A NUTRITIONAL EVALUATION OF EFFLUENT GROWN ALGAE AND ZOOPLANKTON AS FEED INGREDIENTS FOR *Xiphophorous helleri*, *Poecilia reticulata* AND *Poecilia velifera*(PISCES : POECILIIDAE)

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By: Warren Mason Potts
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ABSTRACT

The aim of this study was to evaluate the nutritional value of algae and zooplankton grown in an algal integrated ponding system for poeciliids. The available information on the nutritional requirements of poeciliids was compared with a proximate analysis of the algae and zooplankton. The effluent grown algae and zooplankton had a crude protein value of 41.47 ± 0.2% and 36.84 ± 0.3%, a lipid content of 4.8 ±. 0.4% and 11.1 ± 0.8% and a carbohydrate content of 35.13 ± 0.8% and 31.06 ± 0.6% respectively. These values compared favourably with those of the known nutritional requirements of poeciliids.

To test if the nutritional value of the algae and zooplankton in the AIPS was constant, the species composition of the algae and the crude protein content of the algae and zooplankton in the high rate oxidation ponds were measured monthly and bi-weekly for a year respectively. The species composition remained fairly stable for the duration of the experiment and the average protein composition of the algae and zooplankton was 43.4 ± 4.4% and 38.6 ±2.8% respectively. This indicated that the algae and zooplankton in the AIPS provided high protein biomass through the year.

The quality of the effluent grown algal and zooplankton biomass was evaluated by analysing their amino acid composition and comparing it with the amino acid composition of the body tissue of *X. helleri.* With respect to the amino acid composition of the body tissue of *X. helleri,* the amino acid profile of the algae had a 69% and the zooplankton a 63% similarity.

The quality of the pure algal and zooplankton protein was also tested biologically, by feeding them directly to poeciliids during a ten week growth

trial. The algae and zooplankton diets did not result in adequate growth compared to poeciliids which were fed a formulated control diet.

The initial growth trial also evaluated the effect of processing on the chemical composition of the algae and its biological effect on fish fed with it. There were no significant differences in the chemical composition of fresh and sun dried algae and this was reflected in the growth rates of the fish which did not differ significantly. Freeze dried algae had a lower crude protein content than fresh and sun dried algae and a poorer amino acid composition. Fish fed freeze dried algae exhibited poorer growth and survival in comparison to the other treatments.

The gut transit times of *X. helleri* and the gastric evacuation times of poeciliids were determined using colour dyes incorporated in the diet and by sacrificing fish at predetermined intervals after feeding respectively. The gut transit time of *X. helleri* fed a sun dried algal diet and a formulated diet was 225 ± 8.55 minutes and 261.93 ± 10.86 minutes respectively. The gastric evacuation times of *X. helleri, P. reticulata* and *P. velifera* were 7, 9 and 8 hours respectively. Since the maximum amount of food in the hindgut after feeding was detemined to be 3, 5 and 3 hours in *X. helleri, P. reticulata* and *P. velifera* respectively, fish were sacrificed at these times in the digestibility study. The digestibility of sun dried algae was determined using 1% chromic oxide as an internal marker in the algae. The apparent crude protein digestibility of sun dried effluent grown algae ranged from 65%-75% in the three poeciliid species.

Based on the results of the chemical and biological analysis of the algae and zooplankton, conventional diets incorporating algae at 5% and 20% protein inclusion levels were formulated. These diets and a treatment in which zooplankton, reared in the AIPS were fed as a dietary supplement to a formulated diet were fed to poeciliids for twelve weeks. No significant differences in the growth of poeciliids fed any of the test diets were observed.

It was concluded that the algae could be incorporated into poeciliid formulated diets up to level of 20% protein inclusion without any negative effect on the growth of the fish. The chemical and biological evaluation of the algae suggested that it had a similar nutritional quality to soyabean meal. Poeciliid growth was not enhanced with a zooplankton supplement, however a possible low feeding rate by poeciliids on the zooplankton as a result of their small size may have affected the result.

The colour enhancement potential of effluent grown algae and zooplankton was evaluated biologically in ten and twelve week growth trials using visual observation (31 people) and by using a chromameter. While pure, sun dried algae significantly enhanced the colour of *P. reticulata,* no significant differences in the colour of poeciliids were observed or recorded when fish were fed diets incorporating sun dried algae up to levels of 20% protein inclusion.

The effect of effluent grown algae and zooplankton on poeciliid health was also tested biologically in the ten and twelve week growth trials. The high mortalities (75%-84%) recorded for *X. helleri* when fed the pure algal and zooplankton diets were considered to be as a result of the nutritional inadequacy of the diets since there were significantly less mortalities in fish fed the formulated control diet and 63% of the fish that died during the course of the trial were emaciated. In addition, fewer mortalities (10%-40%) were recorded in *X. helleri* fed the nutritionally adequate formulated diets in the twelve week growth trial. No symptoms of disease were recorded in the twelve week growth trial and the algae was considered to have no toxicological or pathological effects on poeciliids.

Centrifugation, sand filtration, sedimentation, microstraining and biological harvest were evaluated as methods of small scale algal harvest from the high rate oxidation ponds. Algae could be harvested most efficiently with little

associated labour by sedimentation (without flocculating agents) and by microstraining with 60µm mesh.

Effluent grown algae can replace conventional feed ingredients up to a level of 20% protein inclusion without negatively effecting the growth, colour, health or survival of poeciliids.

CHAPTER 1

INTRODUCTION

Although algae have been successfully mass-cultured, the expense of its production, harvesting and processing has limited its commercial use (Stanley and Jones, 1976). However, if it is harvested as a by-product from integrated wastewater systems, production costs can be dramatically reduced (Rose et al., 1996). The recent construction of a pilot phase algal integrated ponding system (AIPS) at the Grahamstown Disposal Works, provides both low-cost sewage treatment and produces large volumes of algal biomass (Anon, 1997) and zooplankton which feeds on it. This AIPS was constructed as a joint project between the Water Research Commission (WRC), Grahamstown Municipality and Rhodes University and presented an opportunity to use algae and zooplankton as low cost feed ingredients for aquaculture.

Feed cost is considered to be the highest recurrent cost in aquaculture, often ranging from 30% to 60% (De Silva and Anderson, 1995). Fishmeal is the main protein source in compound in aquaculture due to its favourable essential amino acid profile (De Silva and Anderson, 1995). However, the world production potential of fishmeal is limited and its price keeps increasing (Sandbank and Hepher, 1980). Attempts to substitute it with other sources of protein have resulted mostly in poorer growth rates of the fish (Koyama *et al.*, 1961; Hepher *et al.*, 1971, Lovell *et al.*, 1975, Viola, 1975). Microalgae is considered a possible alternative protein source because it is fast growing (Grobbelar, 1979), and has a high protein content 50 - 60% (Tamiya, 1975). Microalgae has also been used as a component in the diets of marine bivalve molluscs (e.g. oysters and mussels), marine gastropods (abalone), larvae of saltwater shrimp (*Penaeus*), some fish species (e.g. *Tilapia,* silvercarp, milkfish) and zooplankton (De Pauw and Persoone, 1988). Thus the microalgae produced as a by-product of the sewage purification system

at the Grahamstown sewage works was considered a possible replacement for conventional protein-rich feed ingredients in fish diets.

Furthermore, the zooplankton which feeds on the algae from the wastewater treatment system was identified as a potential feed source for fish, since organisms such as rotifers (*Brachionus),* copepods (*Tigriopus*), cladocerans (*Daphnia, Moina*) and brine shrimp (*Artemia*) are frequently used as food organisms for rearing many fish species. Zooplankton is considered of superior to formulated feeds for larval fish because it is easily digestible, contains enzymes which promote autolysis and supplies all essential nutrients required by the larval fish (Lavens and Sorgeloos, 1996). In addition, the use of appropriate size zooplankton has enabled the production of fish species with small larvae, that could not be raised before (Dhert *et al.,* 1997).

Although effluent grown algae and zooplankton may be good sources of protein for fish, they pose a potential health risk both to the fish and to consumers of the fish. In addition, the health authorities of many countries are reluctant to approve the use of domestic wastewater or treated human effluents for food fish production (Sandbank and Nupen, 1985). Therefore, the decision was made not to evaluate the algae and zooplankton as potential feeds for food fish, but rather for ornamental fish.

Ornamental fish farming in South Africa offer commercial potential since the world trade in ornamental fish has an estimated value of some US$1.6 billion (Winfree, 1992). Singapore, which is the worlds major exporter of ornamental fish, supplying over 60 countries at a value of $51 million (Britz, 1995) is under pressure due to the declining quality of their product as a result of health problems caused by environmental degradation and poor quality water (Britz, 1995). In South Africa only one commercial ornamental fish operation currently exports fish. In addition, local wholesalers currently import up to 63% of their ornamental fish (Britz, 1995). Dealers reported that the main reason for importing fish was the greater variety of fish offered in international markets and that international dealers were supplying fish more efficiently and regularly than local growers (Britz, 1995). Local aquaculturalists could obtain larger local and

international market shares by decreasing the prices of their fish. By utilising effluent grown algae and zooplankton as feed for ornamental fish, their production of costs could be reduced, which would allow the fish to be marketed at more competitive prices.

An aquaculturalist could also increase his local and international market shares by increasing the quality of the fish. One method of increasing the quality of ornamental fish is by enhancing their colour (Hecht and Britz, 1990). Some species of algae were calculated to have caratenoid levels of up to 4000mg/kg dry product (Tacon, 1990). When fish feed on the algae, these caratenoids could be deposited into their tissues (Tacon, 1990) and this may result in enhanced skin colour. Although very little scientific research has been conducted on colour enhancement of ornamental fish (Noakes, 1992), algae have been found to enhance colour when fed to a variety of fish species (Mori *et al.,* 1987; Henson, 1990; Watanabe, Liao, Takeuchi & Yamamoto, 1990). Zooplankton which feed on algae have also been found to enhance the colour of *X. helleri* (Kruger, 1995). Therefore, an evaluation of the colour enhancement potential of effluent grown algae and zooplankton on ornamental fish was undertaken.

Another aspect contributing to the quality of ornamental fish is their health. Since the algae and zooplankton are cultured in effluent, they may contain substances like pesticides, heavy metals and pathogens (Sandbank and Nupen, 1985). When fish consume the algae, these substances could assimilate into their tissues and affect their health. Therefore, the effects of effluent grown algae and zooplankton on poeciliid health were evaluated.

Poeciliids such as the guppy (*Poecilia reticulata),* the swordtail (*Xiphophorous helleri)* sailfin mollies (*Poecilia velifera)* are traditionally amongst the most popular species in the ornamental fish trade. In South Africa, approximately one quarter of the ornamental fish species produced belong to the family Poeciliidae (Kruger, 1995) and in Singapore, twenty percent of the ornamental fish produced belong to this family (Fernando *et al.,* 1991). These three poecliid species were therefore chosen as subjects for this study.

The objectives of this research were the following:

1) To evaluate the potential of effluent grown algae as a protein source for livebearing ornamental fish

2) To evaluate the potential of effluent grown zooplankton a protein source for livebearing ornamental fish.

3) To evaluate effluent grown algae and zooplankton as colour enhancing agents in livebearing ornamental fish.

4) To determine the effect of effluent grown algae and zooplankton on the health of livebearing ornamental fish.

RESEARCH APPROACH

Although effluent grown algae is considered a cheap alternative protein source because it can be mass produced as a by-product of water purification, the cost of harvesting microalgae from the growth medium has presented one of the technological bottlenecks in its commercial production. An initial pilot study determined that cost effective methods of small scale algal harvest from the high rate oxidation ponds were possible at the Grahamstown Sewage Works (Chapter 3).

It is essential that a feed ingredient at least partially meets the nutritional requirements of the cultured organism. (De Silva and Anderson 1995). Therefore, an understanding of the nutritional requirements of the three poeciliid species was acquired by reviewing all available nutritional information on them (Chapter 4). This information was then compared to the nutritional composition of the algae and zooplankton, which was determined by performing a full proximate analysis and an amino acid analysis (Chapter 4).

Since the composition of feedstuffs are known to vary seasonally, and the most variable constituents in a feed ingredient are the protein and essential amino acid concentrations (De Silva and Anderson 1995), the crude protein values of the algae and zooplankton were monitored bi-weekly for one year (Chapter 4). The nutritional composition and method of algal harvest can be species dependant, and therefore the species composition of the algae was also monitored monthly for one year (Chapter 2).

Since the processing costs of microalgae in a commercial operation may constitute up to 30% of the production costs (Maart 1992), various methods of algal processing at the Grahamstown sewage works were selected after considering the potential cost of the processing and the available resources to the aquaculturalist at the Grahamstown Sewage Works. (Chapter 5). Previous literature suggested that different methods of

processing may effect the nutritional composition of the algae and therefore a chemical analysis of the algae (processed in three different methods) was performed (Chapter 5).

Although a chemical analysis gave an indication of the nutritional value of the effluent grown algae and zooplankton, a biological evaluation was essential to ascertain the response of the fish to the feed ingredients.

Since the nutritional effectiveness of a feed is firstly determined by its palatability, the initial stage of the biological evaluation was a three week palatability test to determine whether the fish consumed the algae in various forms (Chapter 5).
The second stage of the biological evaluation measured the growth, feed utilisation, colour and physical condition of poeciliids fed three pure algal diets (each processed using different methods), and a pure zooplankton diet for a ten week period (Chapter 5).
The third stage of the biological evaluation determined the apparent crude protein digestibility of sun dried algae by *X. helleri, P. reticulata* and *P. velifera* (Chapter 6).

Due to the nature of the algal and zooplankton culture medium, these feed ingredients could effect the health of the fish. Therefore a full health screening test was run on the fish at the end of all growth trials to test for pathological symptoms or signs of toxicity.

Based on the results of the first two nutritional studies and the digestibility study, the effect of effluent grown algae as a partial replacement for fish meal and soya meal and the effect of the zooplankton as a dietary supplement to a conventional diet were evaluated (Chapter 7).

CHAPTER 2

A DESCRIPTION OF THE ALGAL PURIFICATION SYSTEM AT THE GRAHAMSTOWN SEWAGE WORKS AND A STUDY OF THE ALGAL SPECIES COMPOSITION OF THE HIGH RATE OXIDATION PONDS.

Wastewater treatment involves the removal of pollutants from wastewater for safe and nuisance-free disposal (Oswald 1988). There are a number of problems associated with conventional waste water treatment methods. These include high maintenance, bad odours, a need for sludge disposal, temperature sensitivity, air pollution, wildlife poisoning and insect attraction (Oswald 1988).

Technology developed by Oswald since the 1950's culminating in an algal integrated pond system (AIPS) has been further adapted to suite South African conditions by Oswald and Green (UCLA, Berkley) and is in pilot phase at the Grahamstown sewage works (Anon, 1997). The system formed part of a strategy of South Africa's Reconstruction and Development Programme to develop small scale, low-cost systems, and alternatives to large industrial works, that can provide the required services within the means and the needs of developing communities (Anon, 1997). Compared with conventional sewage treatment systems, this system reduces the cost of construction and operation by about 50% (Anon, 1997). In addition, the AIPS decreases bad odours (Gibbs, 1995) and drastically reduces sludge production (Anon, 1997). The AIPS also produces large volumes of algal biomass which have a range of commercial uses including animal and fish feed additives (Anon, 1997).

A schematic diagram of the integrated algal-bacterial system at the Grahamstown Sewage Works is shown in Figure 2.1. A number of distinct sections are present and run in series. Each is designed to promote specific physical, chemical and microbiological

activities. This particular pilot plant was intended to purify the waste of between 500 and 1000 people (Gibbs 1995).

Raw sewage influent is introduced at the bottom of the fermentation pit. The fermentation pit (A) which is excavated on the floor of the primary facultative pond (B), has a function similar to that of an anaerobic digester in a conventional sewage treatment system (Anon, 1997) (Figure 2.1). To eliminate the handling and disposal of sludge, the volumetric capacity of the fermentation pit is 15 times the standard per capita capacity of conventional sewage sludge digesters (Anon, 1997). This ensures complete fermentation of volatile solids to biogas (methane) which is subsequently recovered in a gas collector and piped to a gas meter. At present, this methane is released into the atmosphere.

The primary facultative pond (B) is 3.5 meters deep and of a conical shape. The hydraulic residence time of these ponds is 20 days and together with the fermentation pit provides up to 60% reduction of the biological oxygen demand load to the system (Anon, 1997).The odour of these ponds is controlled by oxygenated water and algae pumped in small amounts from the high rate oxidation ponds (HROP).

The HROP (C) are raceway shaped and characterised by shallow depth (40cm), and continuous flow mixing obtained via paddle wheels. These high rate ponds host "ALBAZOD" (algae, bacteria, zooplankton and detritus), which oxidises the remaining soluble organics, provides surplus dissolved oxygen and creates a high pH value. To ensure oxidation of the influent load, the concentration of the algal cells in an HROP expressed in mgL^{-1} is normal set to equal the influent BOD (Anon, 1997). The algae in the HROP and zooplankton which feeds on it were the main focus of the study.

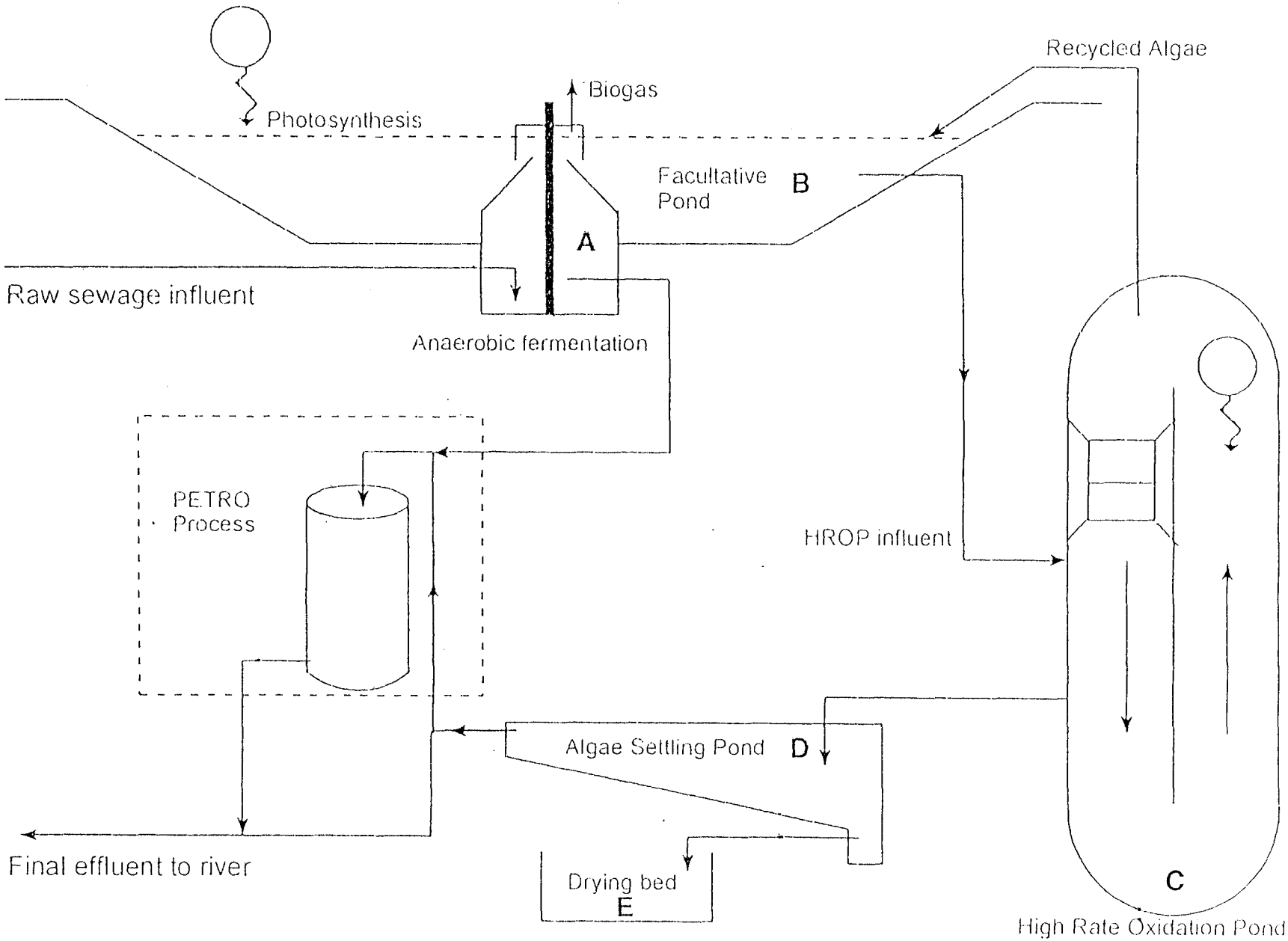


Figure 2.1. A schematic diagram of the algal integrated ponding system at the Grahamstown Sewage Works (from Anon, 1997)

The algae settling ponds (D) are an adaptation related to cell separation. The algal water from the HROP into the settling ponds where the algae autoflocculates and settles in a period of hours. The algal slurry can be stored there, or pumped out from below and spread onto the sand beds (E).

The sand beds (E) serve an efficient site for cell separation and algal dewatering. The algal cells are trapped by the sand particles, while the remaining water evaporates within an average of seven days. The average algal productivity in the two HROP's is 15 grams.$m^{-2}.d^{-1}$ (Anon, 1997). A 50% harvest of this algae from the HROP's will yield 3.75kgd^{-1} (dry weight) (Anon, 1997).

The water which remains in the settling ponds is pumped into the "PETRO" process (E) which comprises two large trickle filters. A small amount of water from the fermentation pit is released into the "PETRO" process to produce a bacterial culture in the trickle filters. These bacteria serve to further purify the water by denitrification. The "PETRO" process also serves as a site for the final removal of solids from the water. From the influent sewage, the final treated water shows a 91.1% reduction in COD, a 88.0% reduction in TKN, a 100% reduction in ammonia, total coliforms and settleable solids, and a 43% reduction in phosphorous (Anon, 1997).

The final outputs of the system include treated water, algal biomass and biogas. Therefore the system could provide the basic imputs for an aquaculture operation. The constant supply of treated water could be utilised as a culture medium for fish. The algal biomass could be utilised as an additive in the feed of ornamental fish. The zooplankton produced in the system could be utilised as a feed or feed supplement for ornamental fish and the biogas could be converted into a heat source and even electricity. This research project is part of ongoing research to develop the Grahamstown Sewage Works as a possible site for an ornamental fish aquaculture operation.

ALGAL SPECIES COMPOSITION

INTRODUCTION

Algal species composition

Generally, the chemical composition of algae is species dependent. In addition, different algal species are more susceptible to certain methods of harvest (e.g. *micractinium spp.* have spines and are therefore susceptible to microstraining). Therefore, it is essential for an aquaculturalist to have an idea of the algal species composition of the high rate oxidation ponds to simplify harvesting and to obtain an indication of the nutritional value of the algae. The initial study on the high rate oxidation ponds focused on the species composition of the ponds for one year. Since the algal species composition and density may be affected by climatic conditions, the temperature and rainfall were recorded for the duration of the experiment.

MATERIALS AND METHODS

A one litre sample was taken biweekly for one year from the high rate algal ponds at the Grahamstown Sewage Works and the algae identified with the help of Dr Olec Shippin (LIRI Technologies, Rhodes University). The daily rainfall and maximum temperature in Grahamstown was obtained from the Rhodes University Department of Geography.

RESULTS

Operation of the algal integrated ponding system was initiated in January 1996.

Initially *Pediastrum spp.* dominated the algal species composition of the pond (Table 2.2). In April 1996, a mechanical breakdown of the HROP resulted in an algal population crash. After the HROP was repaired, *Scenedesmus spp.* dominated the algal species composition of the pond. A second algal population crash occurred during July and coincided with an increase in the concentration of sewage associated with the

Grahamstown festival. *Scenedesmus spp.* continued to dominate until mid-September when the algal population crashed due to a mechanical breakdown of the pond and was replaced with *Micractinium spp.* Although *Micractinium spp* continued dominating the pond, the algal concentration in the pond was very low during November. From December until the end of the experiment the pond was dominated with both *Micractinium* and *Scenedesmus spp* (Table 2.2).

TABLE 2.2 The dominant algal species in the high rate oxidation ponds between March, 1996 and February, 1997.

Date	**Species composition**
01/03/96	*Pediastrum spp.*
15/03/96	*Pediastrum spp.*
01/04/96	*Pediastrum spp.*
15/04/96	High rate pond not operational due to mechanical failure.
02/05/96	High rate pond not operational due to mechanical failure.
17/05/96	High rate pond not operational due to mechanical failure.
01/06/96	*Scenedesmus spp.*
16/06/96	*Scenedesmus spp.*
02/07/96	*Scenedesmus spp.*
14/07/96	Activated sludge.
01/08/96	*Scenedesmus spp.* and activated sludge.
17/08/96	*Scenedesmus spp.*
02/09/96	*Scenedesmus spp.* and *Micractinium spp.*
18/09/96	High rate pond not operational due to mechanical failure.
01/10/96	High rate pond not operational due to mechanical failure.
14/10/96	*Micractinium spp.*
03/11/96	*Micractinium spp.* (very low concentration of algae)
17/11/96	*Micractinium spp.* (very low concentration of algae)

Date	Species composition
01/12/96	*Scenedesmus spp.* and *Micractinium spp.*
15/12/96	*Scenedesmus spp.* and *Micractinium spp.*
04/01/97	*Scenedesmus spp.* and *Micractinium spp.*
16/01/97	*Scenedesmus spp.* and *Micractinium spp.*
01/02/97	*Scenedesmus spp.* and *Micractinium spp.*
17/02/97	*Scenedesmus spp.* and *Micractinium spp.*

The rainfall was extremely variable throughout the year. An extremely high rainfall was recorded in November (211mm) and there was a downpour in mid-July (25mm), (Table 2.3). Although the maximum daily temperatures were also measured, they did not appear to affect the species composition of the ponds.

TABLE 2.3. The average rainfall in Grahamstown between March, 1996 and February, 1997.

Month	Rainfall (mm)
March	66.0
April	27.1
May	9.0
June	1.9
July	33.5
August	16.8
September	12.4
October	76.2
November	211.7
December	54

Month	Rainfall (mm)
January	24
February	38.9

DISCUSSION

The initial domination of the algae *Pediastrum spp.* was unexpected, since this algal species is not generally associated with sewage pond algae, but rather with non polluted dams and lakes (Palmer, 1980). The presence of *Pediastrum spp.* can be explained since the high rate ponds were fed with purified water from the conventional sewage purification system and not polluted water from the facultative pond in the initial phase of their operation.

By June 16th, a stable population of *Scenedesmus spp.* had established itself, however the algal and zooplankton population crashed in mid-July, 1996. The population crash coincided with an increase in the concentration and volume of effluent as a result of the influx of people for the Grahamstown Festival. In addition, run off caused by a downpour (25mm) on the 12th of July increased turbidity and decreased light penetration and photosynthesis in the high rate ponds and may have contributed to the subsequent algal and zooplankton population crash. The effect of rainfall on the concentration of algae in the pond was evident in November, when very low concentrations were observed in the pond after extremely high rainfall (211mm in November).

From December (1996) to December (1997), no mechanical break down or algal population crashes occurred in the high rate oxidation ponds. This indicates that these ponds are a reliable source of algae and zooplankton. However, the ornamental fish

farmer who relies on a constant supply of algae and zooplankton must be aware that potential population crashes may occur due to events such as the Grahamstown festival or high rainfall and that high rainfall could decrease the density of algae in the high rate oxidation ponds.

CHAPTER 3

HARVESTING MICROALGAL AND ZOOPLANKTON BIOMASS

INTRODUCTION

Algal Harvest

The utilisation of microalgae grown in wastewater as an alternative protein source for fish, largely depends on the economics of the harvesting technique (Soeder, 1978, 1984). The economics of harvesting large amounts of algae for commercial use is beyond the scope of this project and since this pilot algal integrated ponding system has an algal production capacity of only 3.75kg.d^{-1} (Chapter 2), it is unsuitable for the commercial production of algae for use as animal feeds. However, this research is part of ongoing research aimed at developing the Grahamstown Sewage Works as a site for an ornamental fish aquaculture operation. Algae in the HROP's would be available to the aquaculturalist at the Grahamstown Sewage Works at no associated cost, apart from labour. Therefore the time required for harvesting algae on a small scale from the HROP's using a variety of harvesting methods was determined.

Many technologies have been developed for removal of waste grown biomass from high rate pond systems treating municipal effluents. These technologies include centrifugation (Mohn, 1980), sedimentation (Mohn, 1988), flocculation (Eisenberg *et al.*, 1981), floatation, continuous belt filtration, vibrating and stationary screen filtration and sand bed filtration (Richmond, 1986b). In addition, experiments using 'biological' harvesting (harvest of algae by zooplankton which is more easily harvested) have been conducted (Schluter and Groeneweg, 1981; Groeneweg and Schluter, 1981). Six different methods of small scale algal harvest were evaluated experimentally. They were centrifugation, sedimentation, sand filtration, microstraining and biological harvest. A brief description of each method is provided below.

Centrifugation

The centrifugation of microalgal suspensions is expensive (Soeder and Mohn, 1975) due to the high initial investment costs, and relatively high energy requirements (Kawaguchi, 1980). However, centrifugation is one of the most efficient methods of removing algal cells from the supernatant, usually with a near 100% efficiency (Maart, 1992). This high efficiency ensures that all species of microalgae are separated form the supernatant. A centrifuge machine at the Department of Microbiology, Rhodes University was made available for the purposes of this study.

Sedimentation

Sedimentation is expensive due to the high initial investment cost of the large sedimentation surfaces. In addition, these surface increase the space requirements of the algal production system (Mohn, 1988). Sedimentation is most effective with the addition of flocculating agents such as alum, potato starch derivatives and chitosans. Since the algae will be utilised in fish diets, flocculating agents such as potato starch derivatives and chitosans which are recognised to be toxicologically safe when used as a human food source are suitable for initiating algal sedimentation (Mohn, 1988). At the settling ponds of the algal purification system at the Grahamstown sewage works no flocculation agents are added and the algae is subject to gravitational settling. The resulting algal slurry can be pumped out and collected, or spread onto the sand beds for sun drying.

Filtration

A number of different filtration methods including sand filtration, cloth filtration, filter presses, pressure suction filters, vacuum drum filters and diaphragm presses have been evaluated (Mohn, 1988). All filtration methods suffer from the problem of clogging and fouling (Oswald, 1988b) and continual backwash of the filter is required.

In addition to the sedimentation ponds, the algal wastewater treatment system has sand beds constructed for algal processing. The sandbeds are constructed at a fairly high initial investment cost but it combines simplicity with low maintenance cost since they

could provide both algal harvesting (by filtration) and processing (by sun drying). Algal slurry from the settling beds is pumped onto the sandbeds, where excess water gravitates through the sand and evaporates, while the algal particles remain on the sand surface. The period required for sun drying is dependant on the weather conditions, the depth of the algae and the percentage solids of the wet weight of the algal slurry.

The dried algae can be raked from the sand beds. Unfortunately, the sand may adhere to the sun dried algae and make it unsuitable for use as fish food. However Oswald and Golueke (1968) suggested that coarse screening could remove most of the adhering sand at a small additional cost.

Screening and straining

A number of different types of screens are used for microalgal harvest. These include vibrating screens, rotating screens, microstrainers and cascade screens (Richmond and Becker, 1986). Microstraining produces pure algae reliably and with the least cost (Oswald, 1988b). Since microstraining is particularly effective with filamentous algae (e.g. *Spirulina),* larger algal species and smaller species with extensive setae (Oswald, 1988b), monitoring the species composition (Chapter 2) of the high rate ponds would provide an indication of when this harvesting method would be most effective.

Biological Harvest

The zooplankton, which feeds on the algae in the high rate ponds contains a large proportion of freshwater rotifers (*Brachionus spp.).* Rotifers are a widely used source of food for rearing fish fry (Howell, 1973;1974). Algal food for rotifers is currently produced in defined nutrient solutions which is an expensive process (Soeder, 1978). Rotifers fed other food sources have had a lower nutritional value for young fish than those fed with algae (Watanabe *et al.*,1978). Effluent grown algae could provide a more economical and possibly highly nutritional food source for rotifers. In addition, the filter feeding rotifers convert the algae into a more easily harvested form of biomass (Mitchell, 1986) and Groeneweg and Schluter (1981), after conducting small scale experiments

concluded that algal-bacterial biomass grown in wastewater can be harvested by rotifers which in turn can be fed to fish.

However, if biological harvesting is the sole method of algal harvest used, the amount of rotifers required to harvest the algal biomass would be very large. These rotifer cultures would require large culture ponds and would result in high initial investment costs and an increase in the special requirements of the algal production system. The biological harvest experiment was designed to test the potential of zooplankton culture using high rate pond algal water as a dietary source, and to evaluate the algal harvesting potential of rotifers.

The objectives of the algal harvest experiments were to evaluate the suitability of different small scale algal harvesting methods for use in an ornamental aquaculture operation at the Grahamstown Sewage works.

Zooplankton harvest

The small scale harvest of zooplankton is usually performed by siphoning the content of the culture tank into filter bags with an appropriate mesh size (Dhert, 1996). This is normally performed in submerged filter to prevent damage to the zooplankton which may result in mortality (Dhert, 1996). However the zooplankton in the high rate oxidation ponds provided a unique set of problems since the culture was not a species specific and there was a large size variation between individuals. In addition, the presence of large algal flocs could have caused mesh clogging during harvest.

MATERIALS AND METHODS

Algal harvest

Since concentrations of algal cells in the high rate ponds can change significantly over time, all algal harvest experiments except the biological harvest experiment were conducted within two days.

Centrifugation

In the first experiment, 100l of high rate pond water was taken to the Department of Microbiology, were it was centrifuged in a Beckman J2-21 centrifuge machine for fifteen minutes at a speed of 8000 revolutions per minute. The resulting solid algal concentrate was weighed wet, sun dried on white plastic boards and reweighed.

Sedimentation

Fifty litres of high rate pond water were poured into each of two tapering, fibreglass sedimentation tanks (Height = 100cm; Diameter = 80cm). The algal biomass was allowed to sink to the bottom of the containers for a period of 24 hours, after which a withdrawal tube which reached to the tank bottom was used to siphon out the algae. The resulting sediment was weighed wet, sun dried on white plastic boards and re-weighed.

Sand Filtration

One-hundred litres of the high rate pond water were poured onto a one square meter area of a sandbed at the Grahamstown sewage works and sun dried. The resulting algal flakes were harvested by raking and weighed after most of the sand particles adhering to the algae was removed by screening it through a 500µm aperture sieve.

Microstraining

Fifty litres of the high rate pond water was microstrained through a mesh size of 40µm and the other fifty though a mesh size of 60µm. The algae harvested was weighed wet, sun dried and reweighed.

Biological Harvest

Three hundred litres of high rate pond water was harvested form the high rate oxidation ponds at the Grahamstown Sewage Works on the first day of the experiment. The water was transferred to a 350 litre capacity indoor high rate pond at the Leather Industry Research Institute. Large windows provided the indoor ponds with natural sunlight similar to that of the outdoor ponds. In addition an artificial light suspended above the

pond was set on a timer to simulate the photoperiod and light intensity regime of the outdoor ponds. The temperature of the indoor pond was maintained at 20°C (the average daytime temperature of the outdoor pond).

Once daily, 100 litres of water from the indoor pond was removed form the pond. this water was microsieved using 80µm plankton mesh and the zooplankton which was captured was transferred back to the culture. One hundred litres of outdoor high rate oxidation pond water containing fresh algae was then placed into the indoor pond, after removing the zooplankton by microsieving with an 80µm mesh. At the end of the one-week experiment, all zooplankton was harvested from the indoor pond using a 80µm mesh, and 100l of the indoor high rate pond water was centrafugated 15 minutes at 8000 revolutions per minute. The resulting algal biomass was weighed wet, sun dried and re-weighed.

To test if zooplankton could be cultured with the outdoor high rate oxidation pond water, the density of zooplankton in the indoor pond was calculated daily by counting five samples of 1.0ml each in a Boggorov counter under a dissecting microscope (5x magnification).

To determine the efficiency of each algal harvesting method, the wet and dry weights of the resulting algal biomass were compared to those of the algae harvested by centrifugation (assuming centrifugation resulted in 100% algal cell removal). Although the efficiency of the algal removal by the zooplankton could not be accurately quantified, the amount of algae remaining in the indoor high rate pond gave an indication of their algal harvesting efficiency.

Since an estimate of the time to feed fish daily would be useful to the ornamental fish farmer at the Grahamstown Sewage Works, in each of the above experiments the time required for the actual harvesting process was calculated.

Zooplankton Harvest

Two methods of microstraining was used to harvest zooplankton from the high rate oxidation ponds. Conventional plankton nets of 80µm and 100µm mesh sizes were used to remove zooplankton. The second method of zooplankton harvest used was a technique developed by Mitchell (1986) (Fig. 3.1). Large amounts of high rate oxidation pond water was siphoned from the pond and spread across the top of the screen through a perforated pipe (A). The pond water then passed through a curved screen (B), covered with netting of 80µm. The screen was curved as a result of a frame of 6mm steel rods (C) which were glued to the plankton netting with silicone.

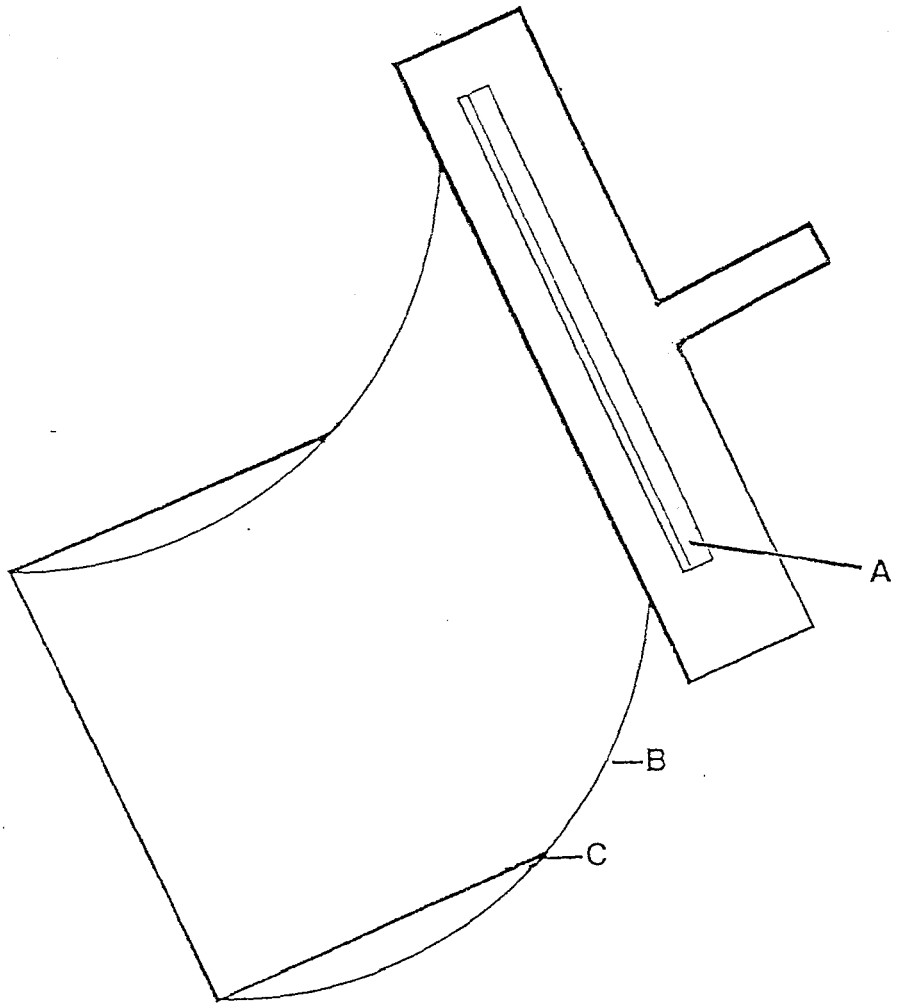


Figure 3.1. Diagram of the screen used for harvesting zooplankton (Mitchell, 1986).

RESULTS

As expected centrifugation yielded the highest dry algal biomass. However since the time taken to process 100 litres of pond water was the highest, it yielded the lowest dry biomass per minute (Table 3.1).

Although sedimentation yielded the second lowest dry biomass, the short time taken to harvest the algae resulted in this method yielding the highest dry biomass per minute. Sedimentation also yielded the highest wet algal biomass (Table 3.1).

TABLE 3.1. The wet weights, dry weights of the algae, approximate labour time and dry algal biomass per minute for five different methods of algal harvesting. Numbers in brackets indicate rating of algal harvest efficiency.

Harvest Method	Wet Weight (g)	Dry Weight (g)	Time (minutes)	DW/t* g/min
Centrifugation	1220	130.2	390	0.33 (5)
Sedimentation	4860	66.7	6	11.1 (1)
Microstraining (60µm)	821[1]	62.1[1]	15	4.14 (2)
Microstraining (40µm)	1051[1]	81.7[1]	25	3.27 (4)
Sand Filtration		122.8	30	4.09 (3)

* DW/t = Dry algal biomass (grams)/ time (minutes). Defined as harvesting efficiency.

** Divided by five for means of comparison.

[1] Mass in 100 litres

The biomass of the sand filtered, sun dried algae after the fairly time consuming raking and sieving, yielded the second highest dry algal biomass and the third highest dry biomass per minute (Table 3.1).

While microstraining with the 60µm mesh yielded a lower wet and dry algal biomass than microstraining with a 40µm mesh, the shorter labour time required to harvest algae with the 80µm mesh resulted in it yielding the second highest dry algal biomass per minute (Table 3.1).

Biological Harvest

The highest density of 330 livefood items per millilitre was recorded on day six of the experiment (Figure 3.1). The wet weight and dry weight of the algae remaining in the supernatant at the end of the experiment was 234g and 23.6g respectively (Table 3.1).

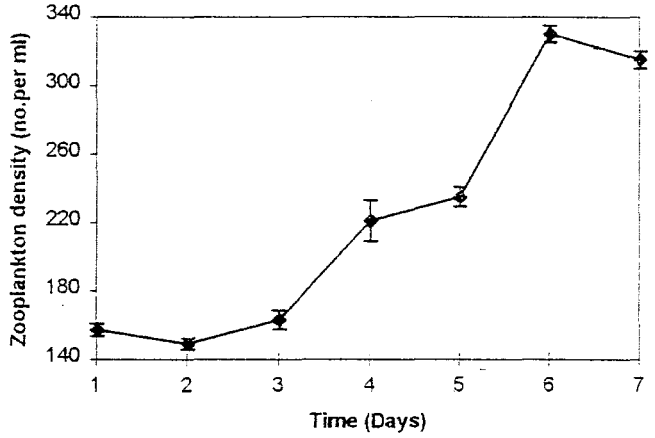


FIGURE 2.3. The density of zooplankton (items /ml) in the experimental indoor high rate pond during the biological harvest experiment.

Zooplankton harvest

The 80µm and 100µm mesh size nets tended to clog with zooplankton and large algal flocs and although not quantified, harvesting became inefficient. In addition, the zooplankton and algae were extremely difficult to separate.

Zooplankton and algal clogging was reduced using a zooplankton harvest apparatus (Mitchell, 1986), since the harvested material was carried to the end of the screen. However, large algal flocs were also harvested by the curved screen and the algae was still difficult to separate from the zooplankton.

DISCUSSION

Algal Harvest

The potential of effluent grown microalgae as an alternative protein source for ornamental fish will not be realised if harvesting of the algae is time consuming for the aquaculturalist.

Since centrifugation resulted in the largest dried algal biomass it was, as expected, considered most effective method of cell separation. However, its labour intensive nature and high electrical, operational costs made it the least efficient method of algal harvest at the Grahamstown Sewage Works.

Although the algae harvested by sand filtration had the second highest dry biomass, the contamination of the algae with sand particles may have significantly increased the mass and resulted in an exaggerated value. In addition, the reasonably long time required for raking and sieving reduced the efficiency of this method of algal harvest. However, this method of harvesting is still efficient since it combines algal harvesting with algal processing. Since algal processing is a major expense in algal production (Hepher *et al.*,1979), sand filtering is particularly economical. However, a more effective sand removal method must be found before the algae harvested and processed using this method will be suitable as ornamental fish food.

Algal sedimentation was one of the least effective methods of cell separation since the resulting algae had the highest wet mass and the second lowest dry mass. However the short time required to harvest algae from 100 litres of high rate pond water indicated that

this method was the most efficient method of obtaining algal biomass and had application for use by the ornamental fish farmer. This method of algal harvest could be more effective with the addition of non-toxic flocculating agents such as potato starch derivatives to the high rate pond water. However, the effect of flocculating agents will still have to be tested. The high moisture content of the algae harvested by sedimentation resulted in an extended sun drying time and since long exposure to light and heat may effect the nutritional composition of the algae (Oswald and Goulueke, 1968). Sun dried algae, harvested using this method must therefore be tested chemically and biologically before it can be recommended for use in ornamental fish food.

The lowest dry algal biomass was recorded in algae that had been microstrained using the 60µm mesh. This was considered the least effective method of cell separation since smaller algal cells escaped the 60µm mesh. The 40µm mesh appeared to harvest the smaller algal cells since the dry mass of the algae harvested was 24% higher than the algae harvested in the 60µm mesh. However, the 40% shorter labour time required for harvesting using the 60µm mesh resulted in it being a more efficient method of obtaining algal biomass and the second most effective method of algal harvest overall. The longer time required to harvesting algae with the 40µm mesh could be decreased if the mesh is prevented from clogging. By decreasing the mesh size to 40µm, the zooplankton harvest apparatus (Mitchell, 1986) may provide a solution to the problem of clogging (See Fig 3.1 and zooplankton harvest).

Since all of the dominant species encountered in the pond (Chapter 2), were particularly susceptible to microstraining *(Pediastrum spp.* forms flat disc like colonies (Bellinger,1980), *Scenedesmus spp.* are a large algal species (Oswald, 1988), and *Micractinium spp.* form small colonies and have fine tapering spines (Bellinger, 1980)), this harvest method could be efficient throughout the year.

Since the zooplankton survived and reproduced in the indoor high rate pond during the one week experiment, the results of the biological harvest experiment suggest that effluent grown algae is a suitable food source for small scale production of zooplankton

in the short term. This has application in aquaculture, since single species zooplankton cultures which may be required for larval culture could be maintained on the high rate oxidation pond algae. Since the algae produced for mass culture of zooplankton is commonly produced in defined nutrient solutions and is an expensive process (Soeder, 1978), this may result in a cost saving for the aquaculturalist. Besides this possible cost saving for the aquaculturalist, the zooplankton also removed algae from the high rate pond water. Although the mass production of zooplankton is labour intensive, the potential benefits of a constant supply of zooplankton to ornamental fish need to be considered. For example, the mass production of smaller zooplankton species such as rotifers could allow the production of fish species, with small larvae, never before produced in this country.

Zooplankton Harvest

Although not quantified, the harvest of zooplankton using 80µm and 100µm mesh nets was not very efficient. While the use of the zooplankton harvest apparatus (Mitchell, 1986), was more efficient, the algal flocs contaminating the zooplankton was problematic. However, if small scale zooplankton harvest is required and large algal flocs are clogging the net, water can be collected from the high rate ponds in 25-100 litre containers and left standing for 24 hours. Sedimentation of the algal flocs will allow zooplankton to be collected unhindered, by siphoning the surface water from the containers.

CHAPTER 4

CHEMICAL EVALUATION OF THE NUTRITIONAL VALUE OF EFFLUENT GROWN ALGAE AND ZOOPLANKTON TO POECILIID FISHES.

INTRODUCTION

Algae and algal products have been shown to promote growth in many fish species in aquaculture, (Gupta and Ahmed, 1965; Stanley and Jones, 1976; Matty and Smith, 1978; Sandbank and Hepher, 1980; Appler and Jauncey, 1982; Wong and Tam, 1984; Davis, 1992). However, the success of the algae and zooplankton as feed ingredients relies on them at least partially meeting the nutritional requirements of the cultured organisms. Therefore the available nutritional information on the requirements of the three test species was reviewed, and the chemical composition of the algal and zooplankton biomass was determined in order to evaluate their nutritional value to the test species.

The species chosen for the study were *Xiphophorous helleri,* (swordtails), *Poecillia reticulata* (guppies) and *Poecillia velifera* (sailfin mollies). These Poeciliid species are native to South and Central America (Rosen and Bailey, 1963) and are typical of most of the livebearer species common in the aquarium trade (Thibault and Schultz, 1978).
X. helleri and *P. reticulata* , like most poeciliids are omnivorous (Table 4.1). Dussalt and Kramer (1981) concluded that opportunistic feeding on a variety of animal foods with a major component of benthic algal material may be a common feeding pattern in these fishes. Unlike *X. helleri* and *P. reticulata, P. velifera* is considered the most herbivorous poeciliid in its natural environment (Harrington and Harrington, 1961;1982; Wetzel, 1971), with diets ranging from vascular plants to algae and detritus (Table 4.1).
Due to the omnivorous and herbivorous feeding habits of these fishes, it was concluded that they were suitable candidates for feeding algal and zooplankton biomass.

The most important ingredient of diets in aquaculture is protein, since it promotes growth (Hepher *et al.* 1979). Information from commercial poeciliid operations in Singapore suggest that poeciliids require crude protein values of 15-33% in their diets (Fernando *et al.*, 1991), (Table 4.1). However, these diets rely on regular livefood supplementation (Fernando *et al.,* 1991), suggesting that the protein requirements of the fish may be higher than the recommended formulations suggest. Kruger (1995), investigated the protein requirements of *X. helleri* and concluded that 45% crude protein was optimal for swordtail formulated diets with no supplementary feeding. Thus in order for the diets to at least partially meet the protein requirements of the culture organisms, one would expect the ingredients to have a relatively high crude protein content. Since previous literature has suggested the crude protein content of feed ingredients vary geographically and with time (De Silva and Anderson, 1995), the crude protein content of the algae and zooplankton was measured bi-weekly for a one year period.

Nitrogen free extracts (NFE) is a measure of the soluble carbohydrates present within a feed ingredient (Tacon, 1990). Carbohydrates function as readily metabolisable energy stores and facilitate the transfer of energy throughout the organism (De Silva and Anderson, 1995). Poeciliid diets in Singapore contain between 48 and 77% NFE. Kruger's (1995) optimal swordtail diet contained a carbohydrate content of 21%. To chemically evaluate the potential of effluent grown algae and zooplankton as a dietary ingredient for poeciliids, their NFE values were calculated.

Lipids function as high energy storage molecules or components of cell membranes (De Silva and Anderson, 1995). Singapore commercial poeciliid diets contain between three and seven percent and Kruger's (1995) optimal swordtail diet, a 12% lipid content. These values were compared to those determined for the algae and zooplankton.

TABLE 4.1. Summary of the published information of the dietary requirements of *X. helleri*, *P. reticulata* and *P. velifera*. NFE = Nitrogen free extract.

Species	Natural Diets	Singapore Aquaculture
X. helleri	Omnivorous (preference for plant material)[1]	18-26% Protein, 3-7% Lipid 48-77% NFE 60-80% Wheat bran Regular livefood supplementation[2]
P. reticulata	Omnivorous (Preference for insects)[1]	15.1-33.5% Protein ±5% Lipid 53-63% NFE 70-80% Wheat bran Regular livefood supplementation[2]
P. velifera	Herbivorous (Plants, algae and detritus)[1]	Zooplankton and phytoplankton Bread and wheat bran supplementation[2] 15-19% Protein 59-72% NFE

[1] Dussalt and Kramer ,(1981).

[2] Fernando *et al.*, (1991).

Since amino acids are the building blocks of proteins, the quality of a protein as a feed ingredient is dependent its amino acid composition and the biological availability of the amino acids presented to the fish (Tacon 1990). All fish studied to date require the same ten essential amino acids (De Silva and Anderson, 1995). These are: arginine (arg),

histidine (his), isoleucine (ile), leucine (leu), lysine (lys), methionine (met), threonine (thr), phenylalanine (phe), tryptophan (trp), and valine (val) (Steffens 1989). Although cystine and tyrosine are non essential amino acids, cystine is known to spare part of the methionine requirement in some fish and tyrosine is thought to have a similar sparing effect on phenylalanine (Ketola, 1982). The accurate quantitative dietary requirements for all ten amino acids have only been established for five species of fish and each has been different (Tacon, 1990). The accurate determination of these requirements is a lengthy procedure and requires a considerable experimentation for each fish species (De Silva and Anderson 1995). However, Tacon and Cowey (1985) suggested that there is no difference between the relative proportions of individual EAAs required in the diet and the relative proportions of the same ten EAA present within the fish carcass. Therefore the amino acid composition of the *X. helleri* carcass (Kruger, 1995) was compared to the amino acid compositions of the algae and zooplankton biomass to evaluate their nutritional value.

MATERIALS AND METHODS

Monthly crude protein content.

Once every two weeks, an algal sample (harvested by sedimentation) and a zooplankton sample (harvested by microstraining) were harvested from the high rate ponds. The crude protein value of the samples were determined using the micro-kjeldahl method by measuring the total nitrogen content within the samples, and then converting this figure to a total crude protein value by multiplication with the empirical factor 6.25 (Tacon, 1990).

Since climatic conditions may influence the nutritional value of the algae, the daily Grahamstown ambient temperature for the corresponding year was obtained from the Geography Department at Rhodes University.

Proximate analysis

On the 10th of January 1997, five hundred litres of high rate pond water was collected. Algae was harvested from the sample using the process of sedimentation, while zooplankton was harvested from the remaining supernatant using a 80µm mesh.

The algal and zooplankton samples were oven dried at 105°C for 24 hours to determine their moisture contents (Tacon, 1990).

The ash content of the algae and zooplankton were determined after placing them in a combustion furnace at 450°C for 24 hours (Tacon, 1990).

The crude protein content of the algae and zooplankton was determined using the micro-kjeldahl protein analysis method. The crude lipid contents of the algae and zooplankton were determined using the lipid solvent (chloroform) extraction method (Tacon, 1990).

The crude fibre and digestible carbohydrate content (Nitrogen Free Extract) of the algae and zooplankton were determined by subtracting the sum of the moisture content, ash content, crude protein content and crude lipid content from 100% (Tacon 1990).

Oven dried, frozen samples of algae and zooplankton were sent to the University of Natal (Pietermaritzburg) for an amino acid composition analysis. Tryptophan and cystine values were not determined. The similarity of the amino acid values of the whole body tissue of X. *helleri* and the algae and zooplankton were calculated using a modified form of essential amino acid ratio (A/E ratio) developed by Ogata *et al.* (1983).

The A/E ratio is defined as:

$$\text{A/E ratio} = \frac{\text{EAA}}{\text{Total EAA}} \times 1000$$

EAA = Essential amino acid value in g/100g dry matter.

Total EAA = All essential amino acid values added (except tryptophan).

To quantify how closely the A/E ratio of *X. helleri* and the algae and zooplankton matched each other, simple regression analyses were performed, and the coefficients of determination (r^2) were calculated.

RESULTS

The crude protein content of the zooplankton and algae varied significantly through the year (Table 4.2). The lowest crude protein values for the algae and zooplankton were 36.1% and 33.3% respectively (Table 4.2). The highest crude protein values of 52.6% were recorded in July, when activated sludge dominated the pond. The highest average maximum temperatures were recorded in December, January and February.

TABLE 4.2. The average maximum temperature in Grahamstown and the crude protein contents (dry matter) of the algae and zooplankton harvested from the high rate ponds between March, 1996 and February 1997.

Month	Ave max Temp (°C)	Algae (%CP)	Zooplankton (%CP)
March	25.25	45.5^{d} ±0.5	38.1^{cdef}±0.4
April	24.29	37.7^{ab} ±0.4	33.3^{b} ±0.1
May	21.29	40.1^{bc} ±0.6	38.7^{def} ±0.4
June	21.29	39.2^{bc} ±0.4	38.5^{def} ±0.5

Month	Ave max Temp (°C)	Algae (%CP)	Zooplankton (%CP)
July	17.33	52.6^{f} ±0.3	52.6^{g} ±0.3
August	18.86	36.1^{a} ±0.5	35.9^{cde} ±0.6
September	24.23	40.5^{c} ±0.3	36.8^{cde} ±0.4
October	22.55	40.2^{bc} ±0.3	35.4^{bc} ±0.1
November	21.97	43.6^{d} ±0.2	38.8^{ef} ±0.2
December	26.18	45.6^{d} ±0.5	No value(expt error)
January	26.73	50.4^{ef} ±0.7	40.1^{f} ±0.4
February	25.77	48.8^{e} ±0.4	36.9^{bc} ±0.2

Proximate analysis and amino acid composition:

The crude protein contents of the algae and zooplankton were 41.5% and 36.8% respectively. The crude lipid contents of algae and zooplankton were 4.8% and 11.1% respectively. The NFE contents of the algae and zooplankton were 35% and 31% respectively.

The body tissue of *X. helleri* had higher histidine, methionine, glutamic acid and glycine values than the algae and higher arginine, histidine, lysine, methionine, serine, glutamic acid and glycine values than the zooplankton (Table 4.3).

TABLE 4.3. The amino acid composition of the algae and zooplankton harvested from the high rate ponds and the whole body tissue of *X. helleri* (Kruger 1995) expressed as percentage protein (g/100g protein). Bold font indicates essential amino acids.

Amino Acid	*X. helleri*	Algae	Zooplankton
Arginine	5.67	5.75	5.45
Histidine	2.31	1.71	1.70
Isoleucine	3.78	4.52	4.76
Leucine	6.83	8.23	8.12
Lysine	7.73	8.15	6.81
Phenylalanine	3.89	4.63	5.13
Methionine	2.60	1.67	1.85
Threonine	3.78	5.46	4.99
Valanine	4.40	6.27	6.26
Tryptophan			
Aspartic Acid	9.27	10.91	10.37
Serine	4.05	4.33	3.81
Glutamic Acid	15.58	11.63	11.80
Proline	4.66	5.21	5.18
Glycine	7.17	6.17	5.82
Alanine	5.71	7.74	6.91
Tyrosine	2.77	3.14	3.46
Cystine			
Crude Protein	54.5	41.47	36.84

There was a relatively low similarity (low r^2 value) between the A/E ratios of *X. helleri,* and those of effluent grown algae and zooplankton (Table 4.4.). In addition the comparison between these ratios indicated that arginine, histidine, lysine and

methionine were limiting in both the algae and zooplankton with respect to *X. helleri* (Table 4.4).

TABLE 4.4. Modified essential amino acid ratios (Ogata *et al.* 1983) for *X. helleri* and effluent grown algae and zooplankton. r^2 = Coefficient of determination with respect to profile of *X. helleri* (Limiting essential amino acids underlined).

Amino Acid	*X. helleri*	Algae	Zooplankton
Arginine	138	128	124
Histidine	57	38	39
Isoleucine	92	100	108
Leucine	167	183	184
Lysine	189	136	132
Methionine	63	37	42
Phenylalanine	95	117	116
Threonine	92	121	113
Valine	108	139	142
r^2 (%)		65.33	62.96

DISCUSSION

Generally, the crude protein values of the algae and zooplankton increased in summer, with its associated longer days and higher temperatures. While the protein contents of the algae and zooplankton did vary significantly through the year, the lowest protein contents during the year were 33.3% for zooplankton and 36.1% for algae. This indicates that the high rate algal ponds provide a reliable source of high protein biomass throughout the year. However, aquaculturalists must be aware that the crude protein content of the algae and zooplankton could be lower in winter. The highest crude protein values were recorded when activated sludge dominated the high rate oxidation ponds. A

possible reason for the high "protein" values of the activated sludge could be the presence of large amounts of non proteinaceous nitrogen in it. Since the crude protein content was calculated by multiplying the total nitrogen content in the sample by an empirical factor of 6.25, the presence of non-proteinaceous may have resulted in an overestimate of the crude protein content of the sludge.

The crude protein contents of effluent grown algae and zooplankton exceeded the crude protein values of Singapore commercial poeciliid diets, but were lower that the 45% crude protein level suggested by Kruger (1995) (Table 4.5). The crude protein contents of the algae and zooplankton are lower than animal products such as fishmeal which has a crude protein value of between 57 and 73% (De Silva and Anderson, 1995), however they are similar to oilseed products such as soya, cottonseed and sunflower which have protein contents between 30.8 and 48.% (Tacon, 1990).

TABLE 4.5. The proximate composition of effluent grown algae and zooplankton, an optimum swordtail diet (Kruger, 1995), and Singapore commercial guppy and swordtail diets (Fernando *et al.,* 1991). All values are expressed as percentage by weight: H_2O - Moisture content; CP - Crude Protein; L - Lipid; NFE - Nitrogen free extract; A - Ash.

	Average composition (% by weight)				
	H_2O	CP	L	NFE	A
Algae (dried)	5.7	41.47	4.84	35.09	12.9
Zooplankton (dried)	10.8	36.84	11.1	31.06	10.2
Swordtail Diet[1]		45.00	12.00	21.00*	
Guppy Diet [2]	53-75	15-34	3-7	48-77	2.8-16.6
Swordtail Diet[2]	9.8-59.1	18-26	±5	53-63	4.9-11.6

* Carbohydrate percentage only.

[1] Kruger, (1995)

[2] Fernando *et al.,* (1991)

The lipid content of algae was similar to those of Singapore poeciliid diets and less that Kruger's (1995) swordtail diet (Table 4.5). While the lipid content of the zooplankton exceeded those of the poeciliid diets in Singapore, it was similar to the lipid content of Kruger's (1995) swordtail diet (Table 4.5). The NFE values of the algae and zooplankton were lower than those of the Singapore poeciliid diets, but higher than the carbohydrate content of Kruger's (1995) swordtail diet.

The proximate composition of the algae and zooplankton, when compared with the Singapore commercial poeciliid diets were favourable, and suggested that they could be tested as sole protein sources in poeciliid diets.

The low degree of similarity between the essential amino acid ratios of the algae (r^2 = 65.33) and zooplankton (r^2 = 62.96) protein when compared to the amino acid profile of the body tissue of *X. helleri* however suggested that the amino acid balance of these proteins as sole protein sources are not adequate for *X. helleri,* and are unlikely to be so for *P. reticulata* and *P. velifera.* The r^2 value for fishmeal and soya oil cake meal when compared to the body tissue of *X. helleri* were calculated to be 96.2 and 69.6 respectively (Kruger, 1995). This suggests that the amino acid profile of pure algae and zooplankton is considerably poorer than that of fishmeal, but not very different from soya oil cake meal. The results of the amino acid analysis suggest that while effluent grown algae could be an efficient replacement for soya meal and other plant proteins, it may not replace fishmeal protein as effectively.

The inadequacy of the amino acid profile of effluent grown algae and zooplankton as a sole protein source for poeciliids was further demonstrated by the arginine, lysine, histidine and methionine levels, which appeared to be limiting. However, if algae and zooplankton were combined with ingredients rich in these amino acids (such as fishmeal), they could be a valuable protein supplement in poeciliid diets.

While a considerable amount of information may be gained by performing a comprehensive chemical analysis, no biological information can be determined. For a feed ingredient to be successful, its nutrients must be biologically available to the animal. Poor palatability, low digestibility and the presence of enzyme inhibitors may render nutrients of the algae unavailable to a fish and result in depressed growth rates. In contrast, live feed is considered easily digestible and may contain enzyme systems which allow autolysing (Lavens and Sorgeloos, 1996), thus resulting in increased growth rates. Therefore, in the next experiments biological aspects such as palatability, growth, protein efficiency and digestibility of the algae and zooplankton to poeciliids were evaluated.

CHAPTER 5

A CHEMICAL AND BIOLOGICAL EVALUATION OF A PURE ZOOPLANKTON DIET AND PURE EFFLUENT GROWN ALGAE PROCESSED BY THREE DIFFERENT METHODS.

INTRODUCTION

A preliminary three week growth trial revealed that *X. helleri* and *P. reticulata* readily consumed fresh and sun dried algae, as well as zooplankton grown in the high rate oxidation ponds. No signs of disease or mortalities were recorded. Thus sun dried and fresh algae were palatable and had no negative effects on poeciliid health in the short term. In addition, the chemical composition of the algae and zooplankton appeared to be nutritionally adequate for the growth of poeciliids (Chapter 4). However, since the chemical analysis does not evaluate the biological response of the fish to the algae and zooplankton, growth, colour enhancement, health and survival of poeciliids fed pure algae and zooplankton diets were measured in this study.

Processing microalgal biomass may constitute up to 30% of the algal production costs (Maart, 1992). Furthermore, different methods of algal processing may alter both the nutritional composition and palatability of the product (Maart ,1992), Therefore, the effect of different algal processing methods was measured chemically (proximate and amino acid composition) and biologically (measuring growth, colour enhancement and health effects of poeciliids fed the algae), to determine the most appropriate method for processing algae for use by prospective ornamental fish farmers at the Grahamstown sewage Works.

Algal processing

Algal processing involves various handling methods beyond the harvesting and thickening of the algae. It may involve drying for storage, the extraction of one or more products such as pigments and oils, or blending and feed manufacture (Oswald 1988). A number of different methods of algal drying exist. Algae may be sun dried, spray dried, drum dried or freeze dried. For small scale production, sun drying on sand beds or on polyethylene surfaces is economical and sun light produces leaf like flakes within hours. However if drying is not rapid, a decrease in the vitamin content (Oswald and Golueke 1967) and digestibility (Sandbank and Hepher 1980) may result, and effect the nutritional quality of the algae. Drum drying has been used extensively and has been found to be an excellent method of processing algae. Unfortunately, the high investment cost and operating costs such as those of heat and labour combine to make this method suitable for large scale algal processing only. Spray drying is an effective method of processing algae as the fine powder which is produced can be stored infinitely in the dark (Oswald 1988). However, spray drying involves expensive large scale equipment. Freeze drying has not been used extensively in algal processing, however Gatesoupe & Robin (1981) used freeze dried algae for rotifer culture and suggested that freeze dried algae is more likely to contain more of the unstable nutritional components than spray-dried algae. Unfortunately, like drum dryers and spray dryers, freeze dryers capable of processing large amounts of algae are expensive.

Three methods of algal processing were selected based on their economical viability and availability to a potential aquaculture operation at the Grahamstown sewage works. In the first method, unprocessed algae was fed to the fish. Since the final step in the algal purification system at the Grahamstown sewage works involves sun drying algae on a sand bed, a second diet of sun dried algae was fed to the fish. The positive effects of freeze drying algae (Gatesoupe and Robin, 1981), provided motivation for this method to be used as the third algal processing method. A freeze drying machine was made available for the study by the Department of Chemistry, Rhodes University. Drum drying and spray drying equipment were not available to the project.

Since different algal processing methods may effect the nutritional quality of algae, the growth of juvenile poeciliids fed the three algal products and a zooplankton diet was measured. In addition, different processing methods may effect the caratenoid content of the algae and therefore, the effect of the three algal products and zooplankton on poeciliid colour was tested.

Fish survival was measured during the experiment and fish health was evaluated at the end of the experiment to determine whether the algae and zooplankton produced any negative side effects in the medium term.

The aims of this study were:

1) To determine the effect processing on algal quality as a feed ingredient for poeciliids.
2) To biologically evaluate the nutritional value of pure algal and zooplankton diets on poeciliids.
3) To evaluate the potential of the algae and zooplankton as colour enhancing agents in poeciliids.
4) To evaluate the effect on health and survival of poeciliids fed the algae and zooplankton.

METHODS AND MATERIALS

Experimental System

A temperature controlled, recirculating system incorporating biological filtration was used for this trial (Figure 5.1). Three replicates for each treatment were allocated to these tanks according to a randomised design. The water temperature was controlled at a constant 27°C for the duration of the experiment. Heating was provided by a 3 kW element which was linked to a solid phase thermostat in the system sump tank. The total volume of the system was 960 litres. The flow rate to the tanks was 0.5l/min and this resulted in 2.7 theoretical water changes per hour. The ambient air temperature in the room was controlled with an air conditioner. Aeration was supplied to each tank via

airstones which were coupled to an air pump. A 12 hour dark : 12 hour light photoperiod was maintained through L18 W/72 Biolux "natural sunlight" tubes (Osram, Germany). These provided a 95% daylight spectrum. All other light sources were excluded by covering the entire system with black plastic boards. Water temperature was measured daily and ammonia, nitrite, nitrate, chlorine, pH and dissolved oxygen values of the water were measured once weekly. All tanks were cleaned weekly.

Experimental Diets

Four diets were used during the growth trial. The first diet, referred to as the control diet was Kruger's (1995) optimum swordtail diet. Three pure algal diets in different forms, one fresh algae, the second sun dried algae, and a third freeze dried algae were fed to the fish as separate treatments. After three weeks, the decision was made to terminate the freeze dried algal diet due to poor growth performances and high fish mortalities, and this was replaced with a pure zooplankton diet.

The control diet was prepared according to the guidelines for experimental feed preparation described by Lovell (1989). All dry ingredients were thoroughly mixed, in a domestic food blender. Oils were added gradually, while mixing constantly. Eighty five ml per 100 grams of water were slowly blended into the mix until the paste had a suitable texture. The dough was kneaded and then processed into thin spaghetti-like strips using a cold-extrusion pasta-maker. The strips were dried out in a convection oven at 30°C for 24 hours and then hammer milled and sieved to a particle size between 200 and 400µm. The diet was then stored at -20°C until required.

For the duration of the experiment, fresh algae and zooplankton were harvested every second day, from the high rate ponds of the algal purification system. The algal water was transported to the Department of Ichthyology and Fisheries Science, Grahamstown in 25 litre containers. The algae was separated from the zooplankton by allowing it to settle for 24 hours, after which the zooplankton was siphoned from the water column and sieved through a 80µm mesh. The zooplankton was then rinsed in fresh water and

fed to the fish. The remaining algal sludge at the bottom of the container was centrifuged in a Beckmann Ja-21 centrifuge machine at 8000 rev/min for 20 minutes and then fed to the fish as fresh algae. The large algal clumps resulting from the centrifugation were fed directly to the fish.

The algae for the sun and freeze dried algal diets was harvested one week prior to the experiment. Excess water was siphoned off and half of the algal sludge was centrifuged at 8000 rev/min for 20 minutes after which it was placed on white flat plastic trays (1m x 0.5m) and left to sun dry for a period of 12 - 15 hours. The second half of the algal sludge was frozen using liquid nitrogen and freeze dried in an Edwards EF4 Modulyo Freeze Dryer for 24 hours. The sun dried algae was hammer milled and sieved to a particle size of between 200µm and 400µm and stored at -20°C until required for feeding. The particle size of the algae after freeze drying was ±75µm.

Chemical Analysis

Each diet was subject to a full proximate composition analysis performed according to the methods described in Chapter 4. The amino acid composition (excluding cystine and tryptophan) of the diets were determined from samples sent to the University of Natal. To quantify how closely the amino acid ratios of *X. helleri* matched the three algal and the zooplankton diets, simple regression analyses were performed, and the coefficients of determination (r^2) calculated.

Experimental fish and Feeding Regime.

The test fish originated from breeding populations of *Xiphophorus helleri, Poecilia reticulata* and *Poecilia velifera* maintained at the Department of Ichthyology and Fisheries Science, Rhodes University. One-hundred and eighty fish of each species between two and three weeks old were selected at random from the broods of various females. These fish were randomly distributed into 36 tanks according to the random design at a stocking density of 1.36 fish per litre. Fish were acclimated in the experimental system for four weeks, during which they were fed finely ground trout pellets to satiation twice daily.

During the course of the trial, feeding was performed twice daily. All fish except those fed zooplankton were fed to satiation. Fish fed zooplankton were fed three livefood items per ml in each tank and the outflows were blocked during feeding to prevent the loss of feed organisms. The mass of the sun dried and freeze dried algae consumed by the fish in each tank was determined by weighing the algae before the experiment and then reweighing it weekly. The amount of fresh algae consumed by the fish in each tank was determined by weighing the algae harvested for each tank before feeding, and subtracting the weight of the algae not fed to the fish in each tank after feeding.

At the beginning of the trial period, and every two weeks thereafter, fish were weighed and measured. All fish were anaesthetised using a solution of 0.2 ml 2-phenoxy-ethenol per litre of water. As the fish were too small to be accurately weighed on an individual basis without incurring undue stress from excessive handling, all fish in each tank were weighed collectively. Fish were captured after being anaesthetised using a small nylon net and excess water was removed by holding the net on a paper towel for ten seconds The fish were then transferred to a pre-measured beaker of water on the scale, and their total mass recorded. The average mass per fish could therefore be estimated. After weighing, the fish were placed on a sheet of graph paper and photographed. The developed photographs were scanned onto a computer using the software package "Deskscan" (Jandel Scientific). The lengths of the fish were then measured and recorded on a spreadsheet using the software package "Sigmascan". Survival was also deduced from this photographic record.

Analysis of length and mass data

The food conversion ratios (FCR) and protein efficiency ratios (PER) were calculated evaluate the performance of fish fed the experimental diets:

Due to the poor survival and initial poor growth of fish in certain treatments, the body mass gain for the FCR's and PER's were calculated from the first time that there was a positive increase in fish mass.

Food conversion ratio (FCR)

FCR = Food Intake (dry mass) / Body Mass Gain (wet mass)

Protein efficiency ratio (PER)

PER = Body Mass Gain (wet mass) / Protein Ingested (dry mass)

All negative FCR's and PER's (where fish constantly lost weight) were excluded from the analyses.

Colour assessment.

Two methods were used to determine the variation in degree of skin pigmentation in the fish at the end of the trial. The first method was a visual assessment of the degree of pigmentation. All the fish from each replicate were pooled and each pool was distributed according to a randomised design in twelve tanks. Constant lighting was supplied to the tanks using 12 desk lamps, all with new 60 watt bulbs. Thirty one people, comprising mostly students studying various degrees were requested, to view the fish to complete a questionnaire which required them to rate the fish in each tank according to their perception of the colour intensity of the fish in each treatment. The rating system varied form category "1" which represented very dull fish, to category "5" which represented very bright fish.

In the second method, 30 fish from each treatment were anaesthetised and colour was measured according to the methods of Wyszeck and Stiles (1967) using a colour analyser (Chromameter CR-200B minolta, Japan, aperture 8mm). Before readings were performed the Chromameter was calibrated with a white plate reference standard (Minolta, Japan). The Chromameter perceives colour in three dimensions, namely hue, chroma and lightness. Hue and chroma are specified by the x and y co-ordinates and the lightness by Y, which is a factor expressed as a percentage based on a perfect reflectance of 100%. The Y, x and y co-ordinates were recorded four times for each individual fish, with the fish being rotated at 90° after each reading (Schmidt and Cuthbert 1969).

Health Evaluation

During the growth experiment mortalities were recorded and dead fish were weighed and measured and examined for any disease symptoms. At the end of the experiment all fish were weighed and measured individually and the condition factor was calculated as follows:

$$\text{Condition Factor (CF)} = \frac{\text{Fish length}^3}{\text{Fish mass}}$$

Fifteen fish from each treatment were sacrificed by administering a sharp blow to the back of the head and thoroughly examined for any diseases, pathological symptoms or physical abnormalities using the procedure shown:

Procedure for health evaluation:

The following procedure to evaluate fish health was performed under a dissecting microscope (2-5x magnification)

1) General surface observation:

- I) Check the colour of the skin, gills and fins.
- ii) Check the skin, fins and scales for abnormalities
- iii) Check for subcutaneous haemorrhagy.
- iv) Check for external fungal infection (visible via light coloured areas)
- v) Check for haemorrhages and/or secondary infections in the mouth.
- vi) Is the fish emaciated? (substantiate with the condition factor)
- vii) Check for symptoms of exophthalmus.
- viii) Check for any skeletal deformations.
- ix) Check for the presence of external parasites.

2) Gill observation:

- i) Check for the presence of fungal growth or necrosis (necrotic areas).
- ii) Check for hyperplasia / hypertrophy / haemorrhages of the filaments and lamellae.

iii) Check for the presence of parasites on the gills.

3) Internal organ observation:

i) Check for internal parasites.

Statistical analysis

Simple regression was carried out on the development of weight and length over time and statistically significant differences in slopes were tested for using an analysis of covariance (ANCOVA)(Zar 1984). The FCR and PER data was analysed using an ANOVA, with multiple range testing (Tukey's) with significance declared at P = 0.05. In addition, a non-parametric Cruscal-Wallace Test was performed on this data to test for significant trends. Observations of the intensity of fish colour were compared by means of contingency table analysis (Zar, 1984). A five by four contingency table was used to determine if significant differences in distribution were present within treatments. A χ^2 analysis in which the distribution of the colour observed in the fish fed control diet was considered to be the expected distribution and was used to test for significant differences in distribution in the treatments. Data from the chomameter colour analysis and data on the health of the fish were analysed using an ANOVA, with multiple range testing (Tukey's) with significance declared at p = 0.05. Data on the chemical analysis of the diets were analysed using an ANOVA, with multiple range testing (Tukey's) with significance declared at p = 0.05.

RESULTS

Water Quality

Total ammonia, nitrite and nitrate remained below 0.01mg/l, 0.25mg/l and 50mg/l respectively. The pH ranged between 7.9 and 8.2 and oxygen levels ranged between 7.7 and 9mg/l.

Proximate analysis and Amino acid composition:

The crude protein value of the control diet was significantly higher than those of the pure algal and zooplankton diets (Table 5.1). The lipid values of the control diet were

significantly higher than those of the zooplankton algal diet. The zooplankton diet had a significantly higher lipid value than those of the algal diets (Table 5.1).

Of the three different algal processing methods the freeze dried algae had the lowest protein, moisture content and ash content, but the highest nitrogen free extract content Sun dried algae had the highest ash content (Table 5.1).

TABLE 5.1. The proximate analysis of the algal, zooplankton and control diets used in the ten week feeding trial (All values are expressed as percentage by weight:, NFE = nitrogen free extract).

	Average composition (% by weight)				
	Moisture	Protein	Lipid	NFE	Ash
Control Diet	6.2^{b} ±0.5	45.62^{c} ±0.3	12.3^{a} ±1.2	25.88^{a} ±0.7	10.3^{a} ±0.3
Fresh Algae (dried*)	5.7^{b} ±0.4	41.47^{b} ±0.2	4.8^{d} ±0.4	35.13^{b} ±0.8	12.9^{ab} ±0.2
Sun Dried Algae	6.7^{b} ±0.4	40.46^{b} ±0.3	5.9^{c} ±0.5	30.14^{ab} ±1.0	16.8^{b} ±0.6
Freeze Dried Algae	3.9^{c} ±0.3	36.96^{a} ±0.4	5.2^{d} ±0.3	44.94^{c} ±0.5	9.0^{a} ±0.3
Zooplankton (dried*)	10.8^{a} ±0.6	36.84^{a} ±0.3	11.1^{b} ±0.8	31.06^{ab} ±0.6	10.2^{a} ±0.3

* Oven dried at 100 °C for 24 hours.

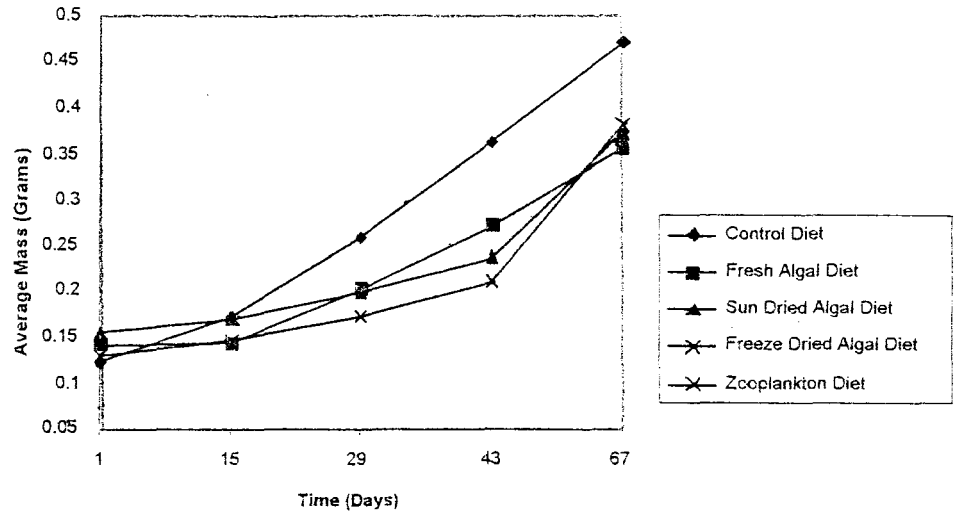
Values in the same column sharing a common superscript were not significantly different from one another ($p>0.05$).

The amino acid profile of the control diet was most similar to the amino acid profile of *X. helleri* body tissue. Freeze dried algae had the lowest arginine, isoleucine, leucine, phenylalanine, threonine and valine values. However, freeze dried algae had an amino acid profile more similar to the amino acid profile of *X. helleri* (r^2=82.46), (Table 5.2) than those of fresh algae, sun dried algae and zooplankton. The amino acid profiles of fresh algae, sun dried algae and zooplankton were similar to each other, but dissimilar to the amino acid profile of *X. helleri* (r^2 = 63.0- 69.57), (Table 5.2).

TABLE 5.2. Amino acid content (g/100g protein), of *X. helleri* and the five diets tested in the ten week growth trial (% dry matter). Bold font indicates essential amino acids. *X.hel* = *X. helleri;* C = Control diet; FA = Fresh Algae; SD = Sun Dried Algae; FD = Freeze Dried Algae; Z = Zooplankton. r^2 = Coefficient of determination with respect to profile of *X. helleri.*

Amino Acid	*X.hel*	C	FA	SD	FD	Z
Arginine	5.67	6.15	5.75	5.99	5.75	5.45
Histidine	2.31	2.44	1.71	1.67	1.87	1.70
Isoleucine	3.78	4.86	4.52	4.51	4.21	4.76
Leucine	6.83	7.97	8.23	8.46	8.26	8.12
Lysine	7.73	8.15	6.13	6.24	6.81	5.82
Phenylalanine	3.89	4.63	5.27	5.22	5.13	5.13
Methionine	2.60	2.61	1.67	1.79	2.04	1.85
Threonine	3.78	4.23	5.46	5.17	4.37	4.99
Valine	4.40	5.39	6.27	6.07	5.72	6.26
Tryptophan						
Aspartate	9.27	10.93	10.91	10.61	11.48	10.3
Serine	4.05	4.01	4.33	4.30	3.50	3.81
Glutamate	15.58	16.46	11.63	12.46	12.83	11.8
Proline	4.66	4.97	5.21	4.97	4.87	5.18
Glycine	7.17	6.06	6.17	6.26	7.23	5.82
Alanine	5.71	5.81	7.74	7.27	6.92	6.91
Tyrosine	2.77	2.88	3.14	3.19	3.22	3.46
Cystine						
Crude Protein	54.5	45.68	41.47	40.46	36.96	36.8
r^2 (%)		94.90	65.33	69.57	82.46	63.0

Fish of all three species grew significantly faster in weight and length than fish fed any of the algae or zooplankton diets (Figures 5.3; 5.4; 5.5; Table 5.3 and 5.4).



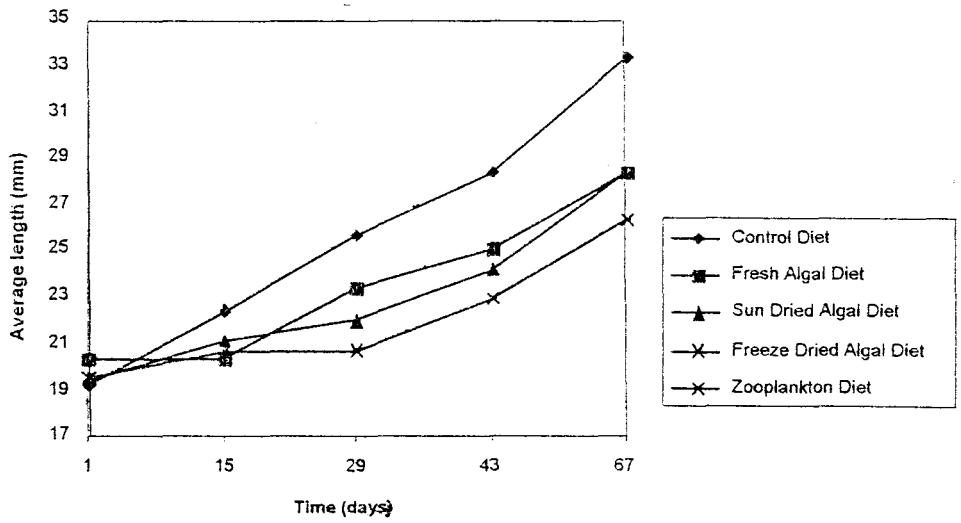
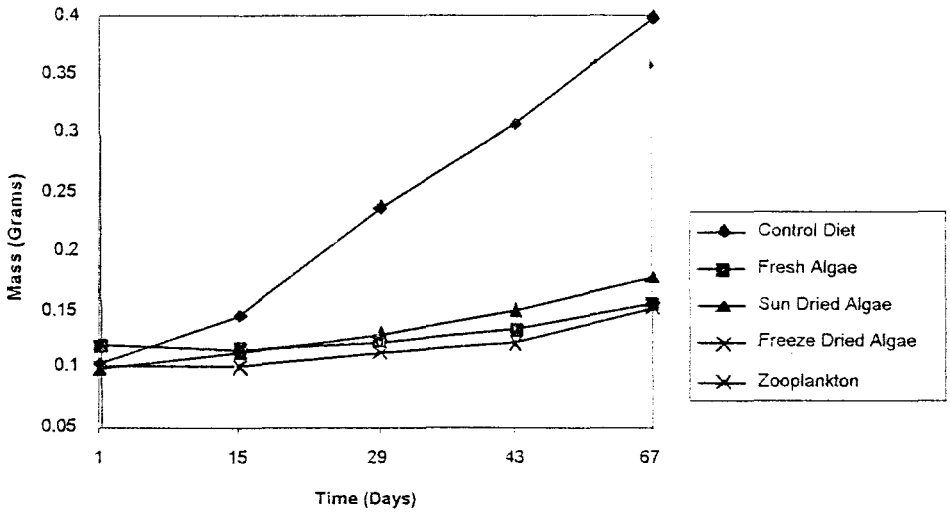


FIGURE 5.3 Growth curves of (mass and length) of juvenile *X. helleri* fed five test diets over the ten week growth trial.



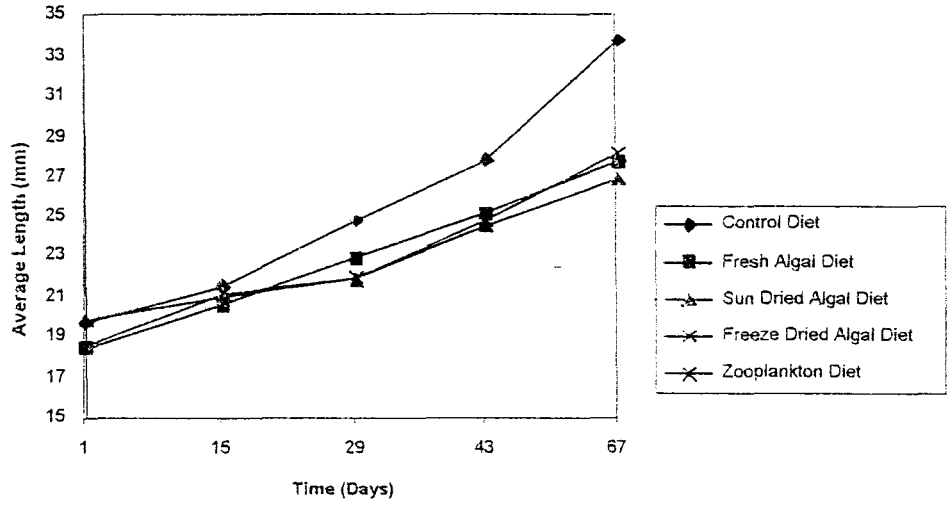
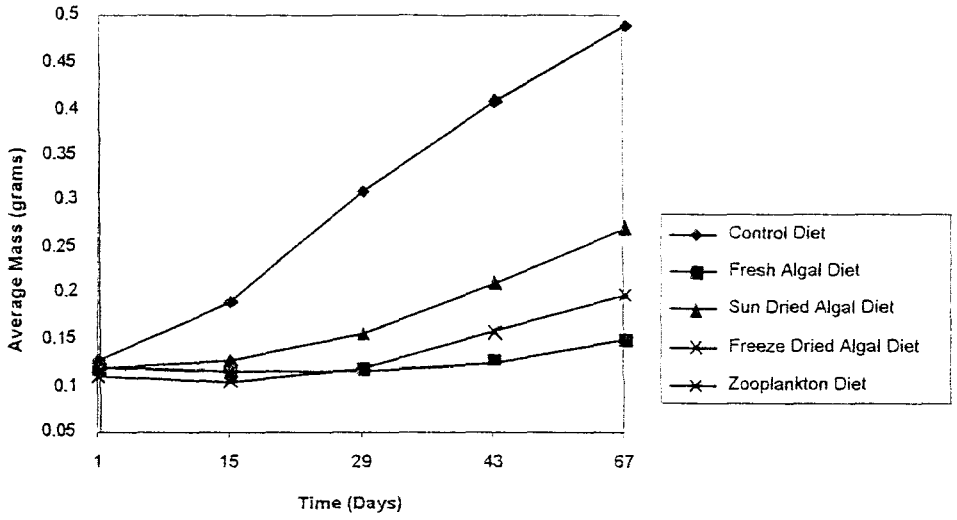


FIGURE 5.4. Growth curves (mass an length) of juvenile *P. reticulata* fed five test diets over the ten week growth trial.



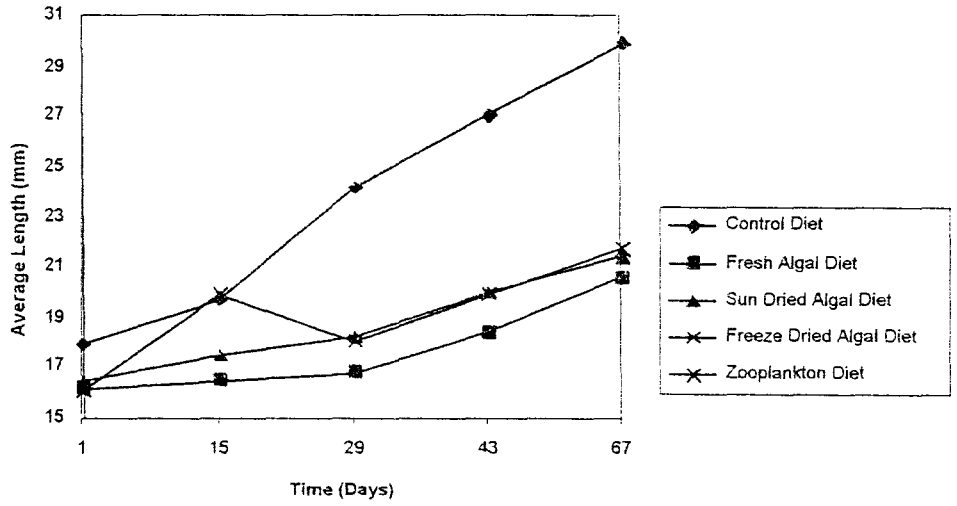


FIGURE 5.5. Growth curves (mass and length) of juvenile *P. velifera* fed five test diets over the ten week trial.

TABLE 5.3 Linear regression equations for the growth curves (mass) of each dietary treatment in *X. helleri, P. reticulata.* and *P. velifera.*

Species	Diet	Regression equation
X. helleri	Control Diet	$y = 0.10 + 0.1300(x)^{a}$
X. helleri	Fresh Algae	$y = 0.08 + 0.0926(x)^{b}$
X. helleri	Sun Dried Algae	$y = 0.13 + 0.0504(x)^{b}$
X. helleri	Freeze Dried Algae*	$y = 0.13 + 0.0162(x)^{b}$
X. helleri	Zooplankton	$y = 0.16 + 0.0902(x)^{b}$
P. reticulata	Control Diet	$y = 0.12 + 0.0753(x)^{x}$
P. reticulata	Fresh Algae	$y = 0.10 + 0.0087(x)^{y}$
P. reticulata	Sun Dried Algae	$y = 0.07 + 0.0196(x)^{y}$
P. reticulata	Freeze Dried Algae*	$y = 0.10 + 0.0083(x)^{y}$
P. reticulata	Zooplankton	$y = 0.07 + 0.0159(x)^{y}$
P. velifera	Control Diet	$y = 0.02 + 0.0945(x)^{m}$
P. velifera	Fresh Algae	$y = 0.10 + 0.0070(x)^{n}$
P. velifera	Sun Dried Algae	$y = 0.06 + 0.0389(x)^{n}$
P. velifera	Freeze Dried Algae*	$y = 0.12 - 0.0069(x)^{n}$
P. velifera	Zooplankton	$y = 0.03 + 0.0320(x)^{n}$

Equations sharing a common superscript do not show significant differences in slope ($P>0.05$).

*Three week dietary treatment

TABLE 5.4. Linear regression equations for the growth curves (length) of each dietary treatment in *X. helleri*.

Species	Diet	Regression Equation
X. helleri	Control Diet	$y = 15.96 + 3.54(x)^{a}$
X. helleri	Fresh Algae	$y = 16.19 + 2.86(x)^{b}$
X. helleri	Sun Dried Algae	$y = 17.55 + 1.74(x)^{c}$
X. helleri	Freeze Dried Algae*	$y = 15.81 + 2.66(x)^{b}$
X. helleri	Zooplankton	$y = 15.95 + 2.30(x)^{b}$
P. reticulata	Control Diet	$y = 15.47 + 3.42(x)^{x}$
P. reticulata	Fresh Algae	$y = 17.40 + 1.93(x)^{y}$
P. reticulata	Sun Dried Algae	$y = 16.72 + 2.09(x)^{y}$
P. reticulata	Freeze Dried Algae*	$y = 18.38 + 1.12(x)^{y}$
P. reticulata	Zooplankton	$y = 15.95 + 1.91(x)^{y}$
P. velifera	Control Diet	$y = 14.30 + 3.14(x)^{n}$
P. velifera	Fresh Algae	$y = 15.64 + 0.49(x)^{m}$
P. velifera	Sun Dried Algae	$y = 14.99 + 1.23(x)^{m}$
P. velifera	Freeze Dried Algae*	$y = 12.08 + 3.92(x)^{n}$
P. velifera	Zooplankton	$y = 17.91 + 0.58(x)^{m}$

Equations for each species sharing a common superscript do not show significant differences in slope ($P>0.05$).

*Three week dietary treatment

There were no significant differences in the slopes of the growth curves of *X. helleri, P. reticulata* and *P. velifera* fed the pure algal and zooplankton diets, except in the length growth curve of *X. helleri* fed the sun dried algal diet which had a significantly lower slope (Tables 5.3 and 5.4). After three weeks, *P. reticulata* and *X. helleri* fed the freeze dried algal diet had low rates of mass and length gain.

During feeding, fish fed the algal diets were initially (±2 minutes) observed to feed readily, after which they began spitting out the algae seconds after ingestion. Although not quantified, a decrease in feeding urgency compared to fish fed the control diet was noted. The large clumps of centrifuged fresh algae were observed to quickly sink to the bottom once placed into the tank and appeared difficult for poeciliids to ingest due to the very large particle size. The sun dried algae and control diet floated on the water surface and were easily ingested by the fish. Although the freeze dried algae floated at the surface, its particle size (±75µm) appeared to small for the fish to ingest and large amounts of it appeared to dissolve into the water. Zooplankton was ingested readily by all species. Generally, after fish were fed zooplankton, the feeding activity was very high for the first two minutes, after which it decreased dramatically and ceased totally after 5 - 10 minutes.

There was large variation in the FCR's and PER's between diets and large variation in the FCR's and PER's in the same diets between species (Table 5.5 and 5.6).
All fish fed the control and zooplankton diets had the lowest FCR's. Swordtails fed the fresh algal diet had a significantly higher FCR than fish fed the control and zooplankton diet. Swordtails fed the freeze dried algal diet had a negative FCR (Table 5.5).

While guppies fed the control and zooplankton diets had significantly lower FCR's than fish fed the fresh algal diet, they were not significantly lower than fish fed the sun dried algal diet. Only one replicate of guppies fed the freeze dried algal diet had a positive FCR (Table 5.5).

There was no significant difference in the FCR for all dietary treatments in mollies. The lowest FCR was recorded in fish fed the zooplankton diet and the highest FCR's were recorded in fish fed the sun dried and fresh algal diets (Table 5.5).

TABLE 5.5. The feed conversion ratios of *X. helleri, P. reticulata* and *P. velifera* fed five test diets over a ten week growth trial.

Diet	Mean FCR (70 days) ±SD		
	X. helleri p=0.09	*P. reticulata* p=0.20	*P. velifera* p=0.14
Control	2.36[a] ±0.1	2.17[a] ±0.24	2.96[a] ±0.25
Fresh Algae	8.16[b] ±2.1	4.61[b] ±0.51	5.12[a] ±0.47
Sun Dried Algae	4.43[ab] ±1.1	3.44[ab] ±1.1	5.20[a] ±0.71
Freeze Dried Algae	97.5 ±0.0*	negative	3.01[a] ±0.5
Zooplankton	1.75[a] ±0.3	2.37[a] ±0.31	1.58[a] ±0.32

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

Values in the same column sharing a common superscript are not significantly different.

*n = 1 (other replicates negative)

X. helleri fed the control and zooplankton diets had significantly higher PER's than those fed the fresh algal diet. Fish fed the sun dried algal diets did not have significantly different PER's from those fed any of the other diets (Table 5.6). After three weeks, only one of the replicates of *X. helleri* fed the freeze dried algal diet had a positive PER.

TABLE 5.6. The protein efficiency ratios (PER) of *X. helleri, P. reticulata* and *P. velifera* fed five test diets over a ten week growth trial.

Diet	Mean PER (70 days) ±SD		
	X. helleri p=0.09	*P. reticulata* p=0.19	*P. velifera* 0.13
Control	0.95^{a} ±0.3	0.93^{a} ±0.1	0.76^{a} ±0.1
Fresh Algae	0.31^{b} ±0.1	0.28^{a} ±0.2	0.30^{a} ±0.1
Sun Dried Algae	0.59^{ab} ±0.1	0.45^{a} ±0.1	0.31^{a} ±0.2
Freeze Dried Algae	0.02 ±0.0*	negative	0.70^{a} ±0.1
Zooplankton	1.32^{a} ±02	0.73^{a} ±0.2	1.21^{a} ±0.2

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

Values in the same column sharing a common superscript are not significantly different.

*n = 1 (other replicates negative)

Colour enhancement

Visual observation:

The visual observation on the colour of the fish, after analysis using contingency tables, revealed an unequal distribution for *X. helleri* (χ^2 = 22.49) and *P. velifera* (χ^2 = 39.49) and *P. reticulata* (χ^2 = 25.72). The distribution of observations to the categories was different in the test diets from the control diet for all species, with higher scores in the categories referring to bright coloured fish in guppies and with higher scores in the areas referring to dull coloured fish in swordtails and mollies (Table 5.7).

Table 5.7. The number of people perceiving three species of poeciliids, fed four test diets to be in a specific colour intensity category after a ten week growth trial (1 = very dull; 2 = dull; 3 = average; 4 = bright; 5 = very bright).

Species	Diet	NUMBER OF OBSERVATIONS 1	2	3	4	5 (degree of colour intensity) →	χ^2 df = 4 (k=1)
X. helleri	Control Diet	0	6	4	19	2	
X. helleri	Fresh Algae	0	4	12	14	1	18.5
X. helleri	Sun Dried Algae	0	10	13	8	0	31.28
X. helleri	Zooplankton	0	2	18	10	1	56.43
P. reticulata	Control Diet	0	8	12	8	3	
P. reticulata	Fresh Algae	0	0	10	16	5	17.67
P. reticulata	Sun Dried Algae	0	0	5	16	10	36.42
P. reticulata	Zooplankton	0	5	9	13	4	5.33
P. velifera	Control Diet	0	2	14	15	1	
P. velifera	Fresh Algae	2	15	11	2	2	97.41
P. velifera	Sun Dried Algae	0	7	19	6	0	20.69
P. velifera	Zooplankton	1	14	15	2	0	84.34

Chromameter analysis

There were no significant differences in lightness, chroma or hue between *X. helleri* fed any of the test diets (Table 5.8).

TABLE 5.8. Colour measurements of *X. helleri* at the end of the growth trial, expressed in lightness, chroma and hue.

Diet	Lightness Mean±SE	Chroma Mean±SE	Hue Mean±SE
Control	19.585^{a}±1.69	0.427^{a}±0.01	0.398^{a}±0.004
Fresh Algae	21.07^{a}±1.74	0 .431^{a}0.01	0.387^{a}±0.01
Sun Dried Algae	22.13^{a}±2.28	0.440^{a}±0.01	0.399^{a}±0.01
Zooplankton	22.30^{a}±2.52	0.423^{a}±0.01	0.393^{a}±0.01

Values in the same column sharing a common superscript are not significantly different from one another (P> 0.05).

P. reticulata showed no significant differences in lightness in fish fed any diet. The chroma values for guppies fed the sun dried algal diet and were significantly higher than those fed the control diet. The fish fed the sun dried algal diet had significantly higher hue values than those fed any of the other diets (Table 5.9).

TABLE 5.9. Colour measurements of *P. reticulata* at the end of the growth trial, expressed in lightness, chroma and hue.

Diet	Lightness Mean±SE	Chroma Mean±SE	Hue Mean±SE
Control	18.62^{a} ±3.63	0.355^{a} ±0.01	0.352^{a} ±0.003
Fresh Algae	16.5^{a} ±3.77	0.399^{ab} ±0.01	0.355^{a} ±0.01
Sun Dried Algae	15.62^{a} ±2.19	0.427^{b} ±0.02	0.374^{b} ±0.01
Zooplankton	13.27^{a} ±1.67	0.402^{ab} ±0.01	0.350^{a} ±0.002

Values in the same column sharing a common superscript are not significantly different from one another (P> 0.05).

P. velifera fed the control and zooplankton diets were significantly darker than those fed the fresh algae. Chroma values for fish fed the control and fresh algal diet were significantly higher than those fed the zooplankton diet. The hue values for fish fed the zooplankton diet were significantly higher than fish fed the control and sun dried algal diets (Table 5.10).

Table 5.10. Colour measurements of *P. velifera* at the end of the growth trial, expressed in lightness, chroma and hue.

Diet	Lightness Mean±SE	Chroma Mean±SE	Hue Mean±SE
Control	27.147^{ab} ±2.08	0.316^{ab} ±0.002	0.331^{a} ±0.00
Fresh Algae	23.261^{a} ±1.57	0.321^{a} ±0.003	0.333^{ab} ±0.004
Sun Dried Algae	33.171^{b} ±1.21	0.324^{a} ±0.001	0.337^{a} ±0.001
Zooplankton	26.155^{a} ±1.59	0.304^{b} ±0.002	0.341^{b} ±0.003

Values in the same column sharing a common superscript are not significantly different from one another (P> 0.05).

Health evaluation:

Condition Factor

There were no significant differences in the condition factor of *X. helleri* and *P. reticulata* fed any of the test diets (Table 5.11). *P. velifera* fed the fresh algal diet had a significantly lower condition factor than fish fed any other diet. In addition, *P. velifera* fed the control diet had significantly higher condition factor values than those fed the fresh and sun dried algal diets, but not significantly better than those fed the zooplankton diet (Table 5.11).

TABLE 5.11. The condition factor of *X. helleri, P. reticulata* and *P. velifera* fed the four test diets at the end of the ten week growth trial.

Diet	*X .helleri*	*P. reticulata*	*P. velifera*
Control	72.50^{a} ±4.54	142.23^{a} ±11.20	81.96^{a} ±3.21
Fresh Algae	89.45^{a} ±3.92	126.71^{a} ±14.30	23.20^{c} ±1.17
Sun Dried Algae	76.38^{a} ±4.67	117.92^{a} ±12.86	72.24^{b} ±2.98
Zooplankton	74.86^{a} ±4.66	129.50^{a} ±15.67	78.70^{ab}±2.12

Values in the same column sharing a common superscript are not statistically different (P> 0.05).

Survival

After three weeks, *X helleri* fed the freeze dried algal diet had a significantly lower survival rate than those fed the control and sun dried algal diet. After ten weeks, the fish fed the control diet had a significantly higher survival rate than those fed any of the other diets (Table 5.12).

TABLE 5.12. Mean survival of *X. helleri* fed five test diets at three weeks and at the end of a ten week feeding trial.

Diet	Three weeks Mean Survival(%) ±SE	Ten weeks Mean survival(%) ±SE
Control	91.7^{a} ±1.2	66.67^{a} ±10.9
Fresh Algae	89.6^{ab} ±2.5	20.00^{b} ±2.9
Sun dried algae	97.7^{a} ±0.5	25.00^{b} ±10.0
Freeze dried algae	73.3^{b} ±5.2	
Zooplankton		16.67^{b} ±1.7

Values in the same column sharing a common superscript are not significantly different (P> 0.05)

P. reticulata fed the control and sun dried algal diets had a significantly higher survival rate after three weeks of feeding than fish fed the freeze dried and fresh algal diets. After ten weeks, fish fed the control diet had a significantly higher survival rate than those fed fresh algae and zooplankton (Table 5.13).

TABLE 5.13. Mean survival of *P. reticulata* fed five test diets at three weeks and at the end of a ten week feeding trial.

Diet	Three weeks Mean Survival(%) ±SE	Ten weeks Mean survival(%) ±SE
Control	91.5^{a} ±3.8	93.33^{a} ±6.7
Fresh algae	60.0^{b} ±5.7	56.67^{b} ±6.0
Sun dried algae	83.3^{a} ±6.1	80.00^{ab} ±5.8
Freeze dried algae	59.5^{b} ±5.8	
Zooplankton		61.67^{b} ±4.4

Values in the same column sharing a common superscript are not significantly different (P> 0.05)

After three weeks of feeding *P. velifera* fed the freeze dried algal diet had a significantly lower survival rate than the fish fed the control and sun dried algal diets. At the end of the experiment the fish fed the control diet had a significantly higher survival rate than those fed any other diet (Table 5.14).

TABLE 5.14. Mean survival of *P. velifera* fed five test diets at three weeks and at the end of a ten week feeding trial.

Diet	Three weeks Mean Survival(%) ±SE	Ten weeks Mean survival(%) ±SE
Control	97.5^{a} ±1.3	93.33^{a} ±6.7
Fresh algae	83.3^{ab} ±4.2	56.67^{b} ±16.7
Sun dried algae	95.6^{a} ±1.5	60.00^{b} ±7.6
Freeze dried algae	68.2^{b} ±6.3	
Zooplankton		43.33^{b} ±15.9

Values in the same column sharing a common superscript are not significantly different (P> 0.05)

Health evaluation

Sixty-three percent of the of all fish which died during of the ten week experiment were emaciated. In addition, secondary fungal infections were detected on 25% on all of these fish. No other signs of ill health were detected.

While signs of subcutaneous haemorrhagy were found on some *X. helleri* fed the pure algal and zooplankton diets at the end of the experiment, none were found on fish fed the control diet. No fish fed the control diet were emaciated, while four percent of the fish fed the pure algal and zooplankton diets were. No other symptoms of ill health were detected in any fish (Table 5.15).

TABLE 5.15. Results of the external body examination after the ten week growth trial in *X. helleri* (Results expressed as a pooled percentage for each treatment:).

Diet	Subcutaneous haemorrhage	Emaciation
Control Diet	0.00^a ±0.0	0.00^a ±0.0
Fresh Algal Diet	2.22^a ±1.1	7.00^a ±3.6
Sun Dried Algae Diet	1.11^a ±1.1	3.33^a ±1.9
Zooplankton Diet	1.40^a ±1.0	2.22^a ±2.2

Values in the same column sharing a common superscript were not significantly different from one another (P>0.05).

Eight percent of *P. reticulata* were found to have signs of subcutaneous haemorrhagy, however there were no significant differences between treatments. Although there was no significant difference, six percent of fish fed the pure algal diets were found to be emaciated, while no fish fed the control diet and zooplankton diet were (Table 5.16).

TABLE 5.16. Results of the external body examination after the ten week growth trial in *P. reticulata* (Results expressed as a pooled percentage for each treatment).

Diet	Subcutaneous haemorrhagy	Emaciation	Exophthalmus	Scoliosis
Control Diet	5.44^a ±2.3	0.00^a ±0.0	0.00^a ±0.0	1.11^a ±1.1
Fresh Algal Diet	6.67^a ±1.9	5.56^a ±1.1	2.22^a ±2.2	1.11^a ±1.1
Sun Dried Algal Diet	13.33^a ±3.8	6.67^a ±1.92	1.11^a ±1.1	1.11^a ±1.1
Zooplankton Diet	7.78^a ±4.4	0.00^a ±0.0	1.11^a ±1.1	2.22^a ±2.2

Values in the same column sharing a common superscript were not significantly different from one another (P>0.05).

Less than one percent of guppies were found to have exophthalmus and scoliosis, but there were no significant differences between treatments. No other sign of ill health were found in *P. reticulata* (Table 5.16).

Although signs of subcutaneous haemorrhagy were found on the skin surface of *P. velifera*, no treatment had significantly more than another. Although *P. velifera* fed the control diet were not emaciated and while 6.8 percent of fish fed the pure algal and zooplankton were, the differences were not significant. While two percent of mollies fed the control diet had exophthalmus, this was not significantly different from the fish fed the other diets. No other disease symptoms were recorded (Table 5.17).

TABLE 5.17. Results of the external body health examination after the ten week growth trial in *P. velifera*. (Results expressed as a pooled percentage for each treatment).

Diet	Subcutaneous haemorrhagy	Emaciation	Exophthalmus
Control Diet	5.55^{a} ±2.9	0.00a ±0.0	2.22^{a} ±2.2
Fresh Algal Diet	3.33^{a} ±1.9	6.67^{a} ±1.9	0.00^{a} ±0.0
Sun Dried Algal Diet	6.67^{a} ±1.9	7.00^{a} ±3.6	0.00^{a} ±0.0
Zooplankton Diet	6.89^{a} ±1.7	6.67^{a} ±1.9	0.00^{a} ±0.0

Values in the same column sharing a common superscript were not significantly different from one another (P>0.05).

DISCUSSION

Growth Trial

The significantly lower growth rates of all species fed the pure algal and zooplankton diets is consistent with a number of other studies involving single ingredient feeds (Stanley and Jones, 1976). A number of possible explanations may exist for this

phenomenon and for the lower survival rates of the fish fed the algal diets. These include nutritional imbalances, such as low crude protein, lipid or carbohydrate contents or incorrect balance of essential amino acids, low digestibility of the feed ingredient (Wong *et al,* 1984) and anti nutritional factors.

The average crude protein contents of the pure algal and zooplankton diets were lower than the control diet (Table 5.1), suggesting that the low crude protein content of the single ingredient feeds may have contributed to the depressed growth rates. However in Singapore, poeciliid diets have lower crude protein contents than the pure algal and zooplankton diets and promote adequate growth (Chapter 4). Since almost all of the essential amino acid values of the pure algal and zooplankton diets were lower than those of the control diet, an incorrect balance of essential amino acids may explain why the fish fed the control diet had a significantly higher growth rate.

The lipid contents of the algal diets were also lower than those of the control diet. Since lipids function as high energy storage molecules (De Silva and Anderson, 1995), this may also have contributed to the depressed growth rates. The NFE content of the algal and zooplankton diets were considerably higher that of the control diet. Carbohydrates function as readily metobolisable energy stores and molecules which transfer energy throughout the organism as structural components (De Silva an Anderson, 1995). The basic units of carbohydrates are monosacharides (Tacon, 1990). Monosacharides occur primarily in nature in their circular form or in polysaccharides (Tacon, 1990). One important polysaccharide is cellulose, and is the primary structural component of algal cell walls. While under conditions of food deprivation, fish absorbed plant cell wall material (Horn, 1989), for practical purposes cellulose is considered unavailable to fish (Tacon, 1990). Therefore, much of the carbohydrate content of the algal diets may have been unavailable to the poeciliids and were not used for energy.

The chemical composition of the fresh and sun dried algal diets were very similar and this was reflected in the growth rates of the fish. This indicates that sun drying the algae does not have a significant effect on its nutritional value to poeciliids.

Fish fed the zooplankton diet were not fed to satiation. While fish fed actively on the zooplankton for the first two minutes after feeding, feeding activity decreased rapidly after this time. This suggested that most of the zooplankton was consumed in the first few minutes after feeding and that the fish may not have been satiated. Therefore, a combination of the low feeding rate and the poor protein content and amino acid composition of the zooplankton may have resulted in the significantly lower growth rates of these fish.

However, although all fish species fed the zooplankton diet grew at a significantly lower rate than fish fed the control diet, the FCR's of guppies and mollies fed the zooplankton diet were lower than those fed the control diet. This suggests that these fish were able to utilise zooplankton efficiently for growth and that the low feeding rate was a more likely explanation for the poor fish growth rather than the nutritional inadequacy of the zooplankton.

The significantly higher growth rates of poeciliids fed the control diets were complemented by these fish also having lower FCR's than any of the fish fed the algal diets. This suggests that fish fed the algal diets were unable to utilise these diets as efficiently as the control and zooplankton diets for growth. In all species, fish fed the freeze dried algal diets had the poorest FCR's. While the percentage protein of the freeze dried algae was significantly lower than the other algal diets, the amino acid composition of freeze dried algae was more favourable with respect to *X. helleri* than the other algal diets. Therefore, an explanation to the poor growth of fish fed the freeze dried algal diets may not be in its chemical composition. Since the nutritional effectiveness of a food organism is also determined by its ingestibility, the small particle size of the freeze dried algae and the subsequent feeding problems experienced by the fish during the growth trial were considered to have an negative effect on the growth of fish fed this diet.

Although not significant due a large standard error, the PER's of all species fed the algal diets were lower than those of fish fed the control and zooplankton diet. This suggests that poeciliids were unable to utilise the protein of algae as efficiently as the protein of the control and zooplankton diet. Since the amino acid profile of the fresh (r^2= 65%) and sun dried algae (r^2=70%) appeared inferior to the control diet (r^2 =95%) with respect to the body tissue of *X. helleri,* the low protein efficiency ratios were expected. Although the amino acid profile of zooplankton (r^2= 63%) also appeared inferior to that of the control diet (r^2 =95%), the PER values for swordtails and mollies fed the zooplankton diet were higher (although not significantly) than those fed the control diet. Thus, despite the apparent poor amino acid composition of zooplankton, poeciliids were able to utilise the zooplankton protein efficiently for growth. This finding is in agreement with De la Noue and Choubert (1985), who suggested that the protein quality of livefeed is excellent, when compared to artificial feeds.

Overall, the nutritional quality of effluent grown algae, after any form of processing was not sufficient to promote optimal poeciliid growth. The similar growth rates, FCR's and PER's, of fish fed sun dried and fresh algae indicate that sun drying has no effect on the nutritional value of algal diets to poeciliids. However, this algae was sun dried over a short period of time (6-7 hours). The algae which is pumped onto the sand beds at the Grahamstown Sewage Works has an average drying time of one week. Since long exposure of the algae to light and heat has been found to reduced the vitamin content of algae (Oswald and Goloueke, 1967), the prolonged drying period at the Grahamstown Sewage Works may effect the nutritional value of the algae to poeciliids, and needs to be tested.

The feeding problems experienced by poeciliids when feeding on centrifuged fresh algae suggests that sun dried algae is better poeciliid food. The high cost associated with the centrifugation, as well as the increased labour required for the daily harvest of fresh algae also makes sun dried algae more cost effective. Freeze drying the algae was considered the worst method of algal processing for use as poeciliid food because of the poor growth of poeciliids after three weeks, the algal ingestion problems of

poeciliids, the significantly lower protein content of freeze dried algae, and the low protein quality with freeze dried algae having the lowest arginine, isoleucine, leucine, phenylalanine, threonine and valine values.

Since zooplankton fed to poeciliids at a feeding level of six items of zooplankton per ml. per day did not support adequate growth, the potential of zooplankton when fed to poeciliids at a higher feeding rate must be considered.

Results of the visual colour observation revealed that *X. helleri* fed the control diet were considered brighter than fish fed the other diets. Choubert and Heinrich (1992), measuring muscle pigmentation of *O. mykiss* using a Chromameter, found that increased pigmentation of the trout muscle caused an increase in chroma and a decrease in hue and lightness in corresponding muscle samples. The chroma meter analysis of *X. helleri* revealed no significant differences in lightness, chroma or hue between fish in any treatment. The apparent conflict of results between the visual observation and chroma meter analysis renders them inconclusive. However, the results of the visual observation may have been biased by the large size difference between fish fed the control diet and the other diets. *P. velifera* fed the control diet were also considered brighter in the visual analysis than fish fed the other diets. Since *P. velifera* are predominantly white fish, one would expect the Chromameter lightness values to express their colour better than the chroma values. However, there were no significant differences in the lightness readings of between *P. velifera* fed the control diet and any other diet. Again, differences in results between the visual and chroma meter analysis may be due to the larger fish (fed the control diet) causing a bias in the visual observation. Both the Chromameter and visual analysis showed that *P. reticulata* fed the fresh and sun dried algae were significantly brighter and fish fed the control diet.

All species fed the algal and zooplankton diets had significantly higher mortalities than those fed the control diet at the end of the experiment. This suggests that the effluent grown algae and zooplankton may have had a toxicological effect on the fish, or the diets may have been nutritionally deficient. Autopsies of the fish during the course of the

experiment revealed that while there were no symptoms of disease in the fish which died during the course of the growth trial, 63% of the dead fish were emaciated. This suggests that rather than the algae and zooplankton having a toxicological effect on the fish, they were nutritionally inadequate. This is supported by the low growth rates, high FCR's and low PER's exhibited by the surviving fish fed the pure algal diets.

Despite the low growth rates and survival observed in fish fed the algal diets, the low cost of algal production (Chapter 1), harvest (Chapter 3) and processing (sun drying) combined with the poeciliid colour enhancement justified further research into the use of sun dried algae as a food source for poeciliids. Since the proximate analysis and amino acid composition of the algae was similar to that of soyabean meal which is frequently used in aquaculture, the microalgae was still considered a prospect as a feed ingredient in formulated diets. Despite the low growth rates of the fish fed zooplankton, its chemical composition suggested that it had potential as a feed supplement.

CHAPTER 6

GASTRIC EVACUATION TIME AND APPARENT CRUDE PROTEIN DIGESTIBILITY OF EFFLUENT GROWN ALGAE IN THREE POECILIID SPECIES.

INTRODUCTION

Digestibility quantifies the extent to which ingested food and its natural components have been digested and absorbed by the animal (De Silva and Anderson 1995). Nutrient digestibility refers to a specified nutrient such as lipid or protein (De Silva and Anderson 1995). Digestibility and absorption of dietary protein varies considerably depending on the protein source (Lovell, 1977; Jobling, 1983). Generally fishmeal is digested efficiently, often exceeding 90% digestibility. However the apparent protein digestibility of various plant products tested is extremely variable. For example, the apparent crude protein digestibility of soyabean meal was calculated to be 79.95% (Sullivan and Reigh, 1995), while the apparent crude protein digestibility of corn gluten meal was calculated to be 96% (Cho *et al.*, 1985). Equally the digestibility a plant protein source by different fish species is variable (De Silva and Anderson, 1995). For example the protein digestibility of soya meal by rainbow trout (*Oncorhynchus mykiss*) was calculated to be 91.5% (Watanabe and Pongmaneerat, 1993), while the protein digestibility of soya meal by channel catfish (*Ictalurus punctatus*) was calculated to be 79.9% (Sullivan and Reigh, 1995). Digestion may also be hindered by the presence of enzyme inhibitors within the protein, such as the trypsin inhibitor in raw soybeans (Tacon 1990).

The digestibility of the protein and availability of the amino acids to the fish becomes important when considering diet formulation. When diets have the same crude protein content but incorporate different ingredients, with different digestibilities, the availability of protein may vary from diet to diet and the performance of the cultured animals may be

different. Therefore, when evaluating effluent grown algae as an alternative protein source for poeciliids, a knowledge of its digestibility should ideally precede feed formulation.

The ratio of the length of the digestive tract relative to body length is known as the relative gut length (RGL), and the mean for a species (mRGL) is indicative of its feeding habit (De Silva and Anderson 1995). Generally, the mRGL is highest in species that feed on detritus and algae and shortest in carnivorous species. Since the three species of poeciliids investigated were known to have different dietary preferences in nature (cf. chpt.4), it was expected that the mRGL would differ between species. This may influence the rate of digestion and the digestibility of the algae by each species. Therefore the mRGL was determined for each species.

Due to the inherent difficulty in measuring true digestibility quantitatively (Jobling 1983), the values in this study represent 'apparent' digestibility and was determined indirectly by means of a marker in the feed. Chromic oxide (Cr_2O_3) has been widely used as a marker in the indirect method of measuring digestibility (Nose 1960, 1961; Inaba *et al.* 1962; Nehring 1963; Cho *et al.* 1974, 1976; Lall and Bishop 1976; Austreng 1977; Atikinson *et al.* 1984; Dong *et al.* 1993 and Irish 1996) . This method circumvents the need to collect all the faeces produced from a meal and is expected to be a suitable method to determine the crude protein digestibility in this study.

Estimates of digestibility in rainbow trout obtained from analysis of faeces recovered after being voided into pond water were consistently about 10% units higher than those from analysis of faeces stripped manually from the fish (Austreng 1977). This indicated leaching of the nitrogen compounds into the water. These results were substantiated by Pierre (undated) who found a 5% difference between the two methods of collection. Austreng (1977) concluded that stripping was the most convenient way of collecting faeces. Stripping fish, particularly small fish does however have inherent problems. The first of these is the contamination of the faeces with nitrogenous compounds from the urine (Austreng 1977). It is also possible that samples squeezed out of the intestinal

segments can be contaminated with digestive juices and small particles of intestinal epithelium (Austreng 1977). The proteinaceous mucous which coats the skin may also contaminate the faeces during stripping. Austreng (1977) concluded that although stripping was the most convenient method of collecting faeces, absorption of protein occurs far backwards in the lower intestine and thus it is advisable to take faecal samples from as close to the anus as possible. This view was supported by De Silva and Anderson (1995) who suggest that sacrificing the fish and dissecting the rectal contents out, is subject to the least error. Thus, in this study the decision was made to kill the fish and to remove faecal samples from as close to the anus as possible.

To obtain a sufficient amount of matter from as close to the anus as possible in these small fish, their rate of digestion had to be predicted.

A variety of terms are used to describe the rate of digestion (De Silva and Anderson 1995): -

Gut transit time:	The time interval between ingestion and the first appearance of faeces.
Gut evacuation time:	Time taken for the entire quantity of ingested food to be voided
Gastric evacuation time :	Time taken between ingestion and emptying of the stomach.

The alimentary canal of poeciliids and carp like species can be distinguished from other aquaculturally important species such as the cold water salmonoids and catfish by the absence of a stomach. Thus the measurement of gastric evacuation time is not possible in these fish.

To determine the time after feeding which would yield the largest faecal samples, as close to the anus as possible, both the gut transit time and the gut evacuation time were estimated using different techniques.

Many investigators have used X-radiography to monitor food movement through the digestive system (Molner and Tolg 1960; Goddard 1970, 1974; Edwards 1971, 1973 Jobling et al. 1977; Fange and Grove 1979; Talbot 1985). X- radiography does have inherent problems. It generally involves stressing the fish due to constant handling, and in some cases force feeding (De Silva and Anderson 1995). Jorgenson and Jobling (1987) found that the marker in the feed of Arctic charr *Salvelinus alpinus* did not appear to pass through the stomach at the same rate as other food components. In addition the digestive tract of certain fish species may be too long and coiled to determine the exact position of the food and marker. X-radiography was therefore evaluated as a method of determining the gut transit and gut evacuation times of *X. helleri.*

Colour dyes have been incorporated into diets and the time at which the dye first appears in the faeces is determined (De Silva and Anderson, 1995). Since previous gastric evacuation studies have suggested that both the composition and the surface-to-volume ratios of the food types are important in determining the patterns of gastric emptying (Jobling 1987), the colour dye experiment was designed to compare the difference between the gastric evacuation time of sun dried algae and a conventional formulated diet. The results of this experiment were also used to determine if sun dried algae or the conventional formulated diet could be used in the following gut evacuation studies.

Brett and Higgs (1970), Elliot (1972) and Peters and Hoss (1974) recovered the stomach contents by sacrificing the fish at predetermined intervals after a voluntary or forced meal. Using this method the amount remaining in the stomach (or intestine in this case) can be estimated as a percentage of the volume, weight (dry or wet), ash-free dry weight or calorific value of the amount ingested (De Silva and Anderson 1995).

By means of the above techniques, the gut evacuation time and gut transit time of the three species of poeciliid was determined. These times were used to estimate the optimum time at which to sacrifice the fish after feeding, to obtain the maximum amount of faecal matter from as close to the anus as possible.

Once sufficient faecal matter was obtained, the apparent crude protein digestibility of sun dried algae could be calculated.

METHODS AND MATERIALS

Experimental diets

The diet for the x - radiography experiment was the same as the control diet in the previous growth trial (Chapter 5), except for the inclusion of barium sulphate at a level of one percent (Table 6.1). Similarly, the first dye experimental diet incorporated one percent of red dye into the control diet. The second dye experimental diet consisted of 99% sun dried algae and one percent red dye (Table 6.1). Pure sun dried algae was used in the fish sacrifice study and starch was used to bind the chromic oxide and sun dried algae in the digestibility study (Table 6.1).

All diets were prepared using the guidelines for experimental feed preparation described by Lovell (1989). The sun dried algae was harvested from the high rate ponds at the Grahamstown sewage works and sun-dried on white plastic boards for a period of eight hours. All diets were hammer milled and the sieved to a particle size of between 200 and 300µm.

TABLE 6.1. Composition of the diets for the x-radiography (R), dye (D1 + D2), fish sacrifice study (S), and digestibility (Dig) studies.

INGREDIENT	DIET				
(% Dry Mass)	R	D1	D2	S	Dig
Fishmeal	45.0	45.0	0.0	0.0	0.0
Soya oil cake meal	24.0	24.0	0.0	0.0	0.0
Fish oil	3.5	3.5	0.0	0.0	0.0
Sunflower oil	3.5	3.5	0.0	0.0	0.0
Starch	15.0	15.0	0.0	0.0	5.0
Mineral Premix	0.2	0.2	0.0	0.0	0.0
Vitamin Premix	0.6	0.6	0.0	0.0	0.0
Vitamin C	0.4	0.4	0.0	0.0	0.0
Barium Sulphate	1.0	0.0	0.0	0.0	0.0
Red Dye	0.0	1.0	1.0	0.0	0.0
Sun Dried Algae	0.0	0.0	99	100	94.0
Chromic oxide	0.0	0.0	0.0	0.0	1.0

Experimental Procedure:

The experimental system was maintained at 26°C in all experiments and was the same system as used in the feeding trials. During the one week acclimation period, fish were fed once daily at 07h00 with the control diet used in the growth trials. During the experiments all tanks were cleaned twenty minutes after feeding to ensure that no left over food or faeces could be consumed during the experiment. Details of experimental procedure such as fish number and stocking density are shown in Table 6.2.

TABLE 6.2 Procedure of the X radiography (Expt 1), Dye (Expt 2), Fish Sacrifice (Expt 3) and digestibility experiments (Expt 4).

	Expt 1.	Expt 2.	Expt 3.	Expt 4.
No. of fish/species	120	120	30	30
Stocking density	1/L	1/L	0.5/L	1/L
Size of fish (mm)	35-60	25-75	25-75	25-75
No. of aquaria	12	12	6	3

Experiment 1

Radiography

The fish were fed to satiation at 7:00 am on the day of the experiment. Exactly one hour after feeding, fish were anaesthetised using 2-phenoxy-ethanol at a concentration of 0.2ml/l. Ten fish of each species were placed on a Picker X-ray machine and one exposure was taken of each. The experiment was terminated after the development of the first x-rays.

Experiment 2

Dye incorporation

At 7:00am on the morning of the experiment half of the fish were fed the formulated diet (D1) and the other half the algal diet (D2). The fish were observed at fifteen minute-intervals until the first appearance of red faeces. The time that each fish first voided faeces was recorded, after which the fish was removed from the tank and sacrificed. The total length, weight and gut length of the fish were recorded.

Experiment 3.

Fish sacrifice

At 7:00am on the morning of the experiment the fish were fed. At hourly intervals after feeding, ten fish from each species and each treatment were sacrificed. Individual fish were sexed, weighed and measured. Each fish was dissected under a Nikon dissecting microscope (2-5x mag)and the entire alimentary canal was removed. The foregut, midgut, hindgut and total length of the alimentary canal was measured. The exact position of the food particles and the length of gut that they occupied were recorded diagrammatically. The length of the foregut, midgut and hindgut that contained food was then used to calculate their percentage fullness for each fish. The gut content of the last 20% of the hind gut was removed. The samples for each species were pooled, wet weighed and dried for 24 hours at 60°C and then reweighed. Fish length and the length of the alimentary canal were used to calculate the mean relative gut length (mRGL).

Experiment 4.

Algal Digestibility

At 7:00 am the fish were fed to satiation with the experimental diet. *X. helleri, P. reticulata* and *P. velifera* were sacrificed 180, 300 and 180 minutes respectively after feeding.

Faecal samples were collected under a nikon dissecting microscope (3x mag.) from the last 20% of the hind gut using the blunt side of a scalpel blade. Samples were pooled, weighed wet, oven drying at 30°C for 24 hours and reweighed.

The pooled samples and samples of the experimental diet were subject to the following analysis:

a) Total nitrogen was measured using the micro-kjeldahl method and protein content calculated as N x 6.25.
b) Chromic oxide (Cr_2O_3) was measured photometrically using an atomic absorption spectrophotometer.

Digestibility was calculated by the following equation: (after Maynard and Loosli, 1969)

Digestion coefficient =

$$100 - \frac{(100 \text{ X indicator in feed (\%)}}{\text{indicator in faeces (\%)}} \text{ X } \frac{\text{nutrient in faeces (\%))}}{\text{nutrient in feed (\%)}}$$

Statistics

Results of the dye experiments were analysed using an analysis of covariance and differences in the gut evacuation time between treatments were analysed using a one way anova.

RESULTS

The water quality in the tanks was similar to that experienced by the fish in the growth trials (Temperature - 27°C ±0.2; Ammonia - 0.10 mg/l ±0.003; Nitrite - 0.18 mg/l ±0.01; Nitrate - 41mg/l ±0.02; pH 7.7; Oxygen - 8.0 ±0.1).

Relative Gut Lengths.

The mean relative gut lengths of *X. helleri, P.* reticulata and *P.* velifera were 2.33 ±0.17, 1.15 ±0.07 and 3.19 ±0.09 respectively. The mRGL's of the three species were not significantly different from one another.

X - Radiography

Fish used in this experiment ranged in length from 2.7cm to 7.2cm and weighed between 0.30g and 2.75g. The form of the alimentary canal of *Xiphophorous helleri* is shown in the radiograph (Appendix 1). The intestine was curled and long (1.983 ±0.69 x body length), with no pyloric ceaca. The passage of food could not be followed using radiography (Appendix 1).

Dye experiment

The gut transit time of *X. helleri* fed the pure algal diet was significantly shorter (225.89 ±8.55 minutes) than fish fed the formulated diet incorporating a dye (261.93 ±10.86 minutes).

Fish Sacrifice

All algae was voided from the foregut after 5, 9 and 6 hours in *X. helleri, P. reticulata* and *P. velifera* respectively. Algae was retained in the midgut up to 7, 9 and 8 hours in *X. helleri, P. reticulata* and *P. velifera* respectively. The gut evacuation times (time at which all food ingested is excreted) in *X. helleri, P. reticulata* and *P. velifera* were nine, ten and eight hours respectively (Table 6.3; 6.4; 6.5).

The maximum amount of food in the hind gut after feeding was found after three five, and three hours in *X. helleri, P. reticulata* and *P. velifera* respectively (Figures 6.3; 6.4; 6.5).

Digestibility

The apparent crude protein digestibility of sun dried effluent grown algae in *X. helleri, P reticultata* and *P. velifera* was 65.45, 65.92 and 74.95 respectively.

TABLE 6.3. The percentage fullness of the foregut, midgut and hindgut in *X. helleri* during the fish sacrifice experiment.

Time (hours)	Foregut (% Fullness) ±SD	Midgut (% Fullness) ±SD	Hindgut (% Fullness) ±SD
0	0.0^{c} ±0.0	0.00^{e} ±0.0	0.0^{a} ±0.0
1	96.4^{a} ±4.0	11.0^{a} ±6.4	0.0^{a} ±0.0
2	72.2^{ab} ±14.2	83.0^{b} ±10.4	74.8^{bc} ±17.6
3	80.0^{a} ±20.0	82.0^{b} ±18	94.6^{c} ±5.4
4	40.0^{abc} ±24.5	36.0^{ab} ±20.6	52.0^{abc} ±19.6
5	0.0^{c} ±0.0	66.7^{ab} ±33.3	90.0^{bc} ±5.8
6	19.8^{bc} ±11.9**	49.0^{ab} ±12.0	76.4^{bc} ±9.1
7	3.3 ±3.3*	46.7^{ab} ±26.7	83.0^{bc} ±11.5
8	1.0 ±1.0*	0.0^{a} ±0.0	27.6^{ab} ±18.8
9	0.0^{c} ±0.0	0.0^{a} ±0.0	4.0. ±0.0*
10	0.0^{c} ±0.0	0.0^{a} ±0.0	0.0^{a} ±0.0

Values in the same column sharing a common superscript are not significantly different from one another.

*n = 1

**n = 2

TABLE 6.4. The percentage fullness of the foregut, midgut and hindgut in *P. reticulata* during the fish sacrifice experiment.

Time (hours)	Foregut (% Fullness) ±SD	Midgut (% Fullness ±SD) ±SD	Hindgut (% Fullness) ±SD
0	0.0^{c} ±0.0	0.0^{e} ±0.0	0.0^{a} ±0.0
1	92.5^{a} ±7.5	55.7^{abcd} ±15.9	0.0^{a} ±0.0
2	94.0^{a} ±4.0	80.3^{abc} ±8.7	8.6^{ab} ±3.7
3	83.0^{a} ±9.4	90.0^{ab} ±7.7	58.6^{bcd} ±16.5
4	92.0^{a} ±8.0	99.66^{a} ±0.3	88.7^{cd} ±10.8
5	63.4^{ab} ±17.5	83.1^{ab} ±10.4	89.3^{d} ±7.7
6	31.6^{bc} ±7.4	45.2^{bcde} ±13.2	85.1^{cd} ±8.4
7	8.6^{c} ±5.7	31.34^{cde} ±13.2	70.0^{cd} ±18.4
8	10.6^{c} ±10.1**	19.28^{de} ±5.9	35.1^{abc} ±18.3
9	4.18^{c} ±3.5**	12.76 ±12.7*	4.24^{a} ±3.7**
10	0.0^{c} ±0.0	0.0^{e} ±0.0	0.0^{a} ±0.0

Values in the same column sharing a common superscript are not significantly different from one another.

*n = 1

**n = 2

TABLE 6.5. The percentage fullness of the foregut, midgut and hindgut in *P. velifera* during the fish sacrifice experiment.

Time (hours)	Foregut (% Fullness) ±SD	Midgut (% Fullness) ±SD	Hindgut (% Fullness) ±SD
0	0.0^{b} ±0.0	0.0^{e} ±0.0	0.0^{a} ±0.0
1	93.2^{a} ±7.5	72.1^{a} ±6.5	0.0^{a} ±0.0
2	73.9^{a} ±8.9	86.8^{a} ±4.1	27.2^{ab} ±4.2
3	7.1^{b} ±4.7	32.5^{b} ±9.7	85.7^{c} ±6.5
4	1.0^{b} ±1.0	4.6^{c} ±4.6	57.0^{bc} ±17.1
5	8.12^{b} ±5.2	16.4^{bc} ±9.5	34.4^{abc} ±20.0
6	2.1^{b} ±1.7**	3.6. ±3.6*	7.5^{ab} ±5.1
7	0.0^{b} ±0.0	0.0^{c} ±0.0	3.5 ±3.5*
8	0.0^{b} ±0.0	6.9 ±6.9*	20.0^{ab} ±18.3**
9	0.0^{b} ±0.0	0.0^{c} ±0.0	0.0^{a} ±0.0
10	0.0^{b} ±0.0	0.0^{c} ±0.0	0.0^{a} ±0.0

Values in the same column sharing a common superscript are not significantly different from one another.

*n = 1

**n = 2

DISCUSSION

The mean relative gut lengths of the poeciliid species appear to reflect their feeding habits (Table 6.6), since the omnivorous guppies and swordtails had a lower mean relative gut lengths than the herbivorous sailfin mollies. The mRGL's were comparable to other species of similar feeding habits (Table 6.6). This apparent adaptation to herbivory would suggest that sailfin mollies may be more efficient at digesting plant material than the other two poeciliid species, and this was supported by the results of the digestibility study which showed that *P. velifera* digested the effluent grown algal protein more efficiently than the other two species.

TABLE 6.6. The Mean Relative Gut Length and feeding habits of fish used in the gut evacuation experiments and other selected species.

Species	Mean Relative Gut Length	±SD	Feeding Habits
X. helleri	2.33	±0.17	Omnivorous
P. reticulata	1.52	±0.07	Omnivorous
P. velifera	3.19	±0.09	Herbivorous
Barbus sharpeyi[1]	2.79 - 3.18		Herbivorous
B.tor[1]	1.24		Omnivorous
Chela bacailia[1]	0.88		Carnivourous

[1] Source: Da Silva and Anderson (1995).

The gut transit time of the sun dried algal diet (D2) in *X. helleri* was significantly shorter than that of the conventional formulated diet (D1). Jobling (1987) suggested that the diet composition and surface to volume ratios may effect gastric evacuation patterns. Since the sun dried algal flakes had a higher surface to volume ratio and a totally different composition to the formulated crumble diet, a combination of these two factors may

account for the difference in gut transit times. Since the gut transit times of poeciliids fed the sun dried algal diet were significantly different to those of poeciliids fed the conventional formulated diet, sun dried algae was used instead of a conventional diet in the following fish sacrifice study.

The gastric emptying times of the three poeciliid species fed sun dried algae were between eight and ten hours and that of guppies and swordtails was longer than that of the more herbivorous sailfin mollies. This result was expected since herbivores are considered to have more rapid patterns of food passage (Smith, 1989).
However, the objective of the fish sacrifice sudy was to determine time of fish sacrifice after feeding. Since the most faeces could be obtained in the hindgut three, five and three hours after feeding swordtails, guppies and mollies respectively, the fish were sacrificed at these times in the digestibility study.

The radiography study revealed that swordtails were not suitable candidates for this method of experimentation due to the combination of their small body size and long (2.33 ±0.17 x body length), coiled alimentary canal. Since the other poeciliid species have similar characteristics one would expect that they would not be suitable candidates for radiography studies either.

The apparent crude protein digestibility of the algal meal was lower than that of fishmeal protein, but it was comparable to that of other algal meals (Table.6.7). The crude protein digestibility of sun dried algae was slightly lower than that of soya meal (Table 6.7), which also has a similar crude protein content and degree of similarity (r^2) between its essential amino acid profile and that of *X. helleri*. The apparent protein digestibility of sun dried effluent grown algae was also lower than that of other plant proteins such as sunflower seeds and corn gluten meal (Table 6.4). However, the comparisons of protein digestibility should be made with caution since these studies involved different fish species. In addition, Hepher *et al.,* (1978) suggested that the digestibility of algae is improved if the algae is drum dried and not sun dried and therefore, the effect of drum drying or spray drying on the digestibility of the algae should be tested.

TABLE 6.7. The 'apparent' crude protein digestibility of effluent grown algae and other selected protein sources.

Feed Ingredient	Fish Species	Apparent protein Digestibility (%)
Effluent Grown Algae	*X. helleri*	65.45
Effluent Grown Algae	*P. reticulata*	65.92
Effluent Grown Algae	*P. velifera*	74.95
Spirulina (Effluent Grown)[1]	*Cyprinus carpio*	69.3
Fish Meal[1]	*C. carpio*	87.6
Algal Meal[2]	*Carassius auratus*	57.3
Soyabean Meal[3]	*Ictalurus punctatus*	79.95
Sunflower Seeds[4]	*Oncorhynkus mykiss*	77
Corn gluten meal[4]	*Oncorhynkus mykiss*	83

[1] Source: Hepher *et al.* (1979)

[2] Source: Nose (1960)

[3] Source: Sullivan and Reigh (1995)

[4] Source: Halver (1989)

The relatively low digestibility indicates that poeciliids may be unable to utilise all of the amino acids in sun dried algal protein. This information as well as the amino acid profile of the sun dried algae and its protein, lipid, and carbohydrate values were considered when the subsequent dietary formulations were made.

CHAPTER 7

EVALUATION OF EFFLUENT GROWN ALGAL PROTEIN AS A PARTIAL SUBSTITUTE TO FISHMEAL AND SOYA PROTEIN AND THE EFFECT OF ZOOPLANKTON SUPPLEMENTATION ON GROWTH, HEALTH, SURVIVAL AND COLOUR OF JUVENILE POECILIIDS.

INTRODUCTION

The omission of fish meal from fish diets, or sometimes even a reduction in its level has resulted in a decrease in growth rate of fish.(Andrews and Page, 1974; Page, 1974; Lovell *et.al.* 1975; Viola 1975). However, since the world production of fishmeal is limited and its price keeps increasing, an extensive research has been performed to reduce the dependency of aquaculture on this single source of protein.

The use of unicellular algae as an alternative protein source has been considered by a number of researchers (Terao, 1960; Ahmed 1966; Reed *et al.*, 1974; Stanley and Jones, 1976; Meske and Pruss, 1977, Appler and Jauncey, 1982; Sandbank and Hepher 1980). In most of these studies, fish were able to utilise the algae as a source of protein, however the use of algae as the sole source of protein in fish diets has usually resulted in impaired growth (Meske and Pfeffer, 1978). This was indicated by the essential amino acid profile of the effluent grown algae (Chapter 4) and confirmed in the growth trial (Chapter 5) in which the growth rates of three poeciliid species fed pure algal and zooplankton diets were significantly lower than those fed a formulated fishmeal and soya based diet. The low growth rates of the fish fed the pure algal diets were attributed to their unbalanced essential amino acid profile, low digestibility and difficulty in ingesting the algal diets. The low growth rate of the fish fed the zooplankton diet were attributed to a low feeding rate. Therefore the decision was made to feed fish the zooplankton as a dietary supplement to a conventional formulated diet, and the algae as a partial replacement for fishmeal and soya meal.

Due to the colour enhancement potential of pure effluent grown algae (Chapter 5) and since the inclusion of small amounts of algae into formulated diets has increased growth, feed utilisation, physical condition and disease resistance of cultured fish (Mustafa and Nakagawa, 1995), it was hypothesised that the inclusion of algae into formulated diets might enhance the colour, growth, feed utilisation, physical condition and disease resistance of poeciliids. Therefore, these parameters were tested in the growth trial.

Due to the potentially low cost of sun dried algae, the cost of a formulated diet would decrease with increasing levels of algal inclusion. However, economically speaking, high levels of algal inclusion into a diet will not be cost effective if the diet does not promote optimum growth. Therefore, before the levels of algal inclusion to be tested were decided, careful attention was paid to the results of the nutritional evaluation of the algae (Chapter 4),the initial growth trail (Chapter 5) and the digestibility study (Chapter 6).

Since the crude protein content, amino acid value and digestibility of the algae were considerably lower than those of fishmeal and poeciliid growth was poor on pure algal diets, it was considered that, sun dried algae should only be evaluated as a partial replacement for fishmeal. However, the crude protein content, amino acid profile, and digestibility of the algae were comparable to that of soya meal, suggesting that the algae could replace soya meal in formulated diets. From the control diet used in the previous growth trial, the fishmeal, soya meal protein combination was replaced in equal proportions of protein. The levels of sun dried algal protein inclusion chosen were twenty and five percent of the total protein content of the diets.

The low growth rates recorded in fish fed a pure zooplankton diet (Chapter 5) were attributed to a low feeding level. This low level of feeding could be avoided by feeding the zooplankton as a dietary supplement to the fish. Since zooplankton supplementation was found to enhance the growth and colour of *X. helleri* (Kruger, 1995), it was hypothesised that and effluent grown zooplankton supplement might increase the growth and colour of poeciliids.

The commercial lifespan (time from birth to sale) of poeciliids in a Grahamstown commercial operation are between three and four months (Williams and Jones pers. comm). This twelve week study could therefore be considered a long term exposure of the fish to the test substance and therefore a health screening would evaluate the effect of effluent grown algae and zooplankton on the health of poeciliids in the long term.

The objectives of this experiment were:

1) To determine the effect of the partial replacement of fishmeal and soya oil cake meal with sun dried, effluent grown, algae on the growth, health, survival and colour of three species of poeciliids.
2) To determine the effect of zooplankton (reared on the algae in the high rate pond) supplementation on the growth, health, survival and colour of three species of poeciliids.

METHODS AND MATERIALS

Experimental System

The experimental system used in this experiment was the same system described in the first growth trail (Chapter 5). Water quality measurements were performed as in the first growth trial (Chapter 5).

Experimental Diets

The first diet, referred to as the control diet was Kruger's (1995) optimum swordtail diet (Table 7.1). Two diets, which incorporated sun dried effluent grown algae were formulated. Algal protein replaced fishmeal and soya protein at five (referred to as the 5% algal diet) and twenty percent (referred to as the 20% algal diet) levels of inclusion (Table 7.1). In the fourth treatment, fish were fed the control diet and a zooplankton supplement (Table 7.1).

Diets were prepared according to the guidelines for experimental feed preparation described by Lovell (1989) and hammer milled and stored as in the first growth trial (Chapter 5). Zooplankton was harvested using the methods described in Chapter 5.

TABLE 7.1. Formulation and calculated proximate composition of the experimental diets used in the twelve week feeding trial. Soya OCM = Soya oil cake meal; Algae SD = Sun dried effluent grown algae.

INGREDIENT	DIET				
(% Dry Mass)	Control	5% algae	20% algae	Control and Zooplankton	
Fishmeal	45.5	43.3	36.4	45.5	-
Soya OCM	24.5	23.27	19.59	24.5	-
Algae SD	0.00	4.88	19.52	0.00	-
Fish Oil	2.67	2.88	3.50	2.67	-
Sunflower Oil	2.52	2.57	2.72	2.52	-
Starch	15	15	15	15	-
Mineral Premix	0.2	0.2	0.2	0.2	-
Vitamin Premix	0.6	0.6	0.6	0.6	-
Vitamin C	0.4	0.4	0.4	0.4	-
Cellulose	6.8	5.42	1.29	6.8	-
	CALCULATED PROXIMATE COMPOSITION				
Crude Protein	45.00	45.00	45.00	45.00	
Lipid	12.0	12.0	12.0	12.0	
Carbohydrate	21	21	21	21	

Vitamin and Mineral Premix (per kg premix): Choline chloride 166g; Copper 0.05g; Manganese 4g; Zinc 11g; Iodine 0.2g; Vitamin A 1300000IU; Vitamin D3 200000IU; Vitamin E 10000IU; Vitamin K3 4500IU; Vitamin B1 1600mg; Vitamin B6 1350mg; Vitamin B12 4mg; Folic acid 580mg; Biotin 55mg; Calcium pantothenic acid 11g; Niacin 19g; Antioxidant 20g; Inositol 58g; Carophyll pink 3,4g. Vitamin C provided as Ascorbic Acid.

Each diet was subject to the same analysis as described in Chapter 5.

Experimental fish and Feeding Regime.

Swordtails, guppies and sailfin mollies, less than one week old from the broods of various females, were stocked at the same density as in the previous growth trial (Chapter 5). The acclimation period was one month. Feeding during the course of the trial was performed twice daily and all fish were fed to satiation. The supplementary zooplankton was fed to fish once daily and the outflows of the tanks were blocked to prevent the zooplankton from escaping out of the tank. The methods used to weigh and measure the fish in the first growth trial (Chapter 5) were used in this growth trial. The FCR's and PER's were calculated using the methods used in Chapter 5. However, the mass and protein mass of the zooplankton supplement was not included in these calculations.

Colour enhancement

The same methods used to determine differences in degree of skin pigmentation in the fish at the end of the first growth trial (Chapter 5) were used in this trial.

Health Evaluation

The experiment was continued for a period of twelve weeks and all mortalities were recorded. All fish which died were subject to a post-mortem. At the end of the growth trial a full health evaluation (described in Chapter 5) was performed on ten fish from each tank.

Statistical analysis

Simple regression was carried out on the development of weight and length over time and statistically significant differences in slopes were tested for using an analysis of covariance (ANCOVA)(Zar 1984). The FCR and PER data was analysed using an ANOVA, with multiple range testing (Tukey's) with significance declared at $P = 0.05$. In addition, a non-parametric Cruscal-Wallace Test was performed on this data to test for significance in trends. Observations of the intensity of fish colour were compared by means of contingency table analysis (Zar, 1984). A five by four contingency table was used to determine if significant differences in distribution were present within treatments.

A χ^2 analysis in which the distribution of the colour observed in the fish fed control diet was considered to be the expected distribution was used to test for significant differences in distribution between treatments. Data from the chomameter colour analysis and data on the health of the fish were analysed using an ANOVA, with multiple range testing (Tukey's) with significance declared at $p = 0.05$.

RESULTS

Water Quality

The water temperature was maintained at 27°C for the duration of the experiment. Total ammonia, nitrite and nitrate were measured weekly and they remained below 0.015mg/l, 0.025mg/l and 60mg/l respectively. The pH was also monitored weekly and remained fairly constant between 7.6 and 8.3. Oxygen levels remained at between 7.7 and 9mg/l.

Proximate analysis and Amino acid composition:

All diet were isonitrogenous and isoenergetic, except the zooplankton supplement diet which had extra protein, lipids and carbohydrates supplied by the supplement (Table 7.2). The moisture and ash contents of the diets were also similar.

TABLE 7.2. The proximate analysis of the five test diets used in the ten week feeding trial. All values are expressed as percentage by weight: (NFE = Crude fibre and digestible carbohydrate (calculated by subtraction)).

	Average composition (% by weight)				
	Moisture	Protein	Lipid	NFE	Ash
Control Diet	8.2	45.92	12.0	21.30	13.3
5% Algal Diet	5.7	45.04	12.4	23.26	13.5
20% Algal Diet	7.7	44.19	11.9	25.61	10.6
Control Diet +	8.2	45.92	12.0	21.30	13.3
Zooplankton	10.8	36.84	11.1	31.06	10.2

The essential amino acid profiles of all the diets except the zooplankton supplement diet were similar (Table 7.3). The 20% algal protein diet had the lowest arginine, histidine, isoleucine, lysine, methionine and valine values and the highest leucine, phenylalanine and threonine values. The control diet had the lowest leucine, phenylamine and threonine values (Table 7.3). The zooplankton supplement diet had the highest essential amino acid values (Table 7.3).

TABLE 7.3. Amino acid content (g/100g protein), of *X. helleri* and the five diets tested in the ten week growth trial. (% dry matter) (bold font indicates essential amino acids). C = Control Diet; 5% = Five percent algal diet; 20% = Twenty percent algal diet; Z = Zooplankton supplement.

Amino Acid	*X. helleri*	C	5%	20%	C and Z
Arginine	5.67	6.65	6.62	6.31	6.65 + 5.45
Histidine	2.31	2.44	2.36	2.23	2.44 + 1.70
Isoleucine	3.78	4.86	4.83	4.73	4.86 + 4.76
Leucine	6.83	7.97	8.01	8.11	7.97 + 8.12
Lysine	7.73	8.15	7.63	7.63	8.15 + 5.82
Phenylalanine	3.89	4.63	4.67	4.74	4.63 + 5.13
Methionine	2.60	2.61	2.60	2.49	2.61 + 1.85
Threonine	3.78	4.23	4.32	4.47	4.23 + 4.99
Valinine	4.40	5.39	5.41	5.37	5.39 + 6.26
Tryptophan					
Aspartate	9.27	10.93	10.91	10.91	10.93 + 10.36
Serine	4.05	4.01	4.17	4.27	4.01 + 3.81
Glutamate	15.58	16.46	16.33	15.87	16.46 + 11.80
Proline	4.66	4.97	4.85	4.90	4.97 + 5.18
Glycine	7.17	6.06	6.03	6.07	6.06 + 5.82
Alanine	5.71	5.81	5.83	5.04	5.81 + 6.91
Tyrosine	2.77	2.88	3.02	2.97	2.88 + 3.46
Cystine					

The modified essential amino acid ratios of the four test diets were similar (Table 7.4). Since the r^2 values for all diets were high, the essential amino acid profiles of all test diets were similar to the essential amino acid profile of *X. helleri* (Table 7.4). There was a decrease in the similarity of the formulated diets with increasing levels of algal inclusion. The r^2 value of the zooplankton supplement was considerably lower than those of the other diets. The modified amino acid ratios for histidine, lycine and methionine were lower, (and thus potentially limiting) than the modified amino acid ratios of *X. helleri* in all diets except the zooplankton supplement diet. The modified amino acid ratio of threonine was lower (limiting) in the control diet than in *X. helleri* (Table 7.4).

TABLE 7.4. Modified essential amino acid ratios (Ogata *et al.*,1983) for *X. helleri* and the five test diets used in the twelve week growth trial. *X.hel* = *X. helleri;* C = Control Diet; 5% = Five percent algal diet; 20% = Twenty percent algal diet; Z = Zooplankton supplement diet. r^2 = Coefficient of determination with respect to the profile of *X. helleri.* (Limiting amino acids underlined)

Amino Acid	*X. helleri*	C	5%	20%	C and Z
Arginine	138	142	142	138	142 and 124
Histidine	56	<u>52</u>	<u>51</u>	<u>48</u>	52 and 39
Isoleucine	92	104	103	102	104 and 108
Leucine	167	170	172	176	170 and 184
Lycine	<u>188</u>	<u>174</u>	<u>169</u>	<u>165</u>	174 and 132
Methionine	63	<u>56</u>	<u>56</u>	<u>54</u>	56 and 116
Phenylalanine	95	99	100	103	99 and 42
Threonine	92	<u>90</u>	92	97	90 and 113
Valine	107	115	116	116	115 and 142
r^2(%)		96.7	95.3	93.5	96.7 and 62.9

Growth Data

The growth rates of swordtails, guppies or mollies did not differ significantly between any of the test diets in terms of both weight and length over the twelve week experiment (Figures 7.1; 7.2; 7.3).

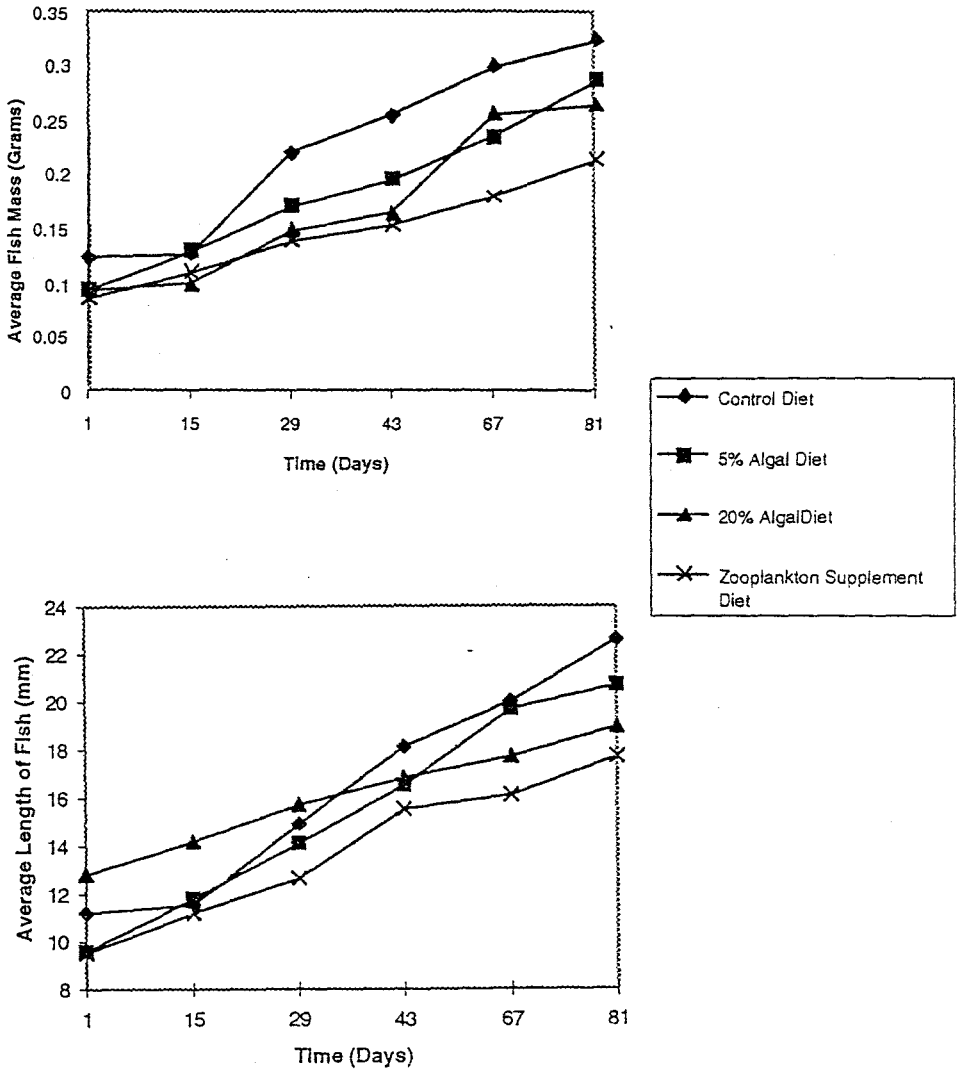


FIGURE 7.1. Growth curves (mass and length) of juvenile *X. helleri* fed four test diets over a twelve week trial period.

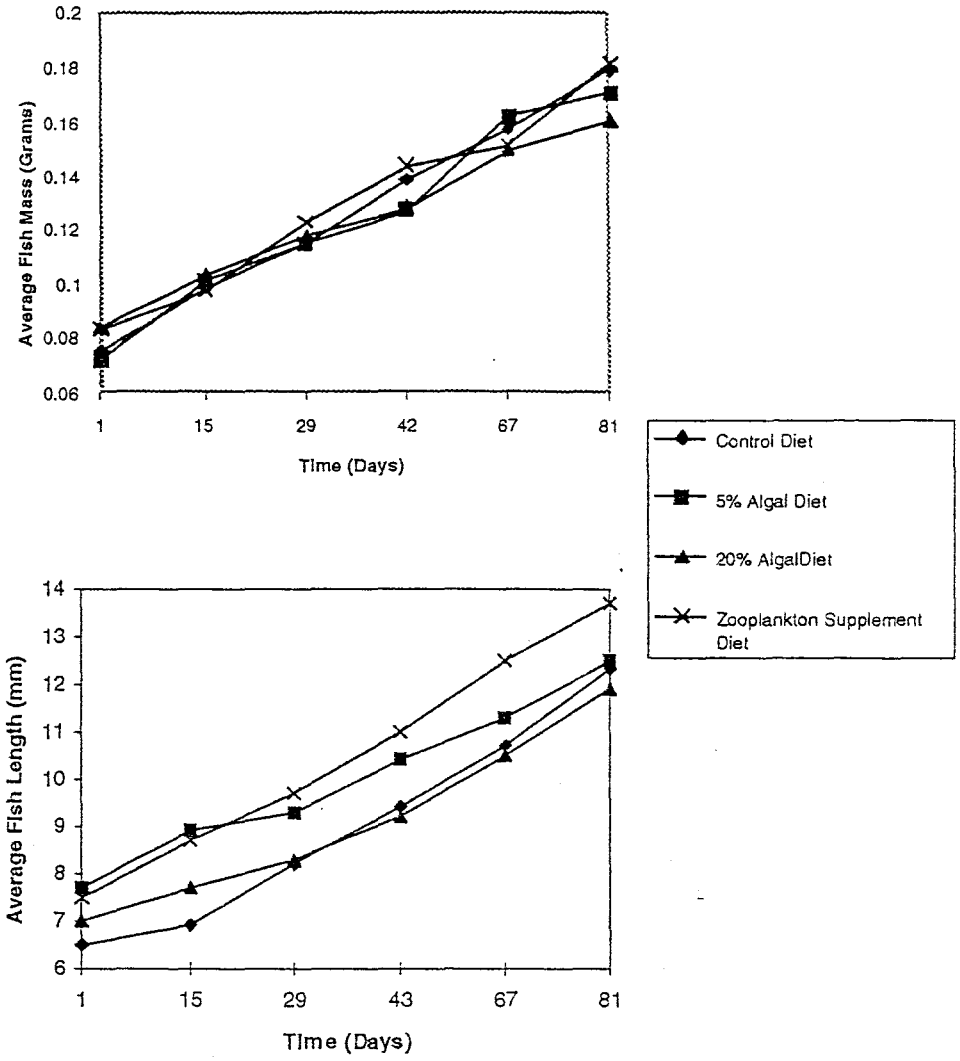


FIGURE 7.2. Growth curves (mass and length) of juvenile *P. reticluata* fed four test diets over a twelve week trial period.

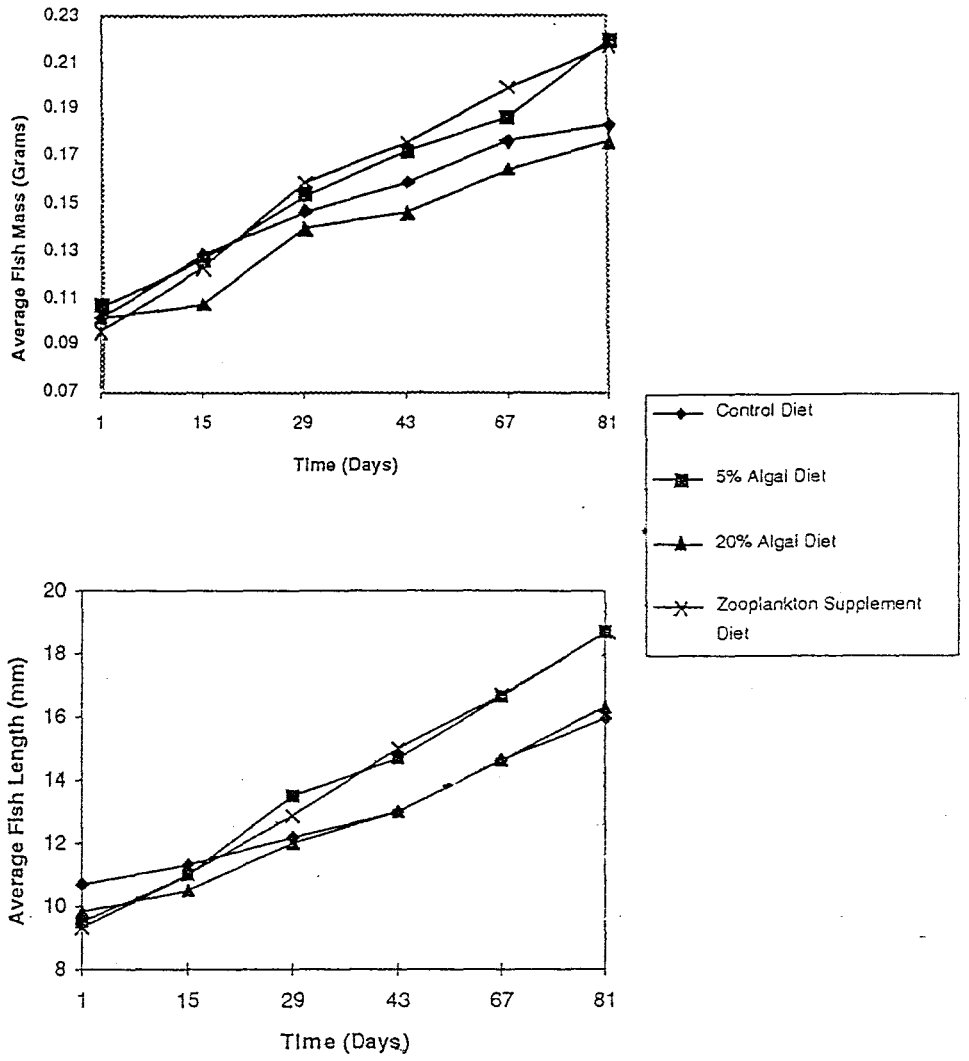


FIGURE 7.3. Growth curves (mass and length) of juvenile *P. velifera* fed four test diets over a twelve week trial period.

The slopes of the mass and length growth curves of all species were not significantly different from one another.

TABLE 7.4. Linear regression equations for the growth curves (mass) of four dietary treatments in three poeciliid species after a twelve week growth trial.

Species	Diet	Regression equation
X. helleri	Control Diet	$y = 0.07 + 0.043(x)^a$
X. helleri	5%Algal Diet	$y = 0.06 + 0.040(x)^a$
X. helleri	20% Algal Diet	$y = 0.08 + 0.046(x)^a$
X. helleri	Zooplankton Supplement Diet	$y = 0.06 + 0.029(x)^a$
P. reticulata	Control Diet	$y = 0.05 + 0.021(x)^x$
P. reticulata	5% Algal Diet	$y = 0.06 + 0.020(x)^x$
P. reticulata	20% Algal Diet	$y = 0.07 + 0.015(x)^x$
P. reticulata	Zooplankton Supplement Diet	$y = 0.06 + 0.019(x)^x$
P. velifera	Control Diet	$y = 0.09 + 0.016(x)^n$
P. velifera	5% Algal Diet	$y = 0.08 + 0.022(x)^n$
P. velifera	20% Algal Diet	$y = 0.08 + 0.016(x)^n$
P. velifera	Zooplankton Supplement Diet	$y = 0.08 + 0.025(x)^n$

Equations for each species sharing a common superscript do not show significant differences in slope ($P>0.05$).

TABLE 7.5. Linear regression equations for the growth curves (length) of each dietary treatment in three poeciliid species.

Species	Diet	Regression Equation
X. helleri	Control Diet	$y = 11.2 + 1.515(x)^a$
X. helleri	5% Algal Diet	$y = 9.6 + 1.409\ (x)^a$
X. helleri	20% Algal Diet	$y = 12.8 + 1.621(x)^a$
X. helleri	Zooplankton Supplement Diet	$y = 9.5 + 1.022(x)^a$
P. reticulata	Control Diet	$y = 6.5 + 0.741(x)^z$
P. reticulata	5% Algal Diet	$y = 7.7 + 0.715(x)^z$
P. reticulata	20% Algal Diet	$y = 7.0 + 0.531\ (x)^z$
P. reticulata	Zooplankton Supplement Diet	$y = 7.5 + 0.669\ (x)^z$
P. velifera	Control Diet	$y = 10.7 + 0.564(x)^n$
P. velifera	5% Algal Diet	$y = 9.5 + 0.775(x)^n$
P. velifera	20% Algal Diet	$y = 9.8 + 0.568(x)^n$
P. velifera	Zooplankton Supplement Diet	$y = 9.3 + 0.881(x)^n$

Equations for each species sharing a common superscript do not show significant differences in slope ($P>0.05$).

All formulated diets were accepted readily by the fish. The fish were fed the conventional formulated diet combined with a zooplankton supplement immediately began feeding on the larger formulated diet food particles and active feeding on the zooplankton was not observed at any stage during feeding.

There were no significant differences in the FCR's and PER's for all dietary treatments within each species (Table 7.6, 7.7, 7.8).

TABLE 7.6. The mean feed conversion, and protein efficiency ratios of *X. helleri* after the twelve week growth trial.

Diet[1]	Mean FCR* p=0.66 (84 days) ±SD	MeanPER* p=0.37 (84 days) ±SD
Control Diet	2.50[a] ±0.11	0.90[a] ±0.04
5% Algal Diet	3.52[a] ±0.92	0.73[a] ±0.20
20% Algal Diet	4.75[a] ±2.43	0.64[a] ±0.33
Zooplankton supplement	2.05[a]±0.11	1.15[a] ±0.21

*Means indicate the average value for all fish in each treatments (3 replicates)

Values in the same column sharing a common superscript are not significantly different.

TABLE 7.7. The mean feed conversion, and protein efficiency ratios of *P. reticulata* after the twelve week growth trial.

Diet	Mean FCR* p=0.15 (84 days) ±SD	Mean PER* p=0.15 (84 days) ±SD
Control Diet	2.30[a] ±0.07	0.97[a] ±0.03
5% Algal Diet	2.60[a] ±0.27	0.915[a] ±0.11
20% Algal Diet	3.24[a] ±0.44	0.71[a] ±0.09
Zooplankton Supplement	2.90[a] ±0.14	0.78[a] ±0.04

*Means indicate the average value for all fish in each treatments (3 replicates)

Values in the same column sharing a common superscript are not significantly different.

TABLE 7.8. The mean feed conversion, and protein efficiency ratios of *P. velifera* after the twelve week growth trial.

Diet	Mean FCR* p=0.08 (84 days) ±SD	Mean PER* 0.09 (84 days) ±SD
Control Diet	10.10[a] ±5.09	0.28[a] ±0.17
5% Algal Diet	3.20[a] ±0.39	0.72[a] ±0.09
20% Algal Diet	5.62[a] ±0.35	0.40[a] ±0.02
Zooplankton Supplement	2.7 ±0.36	0.87[a] ±0.11

*Means indicate the average value for all fish in each treatments (3 replicates)

Values in the same column sharing a common superscript are not significantly different.

Colour enhancement

Visual observation:

The visual observation on the colour of the fish fed the four test diets, after analysis using contingency tables, revealed an equal distribution for *X. helleri* (χ^2 = 6.988), *P. velifera* (χ^2 = 18.301) and *P. reticulata* (χ^2 = 6.5023). The distribution of observations to the categories were similar in the test diets from the control diet (Table 7.9).

Table 7.9. The number of people perceiving three species of poeciliids, fed four test diets to be in a specific colour intensity category after a ten week growth trial (1 = very dull; 2 = dull; 3 = average; 4 = bright; 5 = very bright).

Species	Diet	NUMBER OF OBSERVATIONS					Chi^2
		1	2	3	4	5	
		(degree of colour intensity)					χ^2
		⟶					df = 4 (k=1)
X. helleri	Control Diet	0	5	13	12	1	
X. helleri	5% Algal Diet	0	1	15	14	1	0.84
X. helleri	20% Algal Diet	0	0	15	14	2	6.64
X. helleri	Zooplankton Diet	0	0	15	14	2	6.64
P. reticulata	Control Diet	0	4	10	12	5	
P. reticulata	5% Algal Diet	0	1	8	15	7	3.70
P. reticulata	20% Algal Diet	0	2	5	16	8	6.63
P. reticulata	Zooplankton Diet	0	4	10	12	5	0.00
P. velifera	Control Diet	2	17	11	3	0	
P. velifera	5% Algal Diet	1	8	15	8	1	15.01
P. velifera	20% Algal Diet	1	6	14	11	1	29.77
P. velifera	Zooplankton	2	8	17	6	0	11.03

Chromameter analysis:

The results of the visual observation were substantiated with those of the chromameter analysis (Tables 7.10, 7.11 and 7.12.), with no significant differences in lightness, chroma or hue detected in any of the fish species.

TABLE 7.10. Colour measurements of *X. helleri* at the end of the twelve week feeding trial.

Diet	Lightness Mean ±SE	Chroma Mean ±SE	Hue Mean ±SE
Control Diet	20.68^{a}±1.84	0.437^{a}±0.02	0.383^{a}±0.01
5% Algal Diet	22.13^{a}±2.28	0.440^{a}±0.01	0.388^{a}±0.01
20% Algal Diet	21.07^{a}±1.74	0.442^{a}±0.01	0.379^{a}±0.01
Zooplankton Supplement	22.30^{a}±2.52	0.445^{a}±0.01	0.390^{a}±0.01

Values in the same column sharing a common superscript are not significantly different.

TABLE 7.11. Colour measurements of *P. reticulata* at the end of the twelve week feeding trial.

Diet	Lightness Mean ±SE	Chroma Mean ±SE	Hue Mean ±SE
Control Diet	19.54^{a}±3.14	0.436^{a}±0.02	0.346^{a}±0.02
5% Algal Diet	18.68^{a}±1.97	0.442^{a}±0.03	0.342^{a}±0.01
20% Algal Diet	15.46^{a}±2.98	0.455^{a}±0.01	0.356^{a}±0.02
Zooplankton Supplement	14.73^{a}±1.96	0.457^{a}±0.01	0.344^{a} 0.001

Values in the same column sharing a common superscript are not significantly different.

TABLE 7.12. Colour measurements of *P. velifera* at the end of the twelve week feeding trial.

Diet	Lightness Mean ±SE	Chroma Mean ±SE	Hue Mean ±SE
Control Diet	29.864[a]±3.09	0.322[a]±0.01	0.326[a]±0.001
5% Algal Diet	30.698[a]±2.56	0.333[a]±0.01	0.330[a]±0.00
20% Algal Diet	27.321[a]±1.89	0.326[a]±0.002	0.320[a]±0.003
Zooplankton	26.196[a]±2.89	0.324[a]±0.001	0.329[a]±0.002

Values in the same column sharing a common superscript are not significantly different.

Health evaluation:

Condition Factor

There were no significant differences in the condition factor of *X. helleri, P. velifera* and *P. reticulata* fed any of the test diets (Table 7.12).

TABLE 7.12. The condition factor of *X. helleri, P. reticulata* and *P. velifera* fed four test diets after a twelve week growth trial.

Diet	*X. helleri*	*P. reticulata*	*P. velifera*
Control Diet	85.56[a] ±4.5	136.20[a]±10.5	88.64[a] ±9.4
5% Algal Diet	82.62[a] ±5.7	142.38[a] ±11.3	85.06[a] ±6.5
20% Algal Diet	77.35[a] ±3.9	125.97[a] ±13.5	84.63[a] ±12.6
Zooplankton Supplement	84.36[a] ±6.8	124.39[a] ±9.6	90.25[a] ±10.5

Values in the same column sharing a common superscript are not significantly different.

Survival:

No significant differences in survival were recorded between *X. helleri, P. reticulata* or *P. velifera* fed the different experimental diets (Table 7.13). The survival of fish was higher than in the first growth trial.

TABLE 7.13. Mean survival expressed as a percentage for *X. helleri, P. reticulata* and *P. velifera* after the twelve week feeding trial.

Diet	*X. helleri* Survival(%)±SE	*P. reticulata* Survival(%)±SE	*P. velifera* Survival(%)±SE
Control Diet	70.43[a] ±1.44	90.43[a] ±2.31	79.67[a] ±4.53
5% Algal Diet	63.33[a] ±6.93	93.30[a] ±3.36	91.30[a] ±4.49
20% Algal Diet	58.60[a] ±12.73	98.13[a] ±1.87	82.47[a] ±10.10
Zooplankton Supplement	90.80[a] ±2.91	92.17[a] ±7.83	86.43[a] ±3.25

Health evaluation

No visible signs of ill health were detected during the autopsies of the fish which died during the course of the experiment.

While symptoms of sub cutaneous haemhorrhagy were found on less than ten percent of all fish, no significant differences in the occurrence of these symptoms were present in any treatment. No abnormal growth, parasites, lesions, fungal infections, emaciation, exophthalmus or scoliosis were detected in any of the fish. The gill tissue of all fish appeared healthy and no abnormal growth or parasites were present in the body cavity in any fish.

DISCUSSION

The crude protein, lipid and carbohydrate values of all diets were very similar, and thus

the only nutritional differences that may have existed, were in the quality of the these substances. A comparison of the amino acid requirement of *X. helleri* with the amino acid profile of the proteins in the diet gives an indication of the protein quality. Since all the amino acid profiles were very similar to the amino acid profile of *X. helleri,* the quality of the protein in the diet did not appear to be negatively affected with increasing levels of algal inclusion up to 20%. However, since the amino acid profile of pure algae was inferior to that of fishmeal, higher levels of algal inclusion will result in poorer amino acid profiles. In addition, the digestibility of the algae was found to be relatively low compared to that of fishmeal and soya protein (Chapter 6) and the protein available to the fish in each diet decreased very slightly with increasing levels of algal protein inclusion (Table 7.14).

TABLE 7.14. The protein available to three species of poeciliids in three formulated diets with increasing levels of algal inclusion.

Species	Diet	Available protein* (% by weight)
X. helleri	Control Diet	37.44
X. helleri	5% Algal Diet	36.89
X. helleri	20% Algal Diet	35.11
P. reticulata	Control Diet	37.44
P. reticulata	5% Algal Diet	36.90
P. reticulata	20% Algal Diet	35.15
P. velifera	Control Diet	37.44
P. velifera	5% Algal Diet	37.08
P. velifera	20% Algal Diet	35.86

*Assuming digestibility values of 90% (De Silva and Anderson, 1995) for fishmeal, 80% for soya meal (Halver, 1989) and 65.45, 65.92 and 74.94 for sun dried effluent grown algae in swordtails, guppies and mollies respectively (Chapter 6).

With exception of the slight decrease in available protein in the diets, the nutritional similarity of the diets was reflected in the growth rates of the fish, since no significant differences in the growth rates of any species were recorded. While Appler and Jauncey (1982) reported decreased growth rates in *Tilapia nilotica* when fish meal was replaced with algal meal at a 20% level of inclusion, the inclusion of low levels of algae (±5%) into formulated diets was reported to increase the growth of the nibbler, *Girella punctata,* (Nakazoe *et al.*, 1986) and the red sea bream, *Ascophylum nodosum* (Yine *et al.,* 1986a). However, In the twelve week growth trial, an inclusion of sun dried effluent grown algae into formulated diets up to a level of 20% had no significant effect on the growth of poeciliids.

Poecilliid growth was not significantly enhanced by supplementing the conventional diet with zooplankton. If ingested, the zooplankton would provide supplementary protein, carbohydrates, lipids and amino acids and could be utilised for growth. However, fish were observed to feed actively on the larger formulated diet particles (200-300µm) and not on the smaller zooplankton items (± 90 -120µm). The unblocking of the outflow pipes 15 minutes after feeding may thus have resulted in a loss of much of the zooplankton biomass and may have effected fish growth. This zooplankton may therefore be more suitable for smaller fish. However, this would have to be tested.

There was no statistical difference between the FCR's and PER's in the three species fed any of the test diets. The apparent trends of increased FCR and decreased PER when fish were fed formulated diets with increasing algal inclusions was not statistically significant. Testing for significant trends in FCR's and PER's with increasing levels of algal inclusion would require a growth experiment with at least 4 different levels of algal inclusion and more replicate treatments.

The statistically similar growth rates, FCR's and PER's, of *X.. helleri, P. reticulata* and *P. velifera* as well as the similarity of the nutritional compositions of the diets suggest that the replacement of fishmeal and soyameal protein by effluent grown algal protein, up to 20% inclusion had no detrimental effect on the growth of fish.

No significant difference in the colour intensity was recorded in any fish species fed any of the test diets. This indicates that the inclusion of algae up to a level of 20% did not enhance the colour intensity of the fish.

The survival of poeciliids in this growth trial was high. This was substantiated by the health evaluation in which no symptoms of ill health were recorded. While survival in this growth trial was high, it is in stark contrast to the previous growth trial were the survival of *X. helleri* in particular was low. The nutritional inadequacy of the pure algal and zooplankton diets was proposed as an explanation for the high mortalities. In this study the nutritional requirements of poeciliids were satisfied, therefore suggesting that the low nutritional value of the pure algal diets was a valid explanation for the high mortalities recorded.

Since the four test diets did not produce any significant differences in growth, food conversion ratios, protein efficiency ratios, health, survival or colour of poeciliids, the decision to use one particular diet can be based on cost.

An ornamental fish farmer at the Grahamstown Sewage Works could utilise the algae produced in the algal integrated ponding system by harvesting the algae with a little associated labour (Chapter 3), processing the algae cheaply by sun drying it (Chapter 5) and provided the fish farm formulates its own feed, including it into a formulated diet at a level 20% protein. This could potentially reduce the overall costs of fish feed for an ornamental fish farmer at the Grahamstown Sewage Works.

CHAPTER 8

CONCLUDING DISCUSSION AND RECOMMENDATIONS

The pilot AIPS at the Grahamstown Sewage Works provided algae as a novel feed ingredient for possible use in ornamental fish culture. While the main focus of the study was algae, the presence of large amounts of zooplankton in the high rate oxidation ponds provided an opportunity for research into the potential of effluent grown zooplankton as a feed for poeciliids. A comprehensive chemical and biological evaluation of effluent grown algae demonstrated that the algae produced in the AIPS has potential as a feed ingredient. Algal quality was fairly constant since the species composition of the algae was fairly stable over the experimental period and the algal protein level was consistently high.

Proximate analysis of effluent grown algae indicated that it was a promising protein source for ornamental fish (Chapter 4). The proximate analysis of the most commonly used oilseed namely soya, the cereal grain, sorghum and other algal species were similar to that of effluent grown algae (Table 8.1).

TABLE 8.1. The proximate composition of selected feed ingredient and some algal species.

Ingredient/Species	Protein	Lipid	Carbohydrate
Effluent grown algae[1]	41.5	4.8	35.1
Soya oil cake meal[2]	41.6	5.3	30.1
Sorghum gluten meal[2]	42	4.9	37.6
Scenedesmus dimorphus[3]	50.4	23.1	6.1
Dunaliella salina[3]	40.3	28.1	11.0
Scenedesmus acutus[2]	43.6	10.5	24.4

[1]Chapter 4 ; [2]Tacon (1990); [3]Renaud *et al.* (1994).

Previous studies have suggested that single ingredient feeds seldom promote adequate growth in fish and this was confirmed in the initial growth trial in which pure algal diets were fed to three species of poeciliids (Chapter 5). The poor amino acid balance of the algae in comparison to the experimental fish was considered a prime reason for the poor growth performance. While the amino acid composition of the control diet was very similar (r^2=96.7) to the body tissue composition of *X. helleri,* the amino acid composition of sun dried algae (r^2=65.33) was poor with respect to *X. helleri* but similar to the amino acid composition of soyabean meal (r^2=62.96).

Although, the apparent crude protein digestibility of sun dried algae (65-75%) for the three poeciliid species was calculated to be slightly lower than the digestibility of soyabean meal in another omnivorous species, *Ictalurus punctatus* (79.9%) (Chapter 6), it nonetheless indicated that the algae had potential for inclusion into formulated diets.

The potential of effluent grown algae to poeciliids was confirmed in the second growth trial in which the inclusion of algae into formulated diets at protein levels of up to 20% did not reduce the growth rates or influence the FCR' or PER's of poeciliids in comparison to the fishmeal / soyabean meal control diet.

The results of the initial growth trial (Chapter 5) suggested that the colour of one of the poeciliid species fed a pure sun dried algal diet was enhanced. However, since the colour enhancement results of the second growth trial were inconclusive, the effect of increased levels of algal inclusion into formulated diets and supplementary pure algal feeds on poeciliid colour should be a focus of future research.

Due to the nature of the algal culture medium, a health evaluation of the fish was crucial to the evaluation of the algae as a feed ingredient. The high mortalities of fish fed the pure algal diets may have resulted from the poor nutritional value of the diets fed to poeciliids (Chapter 5). This view was supported when low fish mortalities were recorded in fish fed nutritionally complete diets in the second growth trial (Chapter 7). The

chemical analysis of the effluent grown zooplankton suggested that its chemical composition was slightly inferior to that of effluent grown algae and soyabean meal (Chapter 4). Although the growth rates of fish fed a pure zooplankton diet were poor, the favourable FCR's and PER's of fish fed the pure zooplankton diet suggested that this feed ingredient was fed to poeciliids in insufficient quantities and had potential as a food source for poeciliids (Chapter 5). Possible reasons for the favourable FCR's and PER's were that zooplankton is easily digestible by fish (Lavens and Sorgeloos, 1996) and was fed to the fish at a low feeding rate. However, since a zooplankton supplementation with a conventional formulated feed resulted in similar growth of fish with no zooplankton supplementation, the preference of the larger formulated food particles (200µm-300µm) above the small zooplankton (80-100µm) by the fish may have resulted in much of the zooplankton biomass being lost when the outflows of the tanks were unblocked. Thus the results of the zooplankton experiments were inconclusive, but since the potential of freshwater micro-zooplankton in the hatchery production of freshwater ornamental fish has barely been exploited (Lim *et al.,* 1997), this should be the focus of future research.

For the commercial utilisation of a new feed ingredient in aquaculture, it must at least partially meet the dietary requirements of the culture organism, must have a high production potential, and must be less expensive than conventional feed ingredients. The chemical analysis of the algae and the growth trials incorporating algae into formulated diets provide motivation for the inclusion of algae into formulated diets for ornamental fish. However, the production potential of effluent grown algae must be researched in larger AIPS systems and the cost of harvest and processing algae from these systems should be the focus of future studies. If the objectives of the Water Research Commission (WRC) are met, AIPS systems may become commonplace in South Africa and therefore there is potential for the algae as a food source for other animals as well. However, until it is established that heavy metals, pesticides and pathogens which may be present in the algae (Sandbank and Nupen, 1985), do not effect the culture organisms and their consumers, the potential of the algae for feed for other organisms is limited. Therefore, the focus of future research on the algae should include both growth and toxicological studies on cultured food organisms, such as food

fish. However, until the toxicological effect of effluent grown algae on cultured food organisms are quantified, the utilisation of the algae for ornamental fish diets are a logical choice.

By evaluating the chemical composition of the algae this research has provided a starting point for future research into the growth of other food organisms fed effluent grown algae, and from the biological evaluation of the algae this research has provided a base for research on the effect of effluent grown algae in other fish species.

In terms of evaluating the cost of the effluent grown algae, the results from the small scale algal harvesting studies (Chapter 3) suggest that algae can be harvested from the AIPS with little associated labour and therefore the algae can be utilised as a cheap alternative protein source for an on site ornamental fish farm.

In conclusion, the objectives of this study have been met in that the nutritional value of effluent grown algae and zooplankton to poeciliids has been determined. As part of the ongoing research into the potential of the Grahamstown Sewage Works as a site for an ornamental fish aquaculture operation, the evaluation of the potential of small scale harvesting and processing of the algae has provided a starting point for future research into effective methods of obtaining algal biomass from the algal integrated ponding systems for the aquaculturalist.

APPENDIX 1

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