at harvest.

Growth results were very similar to those encountered by Quynh and Lam (1987) in Vietnam for 14-day old *Artemia* cultured in converted saltpans fertilized with chicken manure. For the same strain of brine shrimp, these authors obtained a final mean length of 7.06±0.67 mm, which is lower than the results obtained with rations 1 and 2 (7.29±1.01, and 7.91±0.98 mm, respectively), but higher than the mean length measured for the control ponds (6.06±1.05 mm). Their trials, however, apart from different fertilization procedures, were conducted under much lower densities (20 ind· L^{-1} as opposed to 200 ind· L^{-1} used in Brazil). This might be a reason why our control groups showed an inferior performance, since culture densities are known to greatly influence the growth of *Artemia* (Jumalon and Robles 1983; Dhert *et al.* 1992). The differences in mean size between the control group and the feeding treatments (rations 1 and 2) demonstrated, however, that the feed had a positive influence on growth.

Survival was relatively low, ranging from 16.04%, in the controls, to 22.19% in the ponds under ration 2. Jumalon *et al.* (1987) have suggested that 50 individuals per liter was the best stocking density for fertilized saltponds. We had expected to maintain a higher standing stock, at least in the feeding ponds, but it seems that some other parameters related to carrying capacity might have been limiting to the brine shrimp population. High temperatures also might have been responsible for a low survival, as will be discussed in section 4.3.4. Final densities ranged from 27.25 ind· L^{-1}, in one control pond, to 59.48 ind· L^{-1} in pond A2, under ration 1. These results seem to agree with the densities suggested above.

Food conversion ratios and efficiencies were calculated taking into account only the biomass increment supposedly caused by the supplemental feeding, i.e., subtracting the biomass values obtained in the control ponds (Table 3.7). The efficiencies obtained were 39.16 and 32.17% for rations 1 and 2, respectively. These values are similar to those achieved by Ronsivalli and Simpson (1987) when feeding brine shrimp for 15 days in raceway tanks with rice bran (40.5), but are nearly twice as efficient as when whey powder was used in the same study (20.5). It seems that malt bran may be efficiently converted into biomass by *Artemia*, similar to the traditionally used rice bran and more efficiently than whey powder.

The biomass harvested at the end of the trials, a combined result of survival and the final individual wet weight, illustrates the differences among

treatments. Average biomass output for ration 1 (970 g) was more than double that of the control groups (413 g) , while ration 2 produced three times more mass (1320 g) than the unfed group.

Biomass yield in the controls averaged 306.17 kg· ha^{-1}· $month^{-1}$. Tackaert and Sorgeloos (1991b) surveyed *Artemia* producing saltfarms in Thailand and found that the most successful operations produced approximately 100 to 375 kg· ha^{-1}· $month^{-1}$. Camara and Rocha (1987) obtained yields of 400-500 kg· ha^{-1}· $month^{-1}$ of *Artemia* biomass in saltponds in NE Brazil, using chicken manure, urea and triple-superphosphate as fertilizers. Jumalon *et al.* (1987) reported a daily harvest of 15-30 kg of brine shrimp per hectare, in a heavily fertilized continuous system (as opposed to the batch system used in our trials) in the Philippines, equivalent to monthly yields of 450-900 kg· ha^{-1}. Our results with feeding rations 1 and 2 shows yields of 718.52, and 977.78 kg· ha^{-1}· $month^{-1}$, respectively. The high yields obtained by Jumalon *et al.* (1987) suggest that heavy fertilization can produce similar results as feeding. The decision in the choice of strategy should therefore be based on prices and availability of fertilizers or suitable residues that can be used as *Artemia* feeds, for every particular region. However, when compared to the average results presented in the literature for fertilized ponds (around 450 kg· ha^{-1}) the yields obtained with supplemental feeding seem to encourage this practice.

4.3.4 Water quality and environmental parameters

During our experiment, temperatures reached a peak of 35 °C at noon in all treatments. Although below the lethal water temperatures reported by Jumalon *et al.* (1987) for *Artemia* cultured in tropical saltfarms (40-42 °C), it may have had an effect on survival. When determining the optimal combinations of temperature and salinity for various *Artemia* strains from different geographical regions, Vanhaecke *et al.* (1984) established a range of 18 to 28 °C for the Macau population as a 90% survival interval, when salinities were between 25 and 120 ppt. They also determined, for the same salinity range, that above a maximum temperature of 31 °C, about 50% mortality would occur.

We experienced higher mortalities for all our treatments. Although a deficient diet may affect environmental stress resistance of the cultured individuals, we believe that diet did not play an important role in survival. Thus the animals cultured under the highest ration showed a good weight gain, but mortalities were equally high in the feeding ponds and the controls. Salinity and pH were kept under optimal ranges. Dissolved oxygen never reached lethal levels for *Artemia*, but the influence of the pronounced daily cycles caused by algal respiration on growth and survival are unknown. For these reasons, we consider temperature as being the single most important environmental factor influencing the survival of the *Artemia* population.

Turbidity was the only measured parameter that showed significant differences between treatments (Fig. 3.9). Lower transparencies experienced in the feeding treatments were obviously caused by the addition of malt bran to the ponds, and these directly correlated with growth, weight gain and biomass

production.

4.3.5 *Artemia* analyses

Proximate composition of adult *Artemia* biomass showed a distinction between two groups: the wild *Artemia* and the ones reared without supplemental feeding on one side, and the samples from the feeding treatments (rations 1 and 2) on the other. Wild brine shrimp and the samples from the control ponds had higher values for crude protein and total lipids, and lower carbohydrate levels. Ronsivalli and Simpson (1987) pointed out that the developmental stage of *Artemia* is more influential in determining its protein and carbohydrate content than dietary factors; thus suggesting a drop in protein content and an increase in carbohydrate levels, with increasing development of the brine shrimp.

The control groups had a lower mean body weight and size, suggesting that a greater portion of its population was not yet fully developed. Unfortunately, no data on size and individual weight was collected from the wild caught *Artemia* to estimate their population development patterns and, thus, to support this hypothesis.

Results for crude protein, ash and carbohydrates were within the ranges compiled by Léger *et al.* (1986) from 15 references. Total lipid values were, however, below the expected range. One hypothesis for the possible low values is the stress suffered by the animals from the time of harvest until processing and freezing. We previously noticed that samples of wild *Artemia* that were frozen immediately after harvest had a higher lipid content than biomass from the same

pond that was transported live, for a period of about eight hours, to the laboratory to be freeze-dried (unpublished data). Harvesting the ponds was a strenuous, time consuming operation, and the lapse between harvesting and processing the samples, usually around six hours in most of the cases, during which the animals were kept alive, might have contributed to the depletion in energy reserves due to this stressful situation.

Amino acids, as opposed to fatty acids, seem to be fairly similar from strain to strain, 'suggesting that it is not environmentally determined in the manner that fatty acids are' (Merchie 1996). A comparison between our results for the profile of total amino acids in cultured and wild brine shrimp, and other *Artemia* strains from different geographical origins (Seidel *et al.* 1980; Trotta et al. 1987) is shown in Table 4.1, and demontrates a high degree of similaritiy among different strains regardless of environment (i.e., inland lakes, tropical estuaries, temperate salterns) or diet.

The content of highly unsaturated fatty acids (HUFA) is probably the most important single factor determining the nutritional value of *Artemia* to marine organisms (Léger *et al.* 1986; Bengston *et al.* 1991). In particular, the levels of eicosapentaenoic acid (EPA, 20:5n-3) and the docosahexaenoic acid (DHA, 22:6n-3) are known to be essential to marine organisms (Kanazawa et al. 1979; Watanabe 1988). Several trials with marine and freshwater fish and shrimp larvae have shown a large variation in survival when fed different strains of *Artemia* (Léger *et al.* 1987). As opposed to freshwater species, most marine species do not have the ability to elongate

Table 4.1 - Amino acid profile for *Artemia* from various geographical locations (g/100 g protein).

Amino acid	*Artemia* source						
	cultured[a]	wild[a]	Australia[b]	Brazil[b]	San Pablo Bay[b]	Utah[b]	Italy[b]
Alanine	6.61	6.96	5.4	4.6	4.2	4.9	4.9
Arginine	7.32	7.15	10.9	11.5	9.8	9.7	9.8
Aspartic acid	10.42	11.15	10.8	11.0	14.1	11.3	11.2
Cysteine	1.51	1.71	ND[c]	ND	ND	ND	ND
Glutamic acid	9.99	10.86	16.3	13.1	10.2	13.5	14.5
Glycine	5.45	6.36	5.7	6.0	7.4	6.0	7.2
Histidine	2.17	2.32	3.8	4.9	3.5	2.7	3.8
Isoleucine	5.18	5.56	4.9	5.6	5.4	6.8	6.4
Leucine	8.26	8.67	7.9	8.9	8.4	10.0	10.1
Lysine	7.39	7.88	10.6	11.7	8.7	9.3	10.7
Methionine	1.81	2.17	2.8	2.2	2.6	3.7	3.7
Phenylalanine	5.04	5.28	7.7	5.1	10.4	8.5	8.5
Proline	5.32	5.30	5.4	5.7	4.9	5.9	5.9
Serine	5.48	6.33	5.9	4.5	7.7	5.4	5.1
Threonine	4.28	4.69	5.5	5.2	6.0	4.8	5.5
Tryptophan	1.27	1.32	ND	ND	ND	ND	ND
Tyrosine	4.21	5.18	7.3	10.5	7.7	6.6	5.4
Valine	6.11	6.52	5.4	5.3	5.5	5.2	3.1

[a] Results obtained in this study.

[b] From Seidel *et al.* (1980).

[c] Not determined.

these essential fatty acids (i.e., EPA, DHA) from lower unsaturated fatty acids (e.g., 18:3n-3), and *Artemia* strains have been classified into a 'marine type', containing high levels of 20:5n-3, and a freshwater type, with low levels of this essential fatty acid (Watanabe *et al.* 1978). Since it has been demonstrated that these fatty acids can be incorporated into *Artemia* when present in sufficient amounts in the diet (Watanabe *et al.* 1982, Léger *et al.* 1985), several enrichment techniques with emulsions have been developed to increase HUFA levels in *Artemia* (Bengston *et al.* 1991).

The Brazilian strain, together with the San Francisco Bay (USA) and Bohai Bay (China) strains have traditionally been known as a 'marine type' *Artemia*, containing levels of EPA higher than 4% of the total fatty acid methyl esters (Léger *et al.* 1986). However, the same authors state that, since the fatty acid profile is directly related to the *Artemia* diet, great variations can be expected. Large fluctuations in EPA content during a year or between years have been reported for the three populations mentioned above (Watanabe *et al.* 1980, 1982), and are as shown in Table 4.2. It should be noted that the strains representing the highest variation in EPA content come from saltworks supplied by estuarine waters (i.e., San Francisco, Macau, China), while inland lakes (i.e., Chaplin Lake, SK, Canada, Great Salt Lake, UT, USA), although displaying lower absolute levels of EPA, show a lesser degree of variation, since these environments are believed to be more stable in terms of water dynamics and algal composition (Lai 1991).

Table 4.2 - Range and intra-strain variability of eicosapentaenoic acid (EPA) in *Artemia* from various geographical origins[a].

Artemia source	EPA range (area %)	Coefficient of variation (%)
San Francisco Bay, CA, USA	0.3 - 13.3	78.6
Great Salt Lake (South arm), UT, USA	2.7 - 3.6	11.8
Great Salt Lake (North arm), UT, USA	0.3 - 0.4	21.2
Chaplin Lake, SK, Canada	5.2 - 9.5	18.3
Macau, RN, Brazil	3.5 - 10.6	43.2
Bohai Bay, PR China	1.3 - 15.4	50.5

[a] as compiled by Léger *et al.* (1986), *in* Merchie (1996).

Our results for EPA content in lipids of both the wild and cultured *Artemia* (2.35 and 2.51%, respectively) are below the range reported for this strain (see Table 4.2). Wild *Artemia* were also expected to have higher levels than the cultured ones, since it was fed exclusively on natural foods which are supposed to present higher in HUFA levels. These low levels, coupled with the absence of DHA, leads us to hypothesise that the wild *Artemia* collected for analysis were underfed, or fed exclusively on natural biota poor in n-3 HUFA.

Low levels of EPA in the cultured individuals can be explained by the absence of EPA in the diet (malt bran). Vos *et al.* (1984), when studying the nutritional quality of *Artemia* produced in fertilized saltpans, found that individuals produced in ponds fertilized with inorganic fertilizer had lower levels of EPA than those produced with organic fertilizers such as poultry manure. In this regard, Jumalon and Ogburn (1987) and Jumalon *et al.* (1987) noticed that ponds fertilized with manure showed consistent blooms of algae rich in EPA (e.g., *Tetraselmis* sp.).

DHA is also recognized as being important to marine species (Merchie 1996), but it is generally absent or present in trace amounts in *Artemia* (Navarro and Amat 1992). The Brazilian strain has been reported to score the highest percentage of DHA observed in *Artemia* (Watanabe 1987). The presence of DHA in our cultured samples seems to agree that algal composition of NE Brazilian estuaries may contain DHA-rich species, since no traces of DHA were present in the feed used.

Arachidonic acid (20:4n-6) is virtually absent in samples from inland lakes, suggesting an association of this fatty acid with coastal habitats (Navarro and Amat 1992). Watanabe (1987,1988) also referred to arachidonic acid as being related to the 'marine type', and the relatively high levels of 20:4n-6 in our samples (more than 4% in the wild *Artemia*) seem to corroborate that opinion.

The two major fatty acids found in the artificial feeds used in this experiment, namely 16:0 (28.08%) and 18:2n-6 (30.40%), were reflected in the fatty acid profile of the cultured *Artemia*. As seen in Table 3.11, these fatty acids were present in high amounts in the samples from cultured individuals, representing values more than twofold higher than those of naturally occurring *Artemia*, suggesting that the fatty acid profile of brine shrimp indeed reflects that of its diet.

5.0 CONCLUSIONS AND RECOMMENDATIONS

Several conclusions could be made based on the results presented in this thesis, as summarized below:

1) Supplemental feeding of *Artemia* in semi-intensive culture ponds was very effective in increasing the biomass production, as compared to fertilized control ponds. Monthly yields in our fertilized control ponds averaged 306.17 kg· ha^{-1}, which is within the range reported in the literature for fertilized *Artemia* ponds, while our feeding ponds averaged up to 977.78 kg· ha^{-1}.

2) Increasing supplemental feeding levels (rations), although not showing marked differences in size and survival, had a direct effect on the weight and consequently in biomass output of *Artemia*.

3) Nutritionally poor by-products and residues could be used effectively as an inexpensive alternative for *Artemia* supplemental feeding.

4) Particle size, moisture content, cost and digestibility were more important in feedstuff selection than nutritional quality. Micronizing samples to the desired size was the most labor-consuming step during processing; drying to allow storage was the highest energy-consuming, and these should be carefully taken into consideration when choosing particular feedstuffs.

5) Malt bran, a by-product of breweries, was efficiently converted into biomass by the *Artemia* (FCE = 39.16%), showing similar results as rice bran, which has been shown to be a suitable feed, and is successfully being used to improve *Artemia* culture in SE Asia.

6) The protein content and especially the amino acid profile in *Artemia* did not seem to be greatly influenced by the diet. Fatty acid profile, on the other hand, was directly related to the fatty acid profile of the diet.

7) The livebearer fish *Poecilia vivipara* was the main predator threatening *Artemia* cultures in evaporation ponds in NE Brazil.

8) Salinity was the cheapest and most effective predator control method for *Artemia* culture in former *salinas*. A salinity level of 90 ppt was a safe limit avoid predation by fish and copepods.

9) Temperature seemed to be the most influential environmental parameter affecting *Artemia* survival, since the other parameters did not reach lethal levels. I believe that measures to reduce pondwater temperatures (e.g., deeper ponds, shading provided by floating devices or palmtree leaves) could reduce mortalities.

10) Initial stocking densities of 200 ind· L^{-1} were apparently too high for this type of environment, even when supplemental feeding was applied. While the main factors governing the carrying capacity in semi-intensive hypersaline *Artemia* ponds are not fully understood, I suggest initial densities of 50-100 ind· L^{-1} for small ponds (< 0.5 ha) and below 50 ind· L^{-1} for larger ponds.

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7.0 PERSONAL COMMUNICATIONS

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8.0 APPENDICES

Appendix A: Formula for organic nitrogen content calculations (Goddard 1992).

$$\% N = \frac{(\text{Vol. of titrant for sample} - \text{Vol. of titrant for blank}) \times \text{Norm. of acid} \times 14.0067 \times 100}{\text{Weight of sample in mg}}$$

Appendix B: Crude fat calculations (Goddard 1992).

$$\% FAT = \frac{W_3 - W_2}{W_1} \times 100$$

where:

W_1 = weight of sample (g)

W_2 = weight of extraction cups with boiling chips (g)

W_3 = weight of cups + dried extracted samples (g)

Appendix C: One-way analysis of variance (ANOVA) for *Artemia* size among the six rations used in the preliminary laboratory trials.

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between groups	7.79E+08	5	1.56E+08	423.413	0.001
Within groups	6.11E+07	166	367884.1		
Total	8.40E+08	171			

Appendix D: One-way analysis of variance (ANOVA) and Scheffe's homogeneous subsets for *Artemia* survival among the six rations used in the preliminary laboratory trials. Percent survival values were transformed into the arcsine of their square roots to reduce deviations from normality.

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between groups	7.697	5	1.539	130.573	0.001
Within groups	2.052	174	1.179E-02		
Total	9.749	179			

	Subset for $\alpha = 0.05$ (arcsine transformations of % survival)			
Ration	1	2	3	4
control	0.1421			
1		0.4386		
2			0.6336	
3			0.6675	0.6675
4			0.6721	0.6721
5				0.7552
Sig.	1.000	1.000	0.864	0.088

Appendix E: Two-way analysis of variance (ANOVA) for the size of *Artemia* reared in laboratory feeding trials with six types of feeds under four different rations.

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Main effects	1.18E+09	8	1.47E+08	1199.689	0.001
Feed	2.18E+08	5	4.37E+07	355.563	0.001
Ration	9.62E+08	3	3.20E+08	2606.565	0.001
Two-way interactions					
Feed - Ration	1.37E+08	15	9.12E+06	74.108	0.001
Explained	1.32E+09	23	5.73E+07	465.614	0.001
Residual	8.56E+07	696	1.23E+05		
Total	1.40E+09	719	1.95E+06		

Appendix F: Scheffe's homogeneous subsets for the size of *Artemia* reared in laboratory feeding trials with six types of feeds under four different rations.

Ration	Subset for $\alpha = 0.05$ (size in mm)			
	1	2	3	4
control	1.9387			
1		2.5605		
2			3.4732	
3				5.0111
Sig.	1.000	1.000	1.000	1.000

Feed type	Subset for $\alpha = 0.05$ (size in mm)				
	1	2	3	4	5
spent grain	2.6203				
brewer's yeast		2.8099			
grain + yeast		2.8706			
malt + yeast			3.3611		
malt + grain				3.5795	
malt bran					4.2340
Sig.	1.000	0.876	0.608	1.000	1.000

Appendix G: Two-way analysis of variance (ANOVA) for the survival of *Artemia* reared in laboratory feeding trials with six types of feeds under four different rations. Percent survival values were transformed to the arcsine of their square roots to reduce deviations from normality.

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Main effects	19.565	8	2.446	354.584	0.001
Feed	5.541	5	1.108	160.673	0.001
Ration	14.024	3	4.675	677.769	0.001
Two-way interactions					
Feed - Ration	4.980	15	0.332	48.137	0.001
Explained	24.546	23	1.067	154.727	0.001
Residual	4.801	696	6.897E-03		
Total	29.346	719	4.082E-02		

Appendix H: Scheffe's homogeneous subsets for the survival of *Artemia* reared in laboratory feeding trials with six types of feeds under four different rations. Percent survival values were transformed into the arcsine of their square roots to reduce deviations from normality.

Ration	Subset for $\alpha = 0.05$ (arcsine transformations of % survival)			
	1	2	3	4
control	0.2618			
1		0.4021		
3			0.4487	
2				0.6513
Sig.	1.000	1.000	1.000	1.000

Feed type	Subset for $\alpha = 0.05$ (arcsine transformations of % survival)			
	1	2	3	4
grain + yeast	0.3007			
spent grain		0.3922		
malt + grain			0.4339	
malt + yeast			0.4629	
brewer's yeast			0.4631	
malt bran				0.5930
Sig.	1.000	0.876	0.608	1.000

Appendix I: One-way analysis of variance (ANOVA) for *Artemia* size among the two rations and control used in the field trials.

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between groups	171.107	2	85.553	83.524	0.001
Within groups	282.707	276	1.024		
Total	453.814	278			

Appendix J: One-way analysis of variance (ANOVA) and Scheffe's homogeneous subsets for *Artemia* survival among the three rations used in the field trials. Percent survival values were transformed into the arcsine of their square roots to reduce deviations from normality.

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between groups	0.301	2	0.150	52.673	0.001
Within groups	0.788	276	2.855E-03		
Total	1.089	278			

Ration	Subset for $\alpha = 0.05$ (arcsine transformations of % survival)		
	1	2	3
control	0.4115		
1		0.4679	
2			0.4893
Sig.	1.000	1.000	1.000

Appendix K: One-way analysis of variance (ANOVA) for water quality and environmental parameters (except turbidity) observed among treatments (control, rations 1 and 2) during the 14-day field trials.

Parameter	Source of variance	Sum of Squares	df	Mean Square	F	Sig.
Temperature	Between groups	2.66E-02	2	1.33E-02	0.002	0.998
	Within groups	1291.326	186	6.943		
	Total	1291.352	188			
Salinity	Between groups	104.000	2	52.000	1.347	0.268
	Within groups	2315.429	60	38.590		
	Total	2419.429	62			
pH	Between groups	3.68E-03	2	1.84E-03	0.092	0.912
	Within groups	1.203	60	2.01E-02		
	Total	1.207	62			
DO (% sat.)	Between groups	0.966	2	0.483	1.271	0.283
	Within groups	70.647	186	0.380		
	Total	71.613	188			
DO (mg· L^{-1})	Between groups	404.952	2	202.476	1.300	0.275
	Within groups	28972.000	186	155.763		
	Total	29376.952	188			
Depth	Between groups	1.556	2	0.778	0.138	0.872
	Within groups	338.762	60	5.646		
	Total	340.317	62			

Appendix L: One-way analysis of variance (ANOVA) and Scheffe's homogeneous subsets for the mean turbidity observed among treatments (control, rations 1 and 2) during the 14-day field trials.

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between groups	192.508	2	96.254	6.12	0.004
Within groups	943.429	60	15.724		
Total	1135.937	62			

Ration	Subset for $\alpha = 0.05$ (turbidity in cm)	
	1	2
1	30.1905	
2	31.5238	31.5238
control		34.3810
Sig.	0.556	0.074