ASPECTS OF THE BIOLOGY OF

Cichlasoma urophthalmus (Günther)

WITH

PARTICULAR REFERENCE TO ITS CULTURE

by

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THIS THESIS IS DEDICATED TO MY

BELOVED WIFE MARIA CRISTINA, MY SON

CARLOS CRISTIAN AND MY DAUGHTER CARLA CRISTINA

FOR THEIR INCREDIBLE PATIENCE AND SACRIFICE

TO MY PARENTS AND PARENTS IN LAW

TO THE MEMORY OF DR. ALEJANDRO VILLALOBOS F.

CONTENTS

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ABSTRACT

This thesis is an investigation of the basic biology, reproductive behaviour, and key physiological and nutritional requirements of the Mexican and Central American cichlid Cichlasoma urophthalmus (Gunther), with particular reference to the potential of native species for aquaculture in the region. C. urophthalmus is a widely appreciated food fish within its native range, commanding good prices and having the advantage in terms of aquaculture that the risk from introduction of an exotic species with the consequent importation costs, diseases and escapes are eliminated.

The dentition, gill rakers and gut structure are indicative of a carnivorous habit and this was corroborated by seasonal examination of gut contents which revealed that the species feeds principally on small animals. Investigation of the species in the natural environment suggest that growth was continuous, with some slight depression in the cooler winter months, reproduction occurred in the second year and the minimum reproductive size was 102cm (50g).

In the laboratory C.urophthalmus grew well between 22.5 and 36.3°C the best growth being achieved at 32°C

and optimum growth and survival (= production) being at 28°C. The species is typically euryhaline and can be acclimated to salinities of 0‰ to 35‰. It can withstand instantaneous transfer from 0‰ to 15‰, and reverse, and have best growth at 15‰, probably due to the energy saving in an isotonic medium. Oxygen consumption rates were similar to other cichlids and C.urophthalmus can survive virtual anoxia for about 2h.

C.urophthalmus is a bottom-spawner and the aggressive features of its reproductive behaviour, described in this work, can probably be minimized in culture. Its fecundity is about 5 to 7 times that of the mouth brooding tilapias, producing 2000-7000 eggs per spawn and it can reproduce at about 23 days intervals, probably throughout the year with management of temperature. Hatching is rapid (61°days) and survival good with simple hatchery techniques. Broodstock requirements, handling and labour costs could be lower than for the mouth brooding tilapias.

The protein requirements of fry were shown to be 39-45% of the diet and this is similar to the tilapias. Diet digestibility was depressed by high dietary fibre content, but was not inhibited by inclusion of soybean (25.81%) or wheat meal (26.7%) in the diets. Although much more research is required. The results reported

here have enabled tentative formulations of diets for aquaculture, based on typically available ingredients.

The features of C.urophthalmus are discussed and compared extensively with those of the other cultured fish, principally the tilapias. It is concluded that C.urophthalmus is a very good candidate for aquaculture development within its range of distribution and that it has strong advantages over introduction of cultured cichlids.

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

VOLUME CONTAINS CLEAR OVERLAYS

OVERLAYS SCANNED SEPERATELY AND OVER THE RELEVANT PAGE.

GENERAL INTRODUCTION

INTRODUCTION.

Aquaculture has been defined as the technology of rearing aquatic organisms under controlled or semicontrolled conditions (Bardach, 1972; Stickney, 1979; Huet, 1986). As a technology to increase the production of aquatic organisms aquaculture is not new. Aquatic farming practices began as long ago as the fifth century B.C. in China, with the culture of carps and were well known during Roman times, with the management of oysters and fish ponds (Bardach et al., 1972 and Huet, 1986). It was not until this century that science became involved with aquaculture, and scientific methods were first applied in salmonids which have been reared for many decades. It is not surprising, therefore, that more scientific information is available for salmonid culture than for any other particular species (Halver 1972, 1976; NRC, 1981; NAS, 1983).

In more recent times substantial efforts have been made to culture other fish species with the aim of increasing the production of food. Thus, currently a number of different species are raised in fresh, brackish and marine waters, in either warm or cold water and much more scientific information on new species with potential is currently available (Bardach et al., 1972;

Smith 1979; Tal and Ziv, 1978; NRC, 1983).

One of the most important aspects for the success of a cultured species is the acceptability of the species by the people. Without that premise, even should all the technical constrains be removed, the venture will ultimately fail. Contrary to popular opinion, undernourished or even starving people will not eat a given food just because it is available. For example, the introduction of fish protein concentrates as a dietary additive, despite a bland presentation as a flavorless and odorless material to the people in developing countries, was a singular failure (Smith, 1979).

It is easily possible to find similar population reactions in the history of aquaculture. For example, among the cyprinids, mainly Cyprinus carpio is widely favoured in Asia and Central Europe, where there are traditions of culture and consumption. These were introduced to the American Continent as long ago as the last part of the eighteen century (Meek, 1904), but only in localized areas have they been accepted with moderate success. No intensive systems for these species have been established and proved successful, and in some American contries they are now proscribed species (Smith, 1979; Caulton, 1984; Contreras and Escalante,

1984). Another example is the channel catfish Ictalurus punctatus which has a very highly developed technology and a successful market in the United States. Despite this it cannot be used in culture in countries such as Israel, although in this particular case the ban is for religious reasons. It is clear then that native species will be well accepted and can be cultured without problem in their original areas because of the consumption tradition. The introduction of the same species in new geographical areas however, has not always been successful and this is principaly due to the preferences of the human population.

Fish from the Cichlid family are distributed naturally in tropical America, Africa and coastal southern India (Lagler et al., 1977). African species have been distributed worldwide because of their high potential for aquaculture in the last 60 years (Coutant, 1984; Pullin and Lowe-McConnell, 1982). However in some countries these African cichlids are now regarded as a pest because they have already escaped and invaded the new environments, often to the detriment of the indigenous ichthiofauna. Importations bring as a consequence the potential introduction of new parasites, and diseases, and worse still their escape from culture facilities can cause alterations in the short or long term to the aquatic environment with potentially irreparable damage (Zaret, 1980; Contreras and Escalante, 1984; Courternay, 1984; Welcomme, 1984;

Payne, 1986).

Before introducing an exotic species it is neccessary to carry out a detailed market study of these preferences. Once it is known that a species will be accepted, then a consequent series of studies on the natural distribution, ecological impact and eco-ethological characteristics could help in the final decision on introduction thus avoiding unfortunate consequences such as unwanted hybridization, competition for food or spawning grounds, or contamination of pure wild strains. It is clearly neccessary to take account of the danger of introductions made without sufficient knowledge of the biology of both the introduced species and the receiving ecosystem, (Philppart and Ruwet, 1982).

For aquaculture purposes Mexico has up to know, introduced 26 exotic species of fish, (Contreras and Escalante, 1984). The effect of many of these species on the native species are clearly identifiable, negative impacts on the native biota, often complicated by other factors and principally manifested by regression of native fishes. In some cases this has been through hybridization with endemic species, but in other cases there have been "quite unexpected biological back-reactions to introduced species" (Contreras and Escalante, 1984). Some introductions have resulted in severe predation of endemic species, bringing these fish

close to extincion, as for example the introduction of Micropterus salmoides at El Potosi, Nuevo Leon, where the major impact was on Megupsilon aporus and Cyprinodon alvarezi (Contreras, 1978). M.Salmoides has also been introduced into Patzcuaro Lake in Michoacan, resulting in a substantial reduction in the ancient fishery of "pescado Blanco" (Chirostoma estor) (Welcomme, 1984). A further example can been seen with the newly introduced, and as yet unidentified, tilapia in the Balsas basin, where the native cichlid, Cichlasoma istlanum acts as a normal host to a nematode parasite. This parasite has attacked the tilapia and has nearly destroyed what was to have been a new fishery (Rosas, 1976). Simultaneously, however, the tilapia almost completely eliminated the native Cichlasoma (Contreras and Escalante, 1984).

In Mexico, interest in the culture of cichlids in the tropical and parts of the subtropical areas has increased as the country has considered alternative ways of producing high quality proteins for human consumption. African cichlids such as Oreochromis and Tilapia species, were first introduced in 1964 (Morales, 1974). These species were popular candidates for culture in spite of the fact that full details of their biology and the technology for culture were not adequately known at that time. Some of these introductions in new man-made dams on the Pacific coast, as well as the Atlantic

coast of Mexico were highly succesful, establishing a new, semi-natural production of tilapia that actually reached some thousands of metric tons per year (Akester, 1985). Probably the major reason for this massive production is because in those newly-created dams there was a lack of indigenous fish species capable of exploiting the explosion of natural food resources as efficiently as the tilapias (Payne, 1986), permitting a rapid flourishing of these African species . These successes were unfortunately marred by a lack of understanding and control of the situation, resulting in uncontrolled releases and a rapid spreading of tilapias throughout the country (Contreras and Escalante, 1984). Further attempts to establish projects on semi-intensive and intensive aquaculture of tilapias have had little real success, due, mainly, to uncontrollable precocious breeding and stunting, followed by management problems of the brood stock, in addition to the generally poorer acceptability of the African species in the local markets. The moderate acceptance of tilapias in many areas of Mexico, is probably due only to the cursory visual similarity of these species with the native cichlids, and all of them are known in the local markets under the common name of "mojarras".

The foregoing reasons, have prompted a search for native Central American species which are well known and

accepted in traditional markets. Such species should have wide ranges of distribution, tolerance to management, and an adequate size for commercial marketing, while it is absolutely desirable to avoid ichthyophagous species. Any suitable native species have the immediate advantage that they are in situ and it is thus not necessary to import eggs, fry or brood stock at high cost. The potential alternatives in Mexico are great as this country is geographically situated in the confluence of the neartic and the neotropical regions, with a consequent wide variety of fish species.

Taking into account all the advantages of resistance, growth, fecundity etc. which characterize the African Cichlids it is tempting to consider that the new world cichlids may have the same advantages plus the regional acceptance and tradition for consumption.

C.urophthalmus (Gunther) is a native species with wide acceptability, good prices in the market and it is part of a regional fishery throughout the southeast of Mexico (Resendez, 1981). In Yucatan the species is well appreciated and it is exploited by marine fishermen as a complementary seasonal fishery. The fish is widely preferred to tilapias due to its firm flesh qualities. These characteristics immediately suggest C.urophthalmus as an alternative for culture in the region and there is

also the possibility that technologies developed for this species could be extended to other promising members of the group.

The fish is bluish-green to brownish in general body colouration with some slight blue in the dorsal area and with seven to eight notable transverse dark bands along the body, and a large dark spot at the base of the caudal peduncle (Plate 1). It has a good growth rate and can achieve an adult body weight of up to 600 g. (Martinez-Palacios and Ross, 1986). The natural distribution of this species extends from the Atlantic slope of middle America from the Rio Coatzacoalcos basin southward into Nicaragua, including the Yucatan Peninsula and Isla Mujeres in Quintana Roo, Mexico (Miller, 1976). The species is found associated with others from the same genus and Petenia splendida (which is one of the few ichthiophagous cichlids represented in the Mexican fauna) and has been able to successfully invade both fresh and brackishwaters, (Miller,1976) either in turbid or transparent conditions. C.urophthalmus prefers lentic enviroments and the populations are often higher in these locations than in lotic waters where populations may be reduced by the amount of food available (Resendez, 1981; Chavez et al., 1983). Normally it is found in places with bottom-vegetation in the form of algae or aquatic macrophytes

Plate 1.

A male of 600g of Cichlasoma urophthalmus showing its characteristic barred colouration.

as well as roots of higher plants such as willows, or mangroves. Frequently they occur in shoals of 50 to 200g individuals, grazing in the middle of lagoons, but when disturbed they immediately move to the shelter of the vegetation (Resendez, 1981 and personal observations). There is a paucity of information of this and other Cichlasoma species in Mexico in terms of aquaculture, and only a few well-detailed zoogeographic and taxonomic studies have been conducted (Miller, 1966, 1976; Bussing, 1976); as well as simple descriptive reports (Resendez, 1981; Chavez et al., 1983).

The objective of the present study was to investigate the potential of the native cichlid, Cichlasoma urophthalmus for aquaculture, based on studies of its natural history, reproductive behaviour, principal environmental tolerances including temperature, oxygen and salinity and basic nutritional requirements.

CHAPTER 1

THE NATURAL HISTORY AND POPULATION BIOLOGY OF *Cichlasoma urophthalmus*

INTRODUCTION.

Cichlasoma urophthalmus is one of the most important and widely appreciated species of the native cichlids in Mexico. It is exploited commercially in artisanal fisheries in the Mexican states of South Veracruz, Tabasco, Campeche (Resendez,1981) and Yucatan, with less importance in the state of Quintana Roo. Few authors have written on the biology of this species. Resendez, (1981) mentioned that this species is found in Laguna del Carmen Campeche, where it is one of the most important fish in the fishery of this lagoon. More recently Chavez et al., (1983) describe some aspects of the life history of various species of cichlids from Tabasco, including C.urophthalmus. They noted that it reproduces in fresh water from May to August, and after a stomach analysis concluded that it principally is an omnivorous fish with a tendency to carnivorous. They also described details of its morphometrical characteristics in the river San Pedro, Tabasco.

The native cichlids such as Cichlasoma sp. and Petenia splendida are popular in the local markets principally because they are traditionally available and are preferred to the tilapias because of the absence of muddy off-flavors. This preference can be

routinely observed in the markets where both groups of species are offered with the native cichlids commanding prices 20 to 40% higher than the tilapias. Unfortunately, the few studies of species of the genus Cichlasoma are quite inadequate to develop basic aquacultural techniques or to asses the feasibility of these native species in aquaculture.

In contrast to many other parts of the world where traditionally a large number of native species have been developed for aquaculture systems, in tropical America and particularly in Mexico those species which have been cultured are invariably exotic species. This is in spite of the particular richness of the neotropical cichlidae with more than one hundred species in the genus Cichlasoma alone (Miller, 1976).

The objective of the present study was to research the basic biology and enviromental conditions of C.urophthalmus in a brackish water lagoon which is exploited commercially, in order to provide data on which to base a series of experimental studies designed to explore the potential of this species for aquaculture.

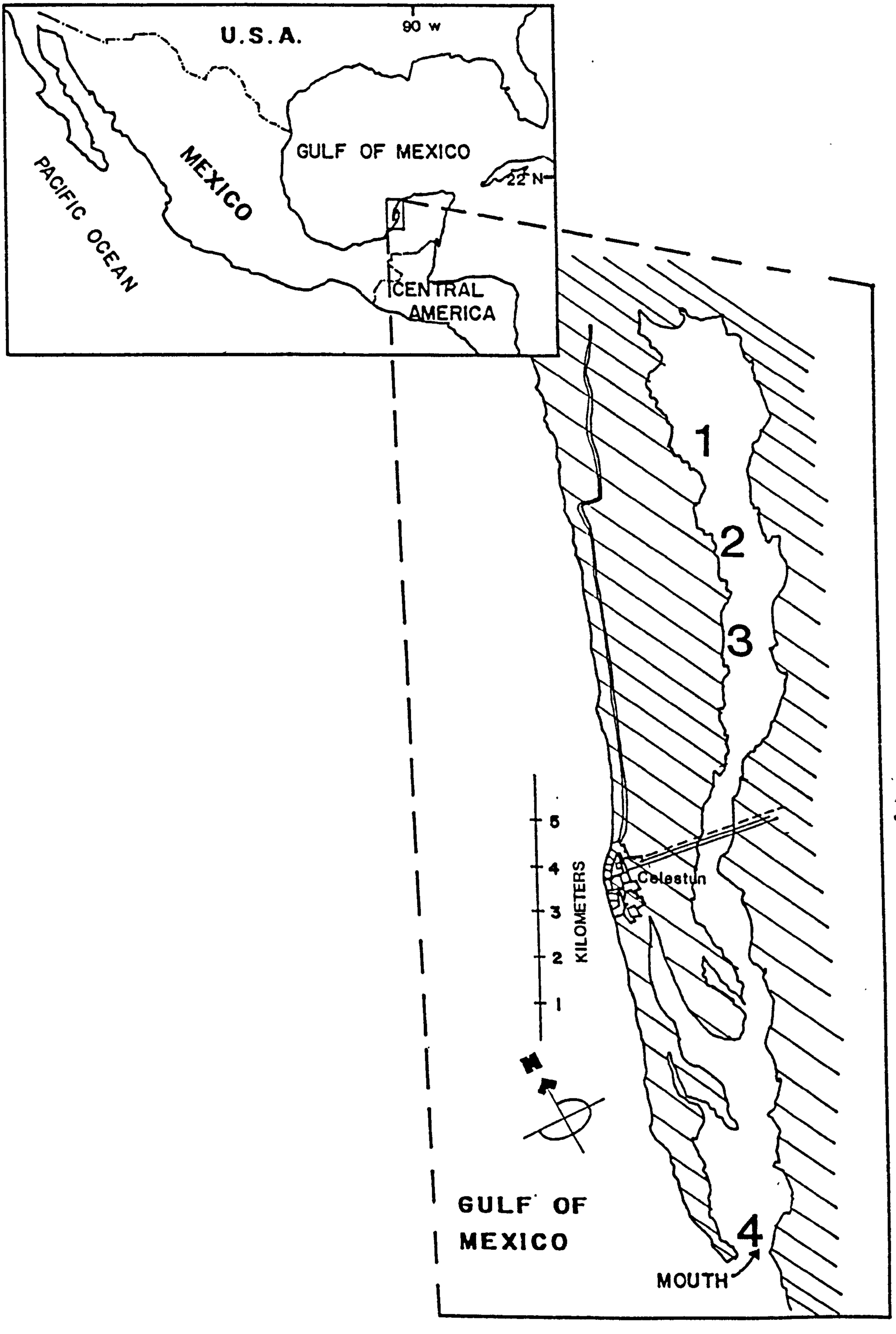
Site description and water quality.

The study area chosen was the Celestun lagoon which is situated on the north-east coast of the Yucatan Peninsula over the carbonate Yucatan platform of the Gulf of Mexico (Lankford, 1977)(Fig.1.1). The lagoon is in the state of Yucatan and has borders in the south with the state of Campeche; it is situated between the parallels 20° 15' 00'' and 20° 58' 00''N and the meridians 90° 15' 00'' and 90' 25° 00''W. The climate in this area is tropical, semidry, with rains in summer, an annual average temperature of 26.5°C and an annual average rainfall of between 40-60 cm. (Garcia et al., 1973). The rainy period in this region is usually from June to October with minor precipitation during October to March, associated with winds from the North, "Nortes".

The Celestun lagoon is classified as Type III, a barred inner shelf, and consequently one of its major characteristics is its protection from the sea by sand barriers which have been formed within the last 5000 years (Lankford, 1977). The lagcon is 20.8 km in length and the width ranges between 0.4-2.3 km. Most of the lagoon is, on average, less than 0.5m in depth, the maximum depth of 2.5m being found in the channel. Fresh water is supplied to this system both by direct rain and by underground springs flowing into the lagoon. This

Figure 1.1.

Geographical location of the Celestun Lagoon in Yucatan, showing the sampling stations used.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5
GULF OF MEXICO
MOUTH

spring water has an important effect on the lagoon producing salinities of 10%. or sometimes less at locations distant from the mouth.

The southern part of the lagoon is open with a 1.3 km wide mouth connecting to the Gulf of Mexico. The area is characterized by wide shallow and muddy areas covered with algae of the genus Chara and by some sea weeds. A tidal channel runs along the length of the lagoon and at the seaward end it is quite branched. This channel permits the circulation of fishing boats during high tides as far as the northern end, but at low tides it is difficult to reach even the middle of the lagoon. The tidal range is up to 50.0 cm (30.cm average) and during low tides most of the lagoon has huge areas exposed.

The lagoon is closely surrounded by extensive mangrove vegetation represented chiefly by Rizophora mangle at the edge of the water and behind this, extensive areas of Avicenia germinans. It is also possible to identify Laguncularia racemosa and Conocarpus erectus but in fewer numbers (Flores, S. Personal communication).

The bottom of the lagoon is soft organic mud,

varying from 30 to 60 centimeters deep, which gives off hydrogen sulphide due to the high amount of organic matter in anoxic conditions. The waters are usually brown in color due the high amount of tannins from the mangrove.

Among the most important commercially exploited marine fish resources of the lagoon are: Lutjanus griseus and L.analis (Pargos), Calamus pennatula (mojarra), Archosaurus probatecephalus (sargo), Lagodon romboides (sargo), Eucinostomus gula (mojarrita), Haemulon plumieri (ronco), Orthopristis chrisopterus (corcovado), Anisotremus surinamensis (burro), Bothus ocellatus (lenguado), Anchoa lamprotaenia (anchoa), Bordiella chrysura (corvina), (Julio Sanchez, personal communication).

Some brackish water fish are also caught in the lagoon and of these the most important is the mojarra castarrica (Cichlasoma urophthalmus). This is fished during winter and spring, when the strong winds ("Nortes") occur thus preventing the fishermen from working at sea. In these conditions the mojarra in this protected lagoon become an alternative fishery and this season may last from November/December to March/April (winter).

The Cichlasoma fishery in Yucatan is not restricted but there is some selection done by the fishermen for a minimum weight of 250-400g.

Water quality parameters.

Throughout the study period changes in water quality parameters were assessed. Water temperature and dissolved oxygen were measured using a YSI Model 57 Oxygen meter, pH was estimated using a Pye-Unicam 9409 pH meter and salinity was measured with a refractometer (American Optical). All water samples were taken from the surface and the stations are indicated in Figure.1.1. The results for seasonal measurements are shown in Table 1.1. The values of these water quality parameters during the sampling period (1985-1986) give an impression of this typical enviroment for C. urophthalmus.

The influence that fresh water has over the lagoon system, as shown in Table 1.1., is quite strong. The inland end of the lagoon always has the lowest salinity, including in the dry season (winter-spring). The middle section of the lagoon is influenced by the ingress of sea water and the salinity gradually increases to full-strength sea water at the entrance of the lagoon.

Table 1.1. Salinity (‰), temperature (°C), oxygen (mg/l) and pH determinations in the Celestun Lagoon along five seasons samplings. Values are means of three observations.

	Spring 1985				Summer 1985				Autumn 1985			
Stations	1	2	3	4	1	2	3	4	1	2	3	4
ToC	26.0	31.2	27.7	28.2	30.0	30.0	29.3	27.2	29.6	29.4	29.3	29.0
S‰	16.0	18.0	16.0	29.6	16.0	19.7	21.6	34.0	7.0	6.5	4.0	22.0
Omg/l	0.84	7.35	7.34	3.92	9.0	-	4.8	3.4	3.9	2.9	1.5	3.4
pH	-	-	-	-	8.2	-	7.7	7.8	8.5	8.4	8.5	8.0

	Winter 1985-1986				Spring 1986			
Stations	1	2	3	4	1	2	3	4
ToC	28.5	28.2	26.4	25.8	29.0	28.0	27.0	29.0
S‰	13.4	17.2	20.08	29.5	17.5	22.1	24.6	40.3
Omg/l	1.6	1.8	1.9	4.5	1.6	2.1	2.0	3.2
pH	8.4	8.5	8.3	8.2	8.0	8.2	8.2	8.3

Temperature is more influenced by the season than the marine waters, and by the depth of the water itself along the lagoon. The oxygen concentration changes little throughout the year.

The basic pH in this lagoon is a clear indication of the calcareous material that is predominant in the area, and is only influenced by the effects of dilution of the rain.

Of all of these parameters, salinity has the greatest influence over the distribution of C.urophthalmus populations along the lagoon. During the present work numerous schools of this species were observed north of the bridge in the brackish water area, but few animals were seen in the high salinity zone. These observations were reconfirmed later by discussions with the fishermen in Celestun who identified the most important area for fishing as a little north of the bridge beyond station number 3 (Figure 1.1). It is, however clear that C.urophthalmus in the Celestun Lagoon is adapted to live in a wide range of salinities and generally low oxygen concentrations (Table 1.1).

Fish capture.

The present part of the study was based on an analysis of 848 fish captured during five seasons between spring 1985 to spring 1986. During all fishing operations in the lagoon no other member of the genus Cichlasoma was recorded. The specimens were caught by angling from an anchored boat and this was the only suitable technique because of the very shallow waters (30-40 cm) and the abundance of these fish at the edge of the mangrove roots. This method is commonly used also by the fishermen in this lagoon. The hook were baited with small prawns (Palaemonetes sp.).

The fish captured were immediately stored in iced-water and transported to the laboratory for further analysis and measurement of individual total weight, evicerated weight, total length, standard length and intestine length. Samples of branchial arches with gill rakers, pharyngeal bones and jaws were taken and the stomachs were preserved in 10% formalin for later analysis.

The fish utilized for identification were maintained in 10% formalin and identified using the keys supplied by Dr.Rush Miller (University of Michigan). Samples of certain animals were sent to Dr. Miller who kindly confirmed their identification.

Maturation of ovaries, development of ova and length of the reproductive season were determined by gross examination of ovaries and ova measurements, and by determination of ovary weights in the seasonal collections. All fish were evicerated in order to determine sex. Both ovaries were then removed from all the females and the mature ovaries were selected and retained for analysis.

The gonads were fixed in bouins fluid (Roberts, 1978) for 24 hrs and later were preserved in 10% formalin. Eggs were measured using a Laborlux 12 Leitz microscope with an eye piece graticule.

Feeding structures.

Dentition.- Cichlasoma urophthalmus has a slightly protrusible mouth and shows a clear line of unicuspid teeth, on both the upper and lower jaws with two less conspicuous rows behind these. These teeth, 2 to 3 on

each side in both jaws, are distinctly differentiated as canines, and in larger animals they are somewhat exposed even when the mouth is closed. The teeth of the first row in the upper jaw are strongest and bigger than those in the lower jaw (Figure 1.2).

Pharyngeal teeth. The lower pharyngeal bone shown in Figure 1.3 is approximately triangular in outline, in common with other cichlids. The central area is occupied by large coarse flat teeth and these are surrounded by rows of fine teeth some of which are very sharp. In general, this conformation suggests an efficient crushing mechanism.

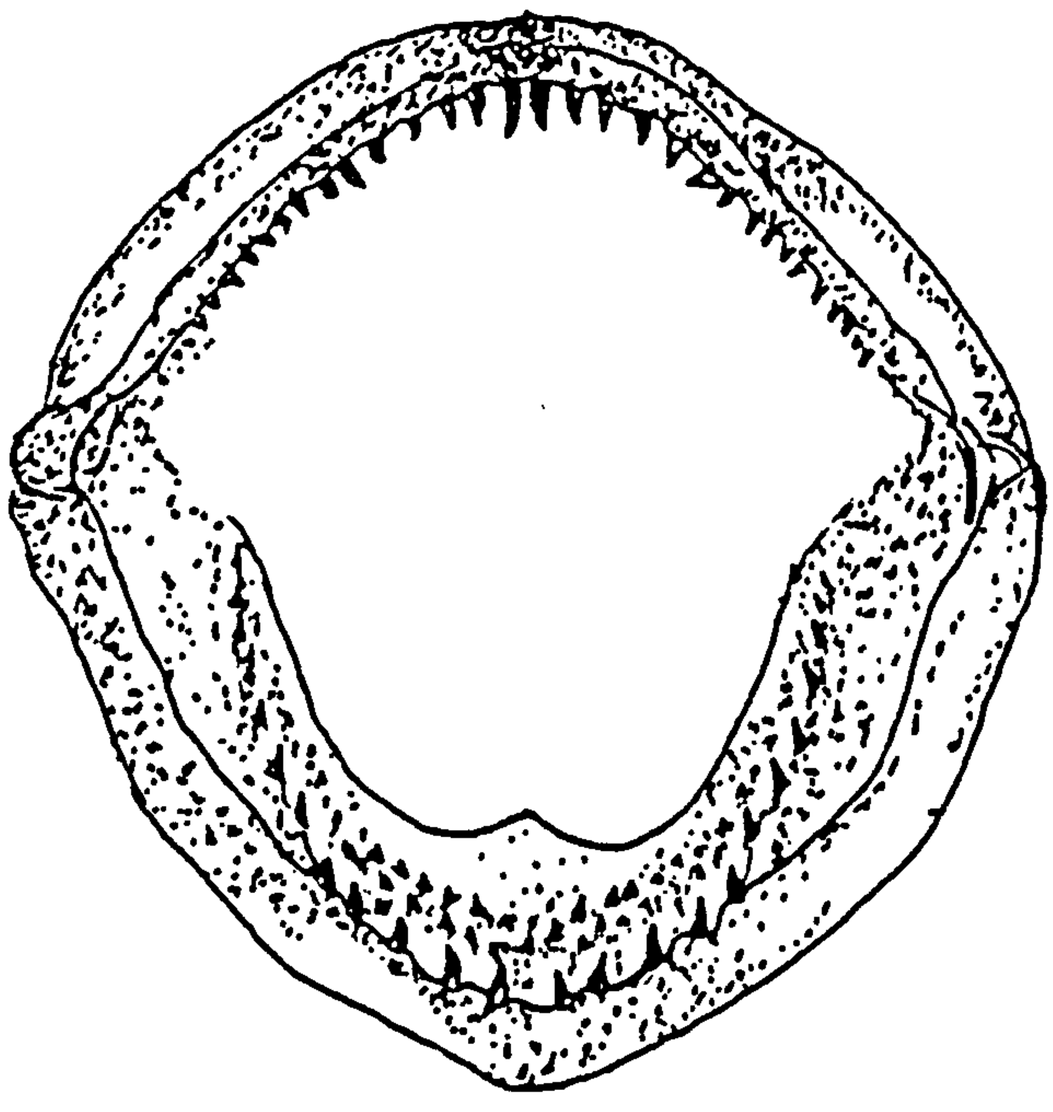
Gill rakers. The branchial arches have conspicuous flat, short and unornamented gill rakers. In the first branchial arch this species usually has 9-11 gill rakers (Figure 1.4).

Gut length. The alimentary tract from (behind the pharyngeal teeth to the anus) is approximately 2.2 times the total length of the fish.

A contrasted X-ray photograph of the intestine, prepared using radio-opaque diets (Plate 1.1), shows the short intestine folded inside the body cavity.

Figure 1.2.

Superior and inferior jaws of C<u>urophthalmus</u> showing details of the unicuspid teeth. The drawings were made by direct observation and features were measured by graduated graticule under microscope.



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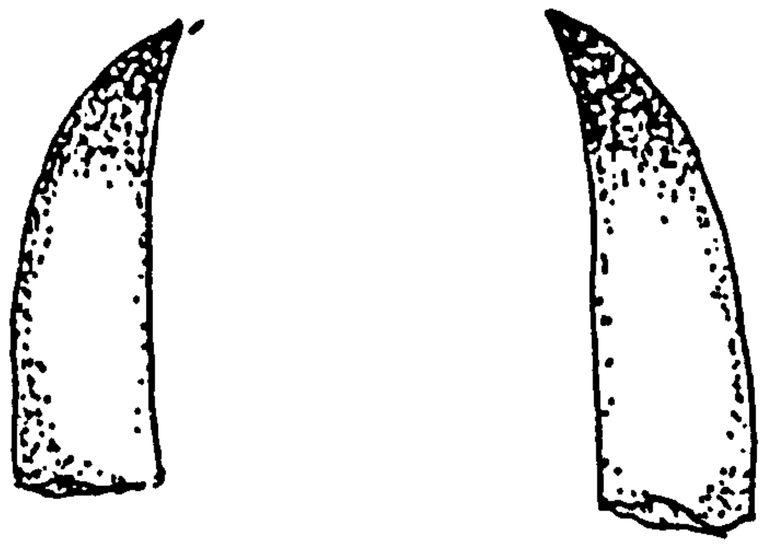

0 1 2 3 mm

Figure 1.3.

Lower pharyngeal bone of C urophthalmus in occlusal view. Drawings were made by direct observation and proportions were adjusted by photography.

Figure 1.4.

First branchial arch of C urophthalmus showing the gill rakers.

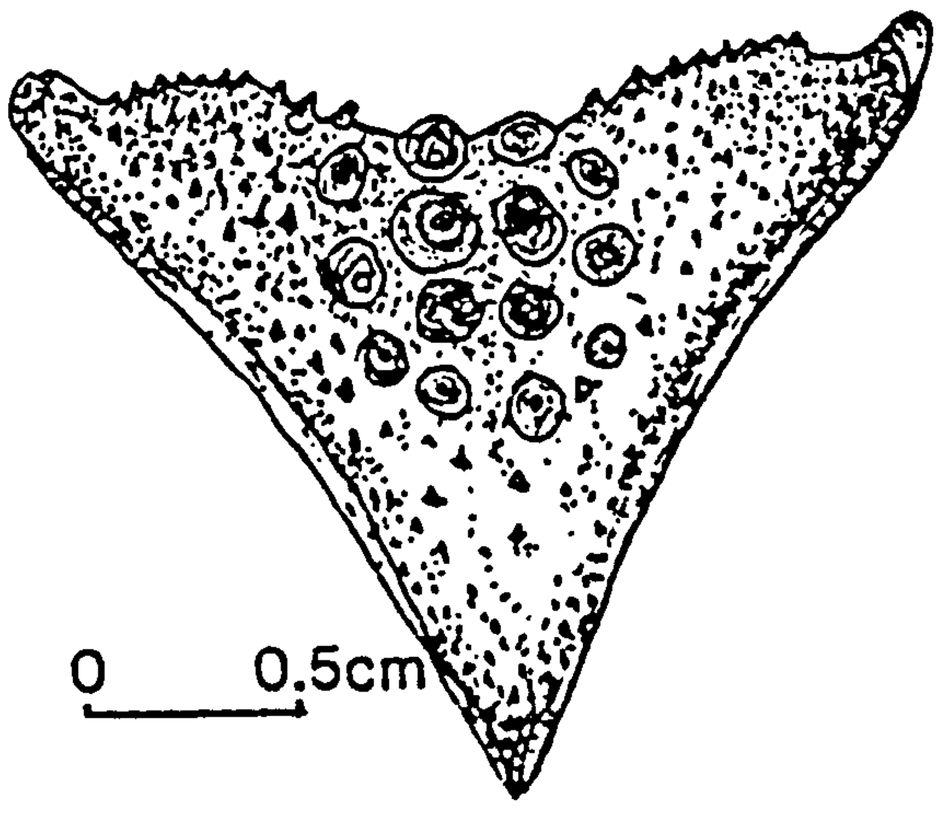
0 0.5cm

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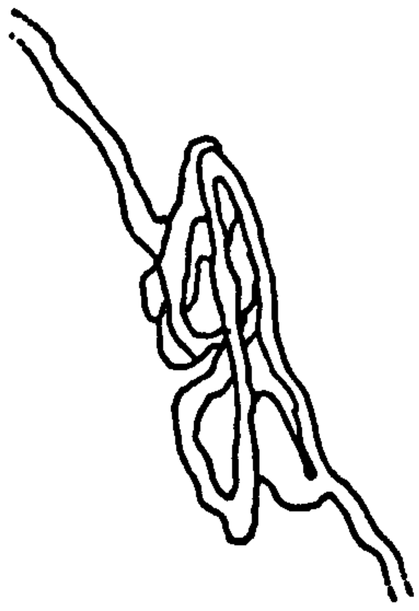
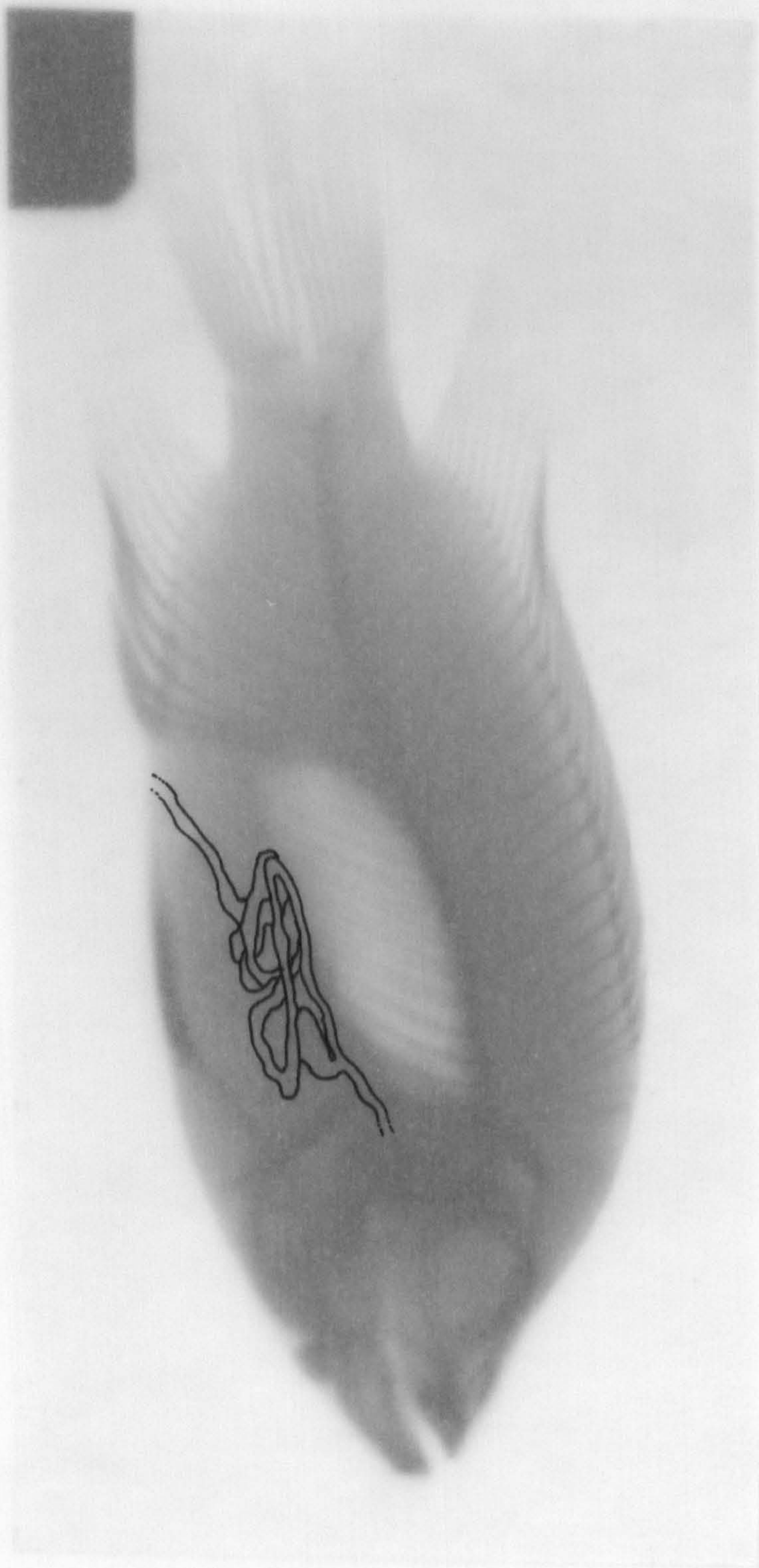
Plate 1.1.

A contrasted X-ray photograph of C.urophthalmus using radiopaque diets. The intestine is over marked with ink to highlight is folding in the body cavity.





There was a highly significant correlation ($r=0.837$, $n=772$, $P<0.001$) between the log10 of the standard length(mm) and the log10 of the total intestine length(mm) and this relationship can be described by the equation:

$$\log IL = 0.3253 + 10157(\log SL). \qquad \text{(Equation 1)}$$

where IL= Intestine length and SL= Standard length. The equation was based on data from 772 fish and the data are shown in Figure 1.5.

The stomach is a simple sac-shape, with very thin walls when full and no pyloric caeca are present. The overall gut structure is thus very similar to many cultured tilapias, but with a reduced intestine length.

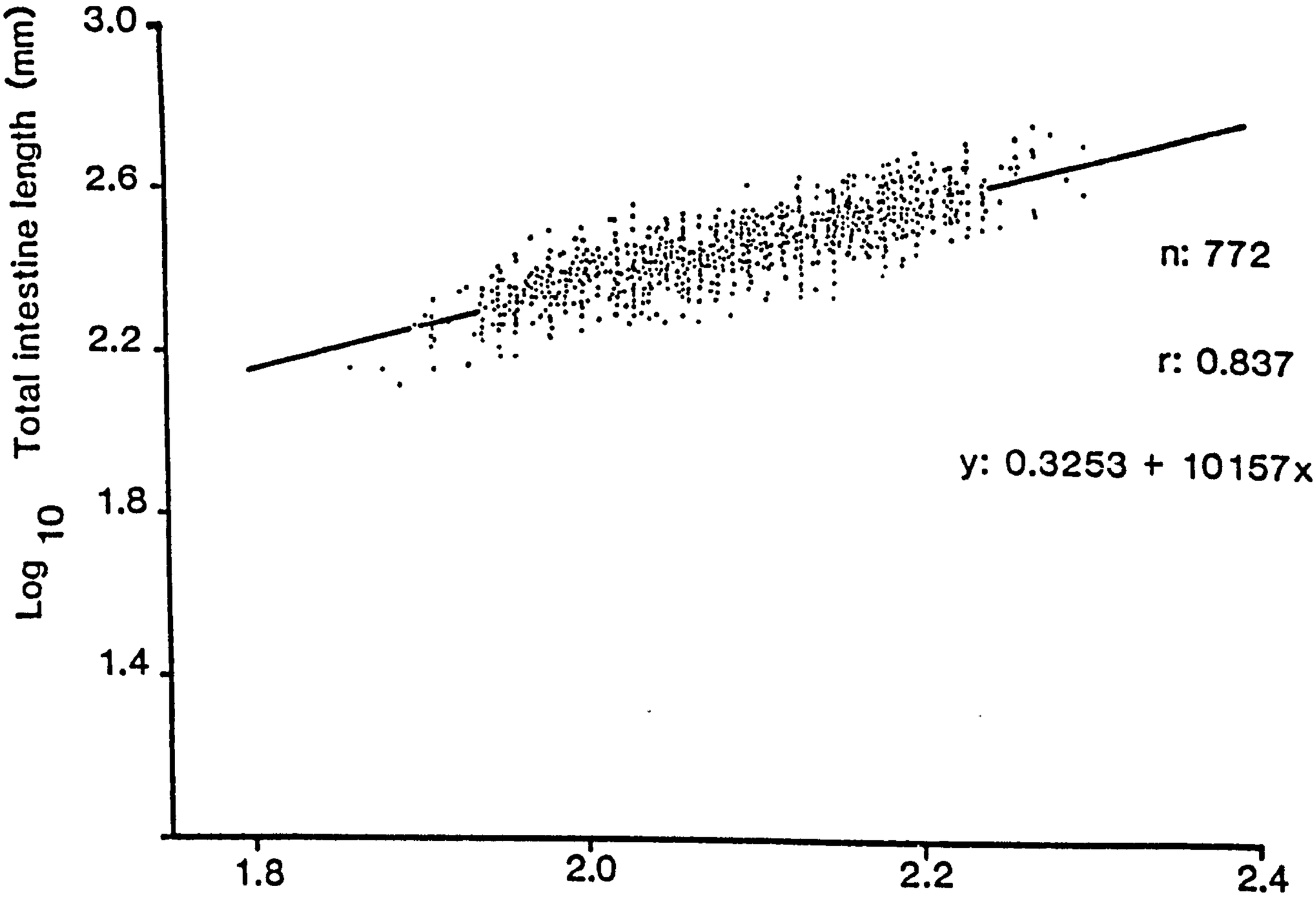
The natural diet of C.urophthalmus.

Studies of the natural diet of C.urophthalmus in the Celestun Lagoon were initiated in the Spring of 1985 and continued until the spring of 1986.

The preserved stomachs were opened and washed carefully with distilled water into a petri dish. The stomach contents of the seasonal samples were then analysed microscopically identifying the major taxonomic groups, such as Penaeids, Algae, etc. Subsequently the relative

Figure 1.5.

Log10 intestine length against log 10 standard length of Cichlasoma urophthalmus from Celestun, Yucatan, Mexico.


Total intestine length (mm)
Log 10
3.0
2.6
2.2
1.8
1.4
1.8
2.0
2.2
2.4
Log 10 Standard length (mm)
n: 772
r: 0.837
y: 0.3253 + 10157x

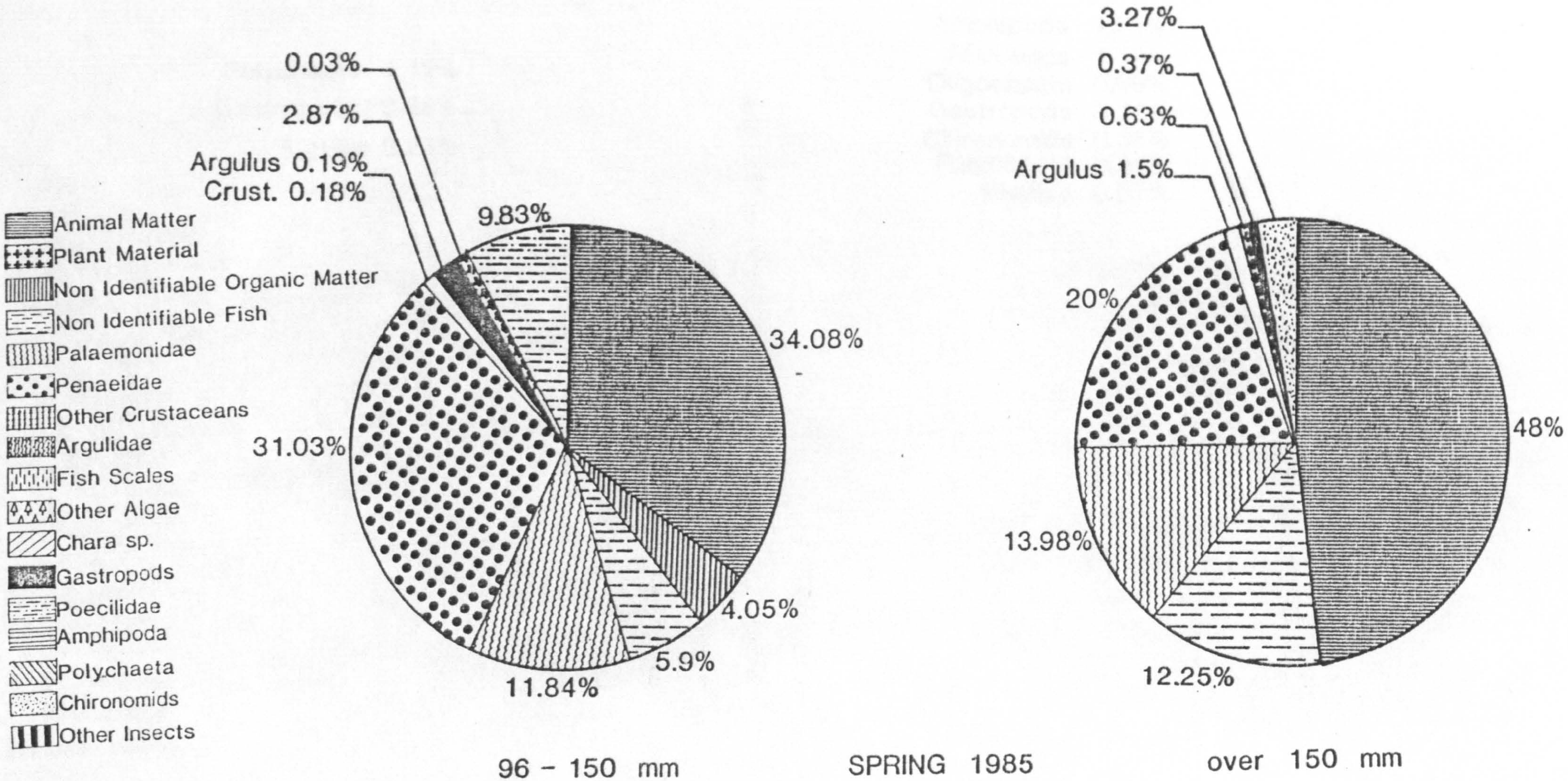
contribution of each of the taxa was assessed quantitatively according to the percentage volume of each particular item in the total stomach contents. The average percentage of these different materials was assessed in the two size clases 96-150 and 151-200 mm standard length. The percent frequency of food items in the diets of these two size classes are shown for all the seasonal collections in Figures 1.6 to 1.10.

The diet was very varied in spring 1985 (Figure 1.6) but the most important components are the palaemonids, penaeids and fish, showing a mainly carnivorous diet. In both size classes a small amount of plant material was consistently found.

In summer 1985 (Fig.1.7) a substantial algal component appeared in the diet of both length classes, with a strong reduction in the penaeid and palaemonid contribution. The only major difference between these classes with the other seasons was the much wider range of species found in the gut. During the autumn 1985 (Figure 1.8) animal matter was predominant in the diet although the penaeid contribution was much reduced in the 96-150 mm size range and was absent in the 151mm range. Minor components such as algae and insects are represented in both size ranges. In Winter 1985 (Figure 1.9), animal matter again contributed more than the half

Figure 1.6.

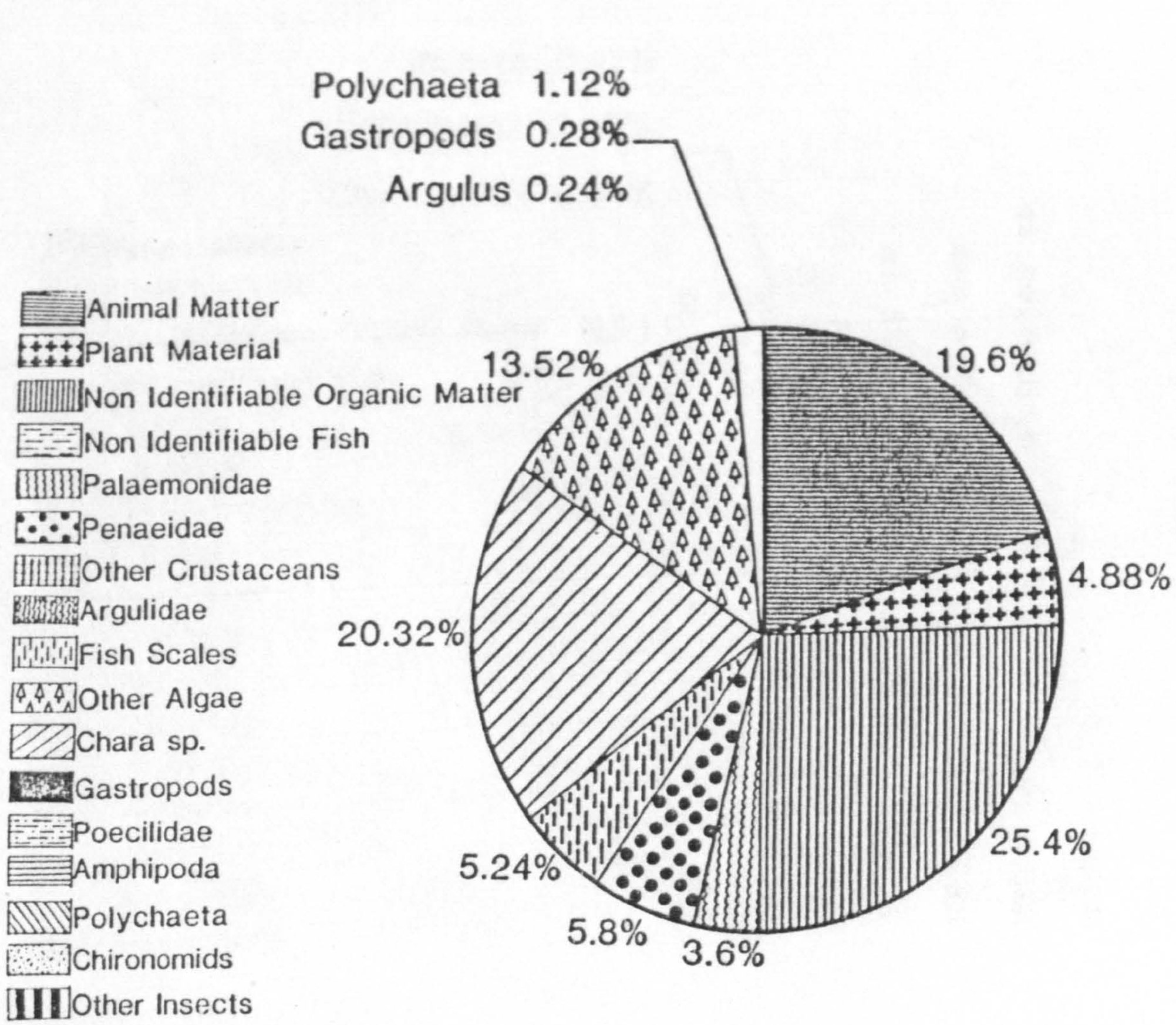
Relative percentage contribution in the diet of two length classes of C urophthalmus in the spring of 1985 in Celestun Lagoon, Yucatan.



96 - 150 mm SPRING 1985 over 150 mm

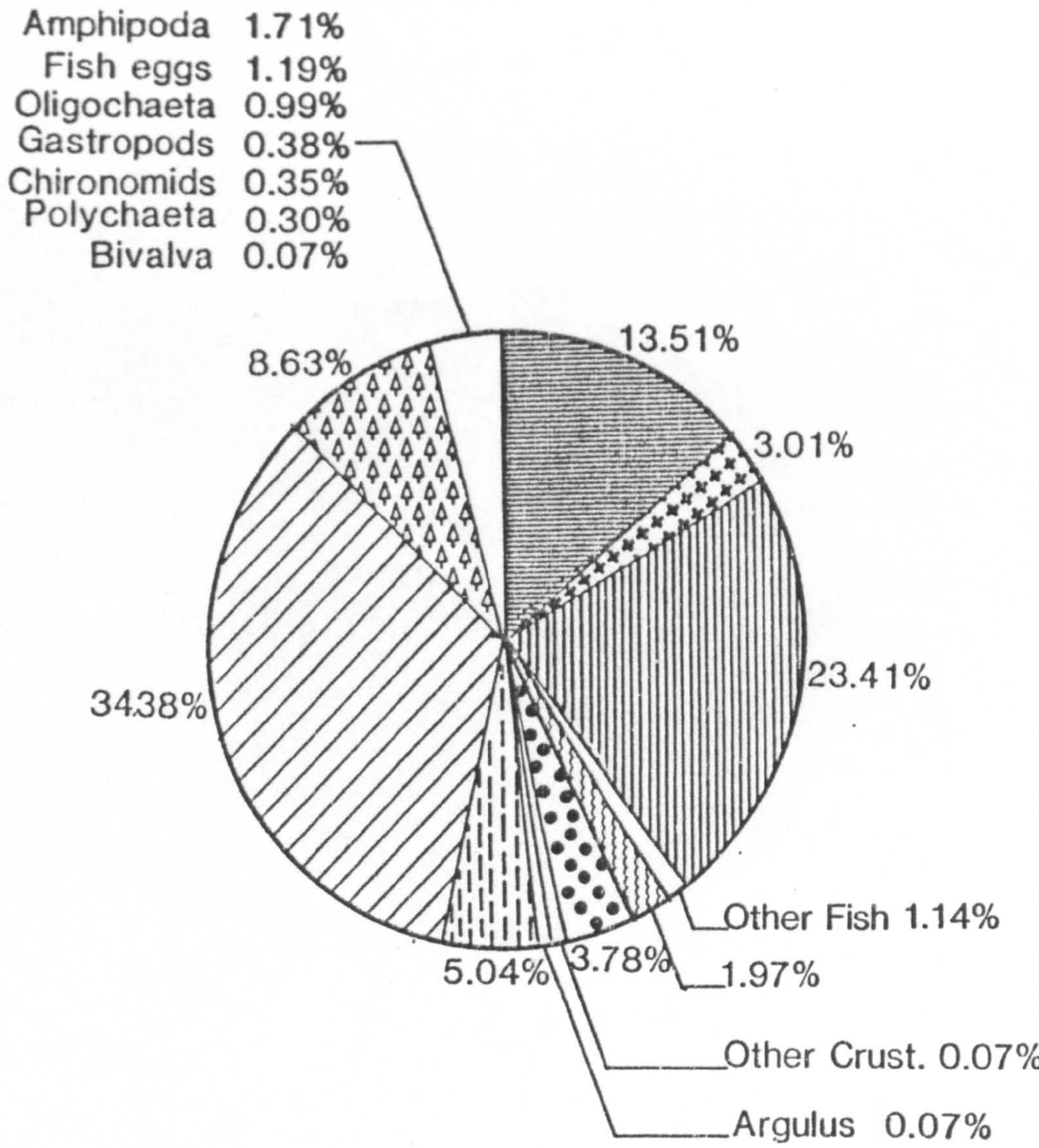
Figure 1.7.

Relative percentage contribution in the diet of two length classes of C urophthalmus in summer 1985 in Celestun Lagoon, Yucatan.



96 - 150 mm

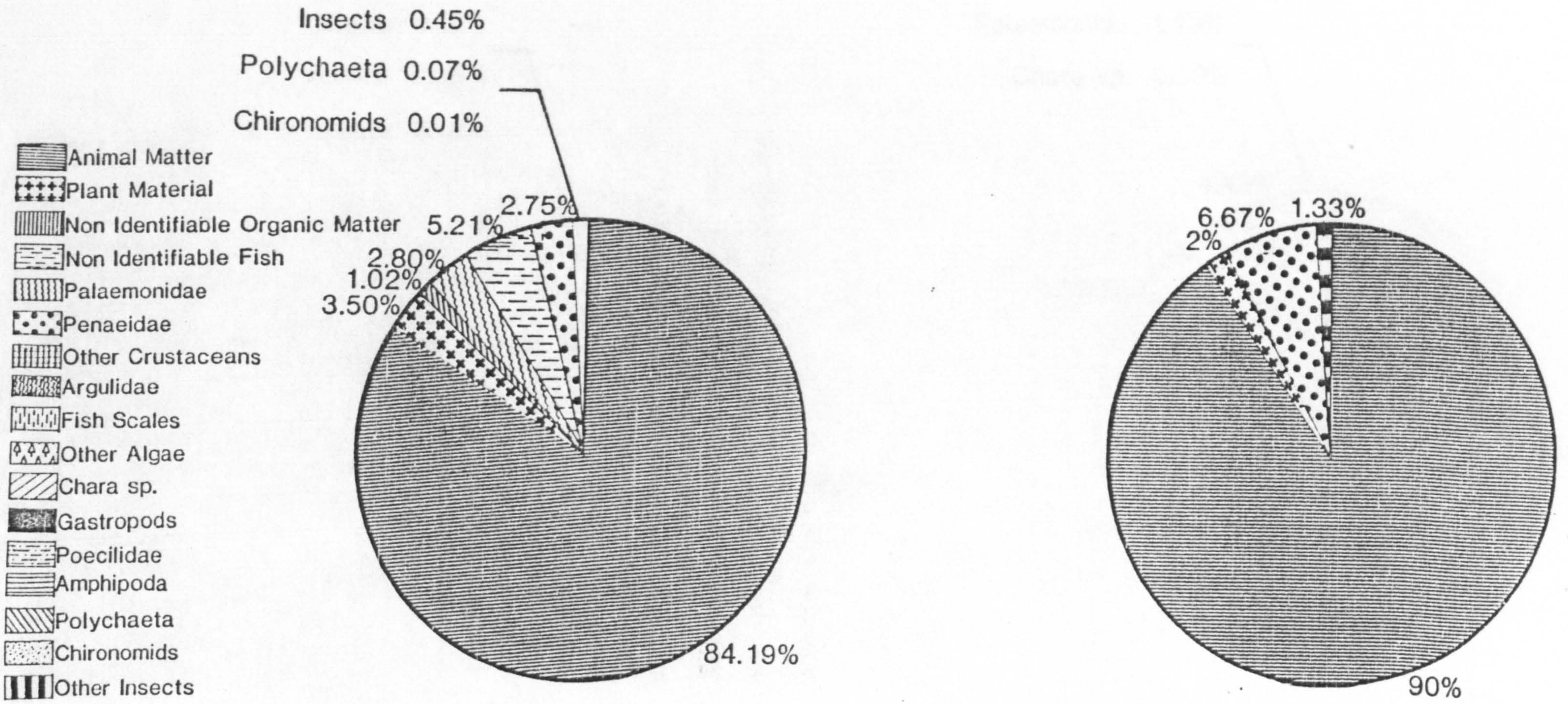
SUMMER 1985



over 150 mm

Figure 1.8.

Relative percentage contribution in the diet of C.urophthalmus in two length classes for autumn 1985 in Celestun Lagoon, Yucatan.



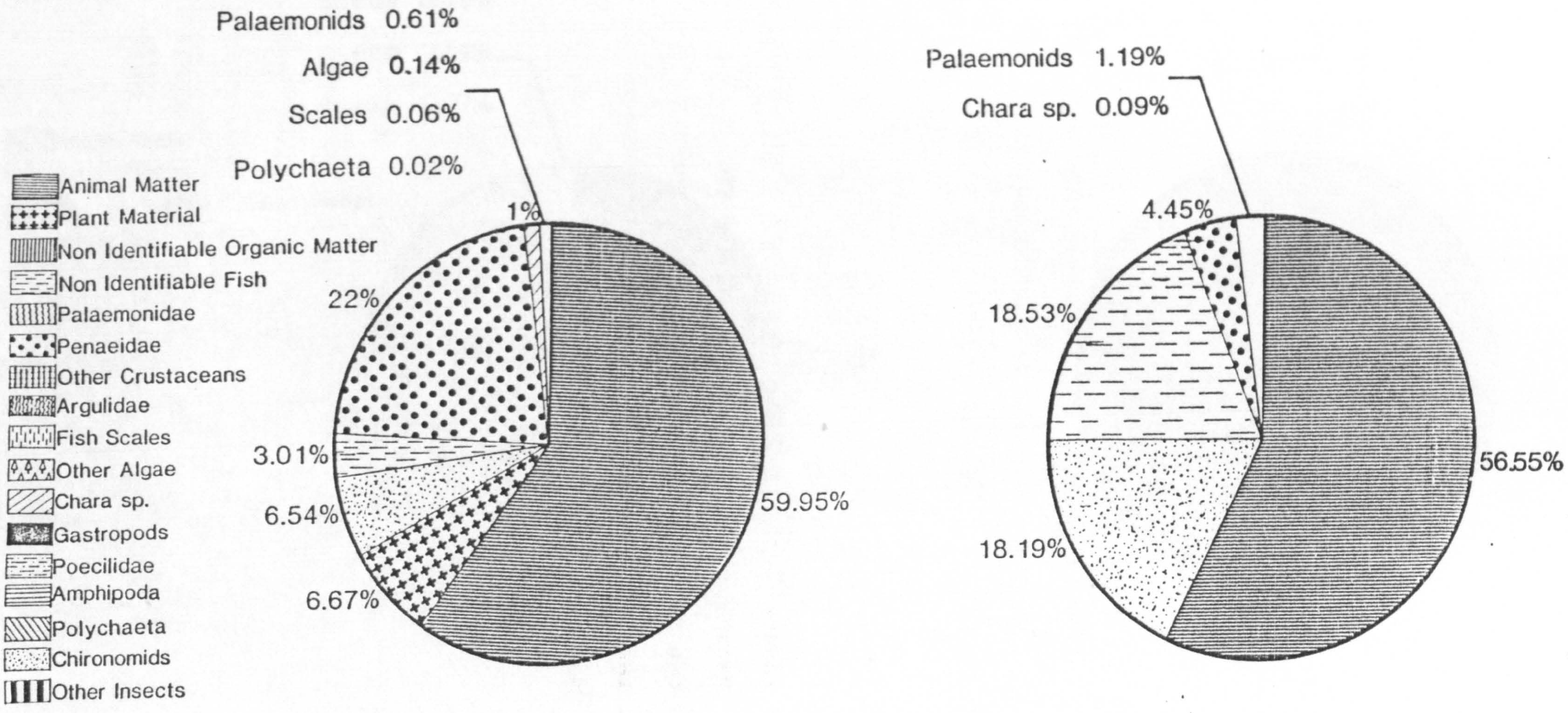
96 – 150 mm

AUTUMN 1985

over 150 mm

Figure 1.9.

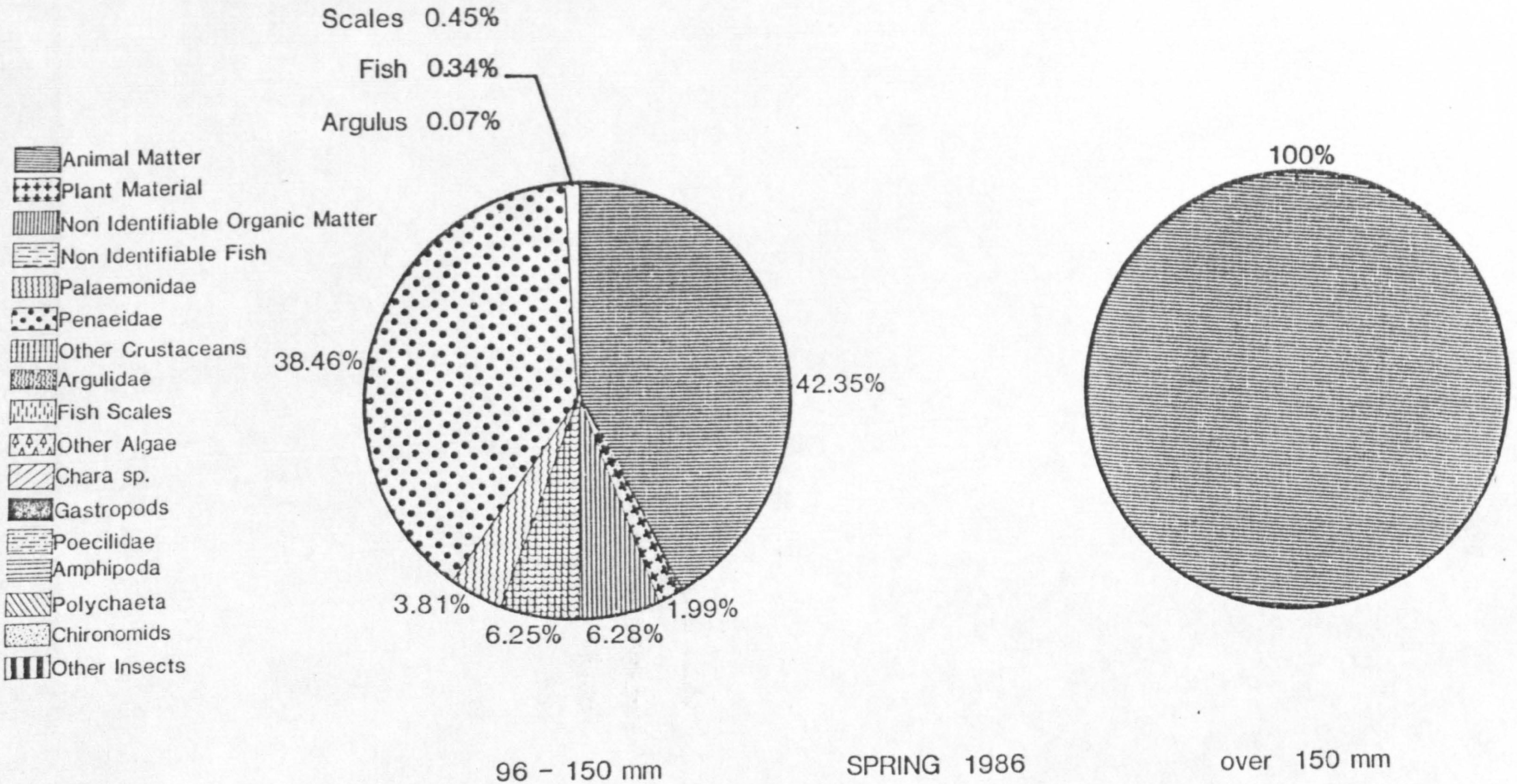
Relative percentage contribution in the diet of C.urophthalmus in two length classes for winter 1985 in Celestun Lagoon, Yucatan.



96 – 150 mm WINTER 1985 – 86 over 150 mm

Figure 1.10.

Relative percentage contribution in the two length classes of C urophthalmus for spring 1986 in Celestun Lagoon, Yucatan.



96 - 150 mm

SPRING 1986

over 150 mm

of the stomach contents in both size ranges. The palaemonids were quite scarce, and the penaeids were the most important fraction in the size range 96-151 whereas fish and Chironomids were the most important in the size range 151mm.

During the spring 1986 (Figure 1.10) in the range 96-151mm the penaeids were the most important animals in the diet, while palaemonids are scarcely represented. In the 151 mm size range only unidentifiable animal matter was found.

Population growth and age structure.

Length frequency histograms of C.urophthalmus were constructed for the five seasonal samples between the spring of 1985 and the spring of 1986 (Figure 1.11).As in most tropical and subtropical species, identification of cohorts is not immediately possible and in order to assist in this interpretation the % frequency of males, females and immature animals two broad size classes is shown for the five seasonal collections in Figure 1.12. The spring 1985 sample shows a relatively clear bimodal distribution. The absence of the lower size groups in summer 1985 suggests that the first group (SL=70-130 mm Figure 1.12 and Figure 1.11) comprises

Figure 1.11.

Seasonal length-frequency histograms for collections of C.urophthalmus between spring 1985 and spring 1986 in Celestun, Yucatan.

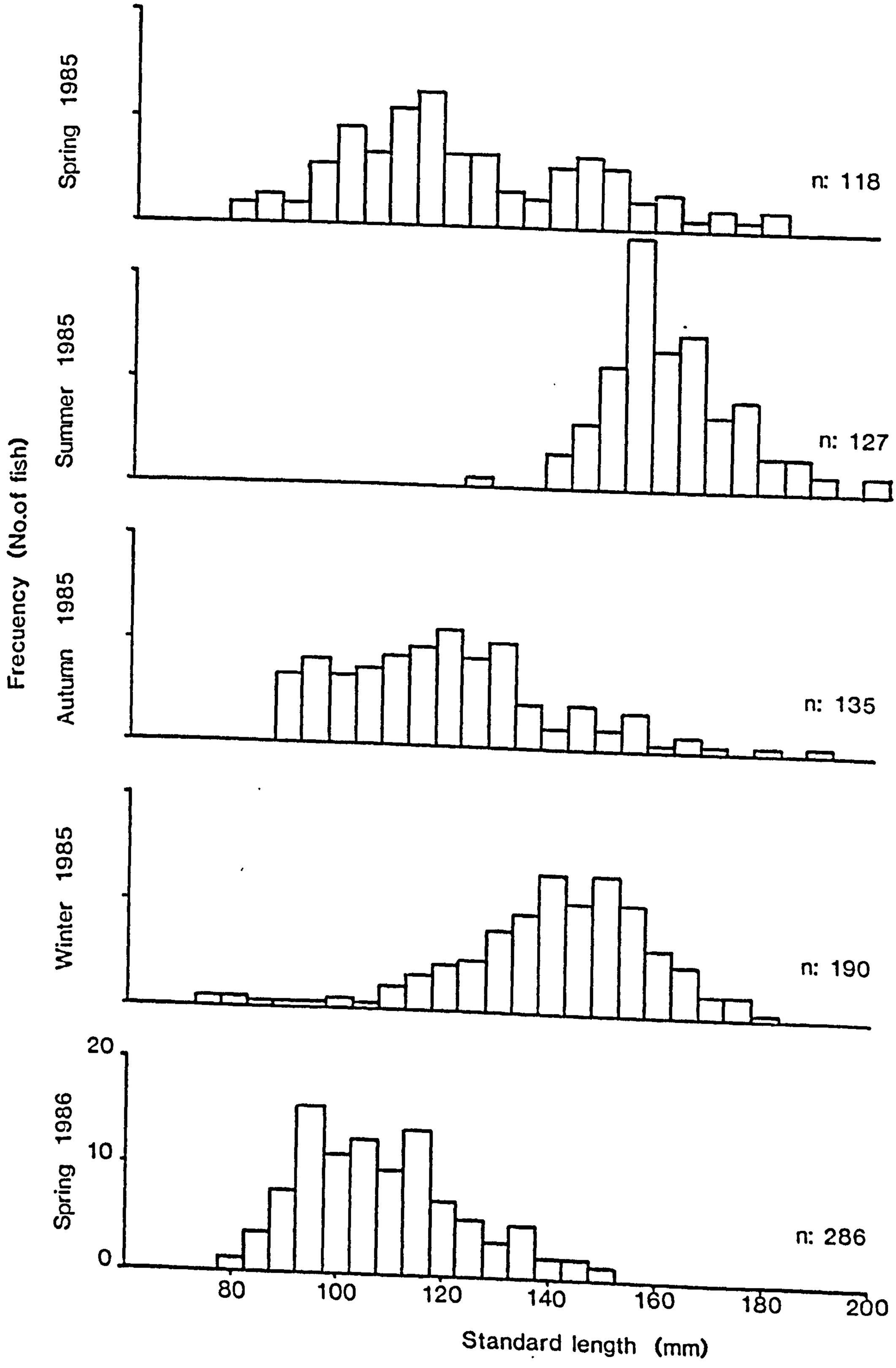
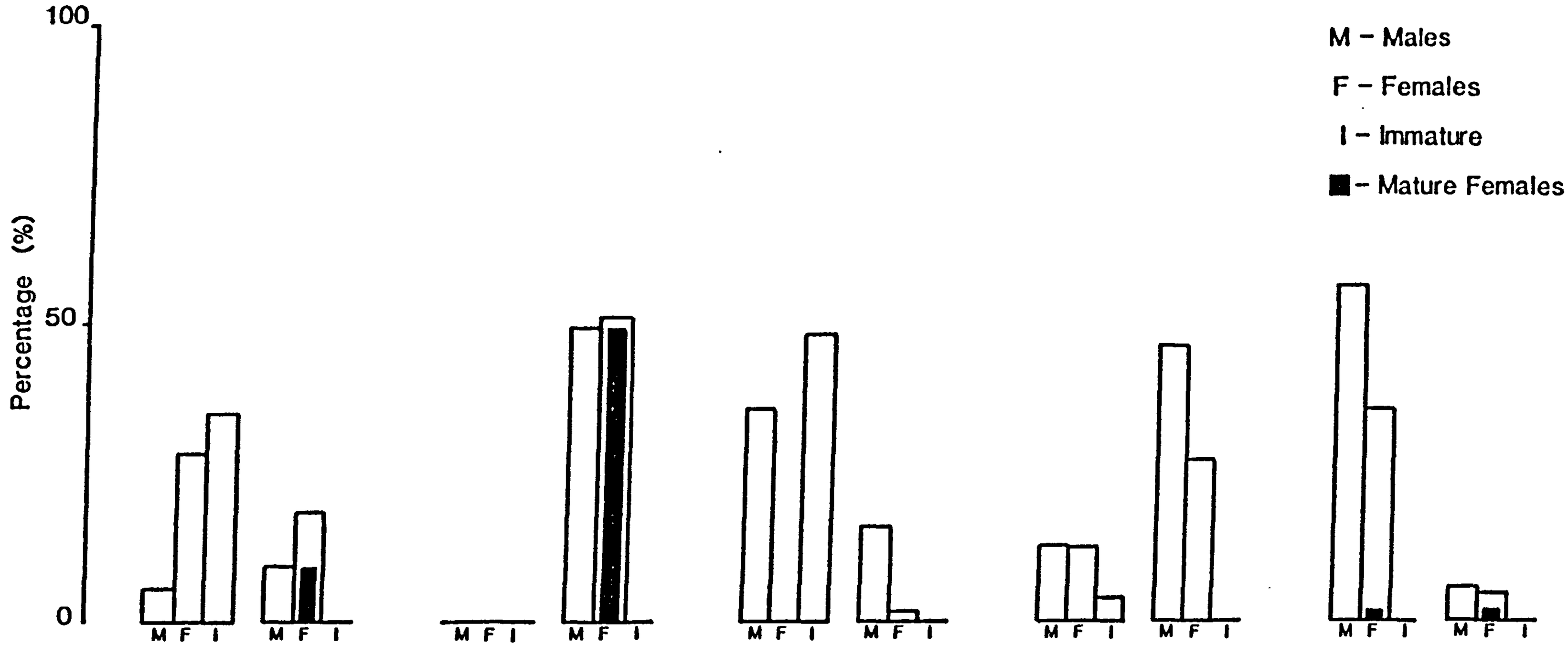

Spring 1985
n: 118
Summer 1985
n: 127
Autumn 1985
n: 135
Winter 1985
n: 190
Spring 1986
n: 286
Frecuency (No.of fish)
20
10
0
80
100
120
140
160
180
200
Standard length (mm)

Figure 1.12.

The relationship between males, females, mature females and juveniles along the five season samplings in Celestun Lagoon, Yucatan.


100
50
0
Percentage (%)
M – Males
F – Females
I – Immature
■ – Mature Females
M F I M F I
70–130 131–200
SPRING 1985
SUMMER 1985
AUTUMN 1985
WINTER 1985
SPRING 1986

animals which have just completed the first spring, while the second group (SL=131-200mm) has already entered their second reproductive year. By summer 1985 the first group (70-130mm) has already grown to occupy the size range of the higher size range, while the percentage of animals occupying this, presumably older, group (131-200mm) increases. Thus the young fish having just completed their first spring probably begin to reproduce in the summer time when the species reaches its maximum reproductive peak (Figure 1.12). During autumn this reproductive group reduces markedly in size, with a notable absence of females (Figure 1.12). It is probable that the females are reduced in this sample as they are involved at this time in care of the young and are thus unlikely to be caught during sampling. Some smaller fish reappeared in the autumn sample and these probably represent residual slow-growing fry from the previous season, mixed with some fast-growing juveniles from the early spring reproductive activity.

Standard lengths and weights were recorded in 413 males and 312 females and from these data lenght-weight relationships were prepared. There was a highly significant correlation between the log of the total weight (Wt) and the log of standard length (SL) in both the males and females. The linear equations expressing these relationships are:

Males: log Wt= -4.71+ 3.18 (log SL). (r= 0.981, n=413, P < 0.01). (Equation 2.)

Females: log Wt= -4.64 + 3.15 (log SL). (r=0.982, n=302, P< 0.01). (Equation 3.)

These data, with the line of best fit are shown graphically in Figure 1.13 a,b. Students t-test showed that there was no significant difference between the slopes of these lines for males and females (P-0.3). An overall lenght-weight relationship, incorporating males, females and juveniles was then recalculated as:

All fish:log wt = -4.52 + 3.14 (log SL) (r=0.98, n=830, p < 0.01). (Equation 4.)

and this is shown graphically in Fig.1.14. Students T-test show no significant diference (P=0.13) between the slopes of the lines for males, females and juveniles.

Sexual characteristics and reproductive performance in the natural environment.

Males and females have quite similar external characteristics, which makes sex determination difficult in juvenile animals and even in adults. During the

Figure 1.13.

Relationship between log body weight and log standard length in males (A) and females (B) of C.urophthalmus in Celestun Lagoon, Yucatan.

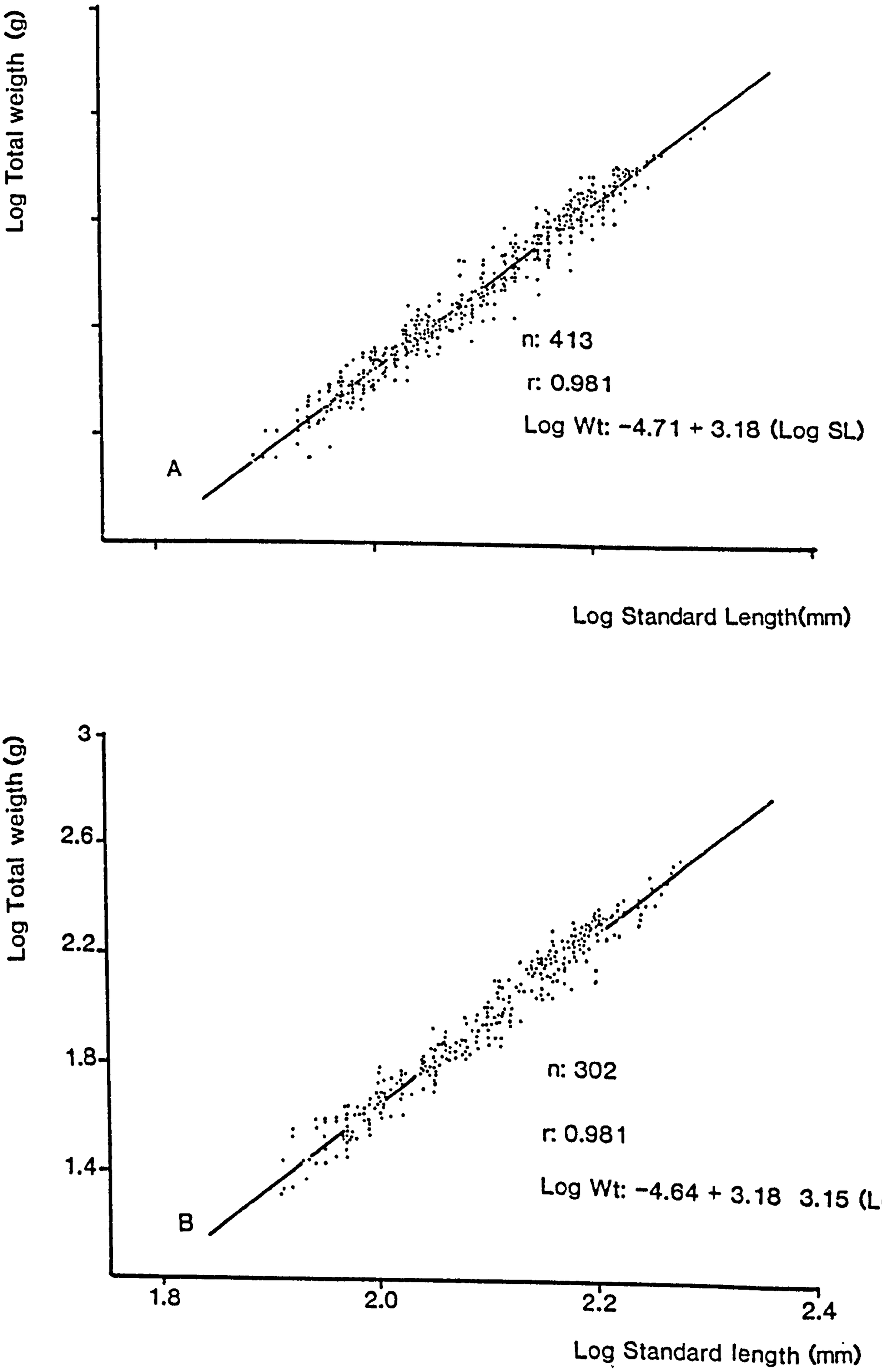

Log Total weigth (g)
n: 413
r: 0.981
Log Wt: −4.71 + 3.18 (Log SL)
A
Log Standard Length(mm)
Log Total weigth (g)
3
2.6
2.2
1.8
1.4
n: 302
r: 0.981
Log Wt: −4.64 + 3.18 3.15 (Log SL)
B
1.8
2.0
2.2
2.4
Log Standard length (mm)

Figure 1.14.

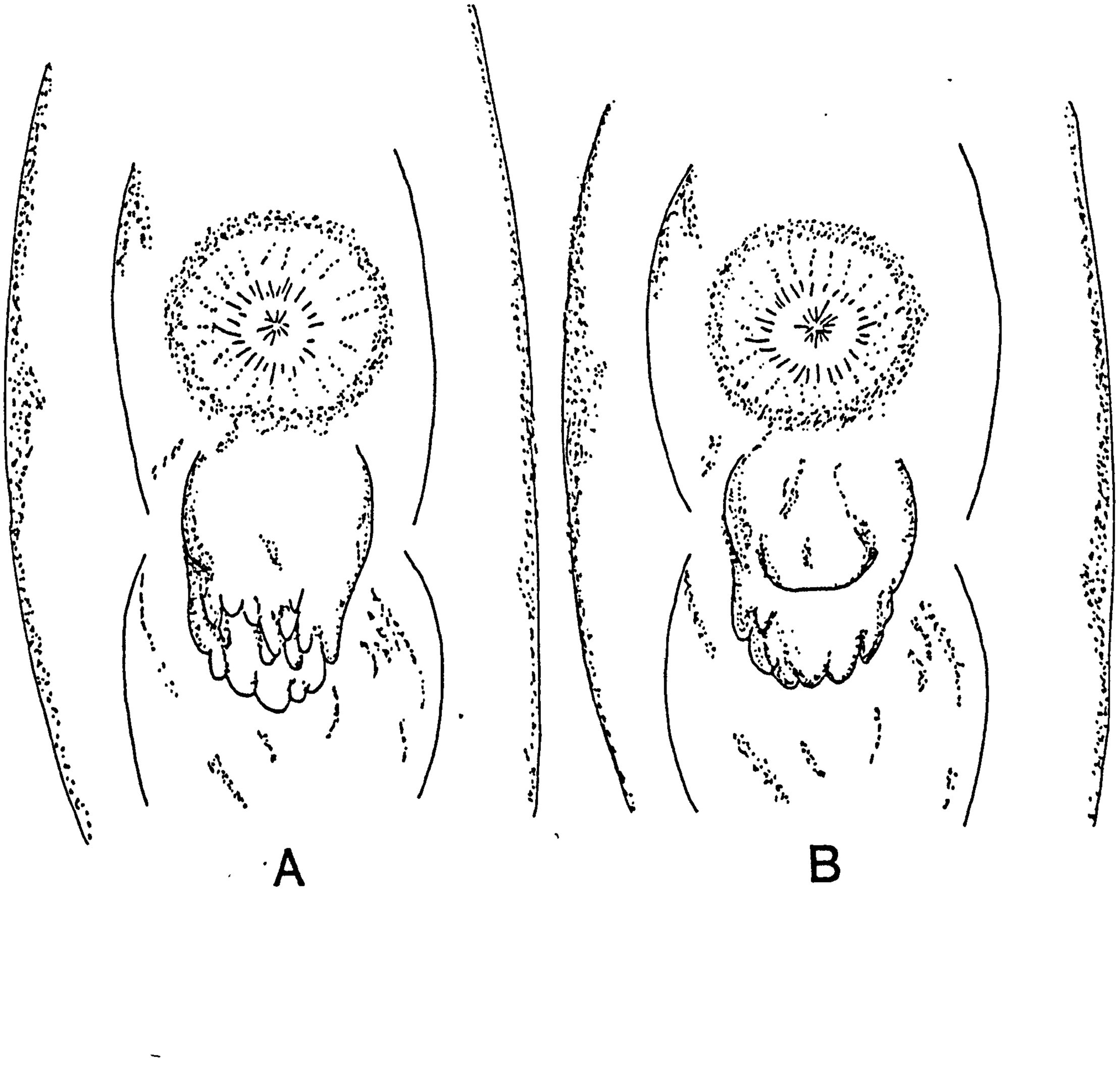
Relationship between log body weight and log standard length (SL) of C urophthalmus in Celestun, Yucatan.

reproductive season the male, as well as the female, developed a strong reddish colouration on the ventral surface which extended posteriorly onto the abdomen. In addition, the striped transversal bars on the body become totally dark and overall the fish becomes quite conspicuous. These characteristics are unfortunatelly of no assistance in distinguishing the sexes, as both sexes take the same colouration (See chapter 2). One useful technique for sex determination is to place a small quantity of ink on the genital orifices. The structures can then been seen in greater contrast, particularly when viewed with a dissecting microscope (Figure 1.15). In the middle of the genital papilla the female has a pear-shape formation with a distinct dividing line separating the distal part of the papilla, and this is contrasted strongly when marked with ink. By contrast, the male genital papilla lacks such a structure. When the fish are adults, near spawning, it is possible to identify the female because the abdomen is swollen and full of eggs, while the male is generally much more slender.

The standard length of the males and females collected was compared and tested using students T-test. The mean length of the males was 134.7 mm and of the females was 135.3 mm and although there appeared to be a slight difference this was not statiscally significant (P=0.15). These data are summarised in Table 1.2.

Figure 1.15.

Schematic drawings showing the anus and genital papilla in males (A) and females (B) of C.urophthalmus. Proportions were taken by direct photography.



A B

Sex ratio.

All the fish captured were dissected to identify the sex by direct observation of the gonads. All individuals capable of producing gametes were considered sexually mature adults. More specifically, females were considered sexually mature if in one ovary they had enlarged ova with yolk deposition. Development of breeding colouration and enlargement of urogenital papillae were also noted.

An analysis of variance performed to test the difference in mean size of adult males and females revealed a significant difference between the seasons ($P<0.01$) and significant interaction between the effects of sex and month (Table 1.2); males were larger in spring 1985, while in autumn the females were bigger than the males. No appreciable difference is noted in the other seasons. The possible explanation for these differences in these two seasons is that the capturing method (angling) was selective on sex.

The F-value for sex suggested that there were non-significant differences in the mean sizes of adult males and females (Table 1.2).

Specimens were taken in spring and summer 1985 and later in spring 1986 and were examined to determine the

Table 1.2.

Mean size (mm SL), number of observations on the mean (in parentheses), and summary table for analysis of variance to test for presence of significant differences between mean size of adult male and female of C.urophthalmus in various collections from Celestun Lagoon, Yucatan. ns=non significant difference at 0.01 level. adjustment of sum of for unequal numbers in the unweighted means analysis of variance was made to sums of squares for sex month and interaction.

Celestun Yucatan

SEASON OF COLLECTIONS

SEX	SPRING 1995	SUMMER	AUTUMN	WINTER	SPRING 1986	MEAN
No.						
MALE	142.11	160.61	123.67	146.78	106.30	134.7
413	(18)	(56)	(64)	(109)	(166)	
FEMALE	126.19	158.80	143.00	139.46	108.9	135.3
292	(56)	(59)	(2)	(70)	(105)	

ANALYSISIS OF VARIANCE OF UNWEIGHTED MEANS

Source	df	SS	MS	F - Value
Sex	1	1400	1400	4.8536 ns
Season	4	258100	258100	894.8 **
Interaction	4	4700	4700	16.294 *
Error	701	202200	288.4	
Total	710	466400		

size at maturity (Figure 1.12). Specimens from autumn 1985 and winter 1985-1986 were not taken because in these seasons only immature animals were collected. Females from spring 1986 were, on average, smaller (SL.average 129.33, Wt.102.77g, N=9, s=16.7398) than the females in spring 1985 (SL.average 146.9, 153.50g, N=10, s=16.8727) and both were smaller than the females in summer 1985 (SL.average 158.19, 193.83g, N=63, s=13.8115). The average size of the mature females from all the seasonal collections was 153.71mm SL.(175.18g) (s=16038, N=80). The minimum size of mature females was found during spring 1986 at 102mm SL.(48.65g) and the maximum size was observed during summer 1985, at 190mm SL.(345.20g).

The sex ratio in summer and winter 1985 was in the ratio 1:1. However in spring 1985 the females were significantly more numerous than the males (Chi-sq=18.7534, P <0.001, df=1), and in autumn 1985 the deviation from the 1:1 ratio was very marked (64 males and only 2 females (chi-square=59.238806, P=0.001 df=1). In spring 1986 significantly more males than females were noted (chi-sq=12.5874, P<0.001, df=1). The proportion of the juvenile population (sexually immature) was very high during the two springs (1985-86) while no juvenils were found during summer.

The lack of females in summer 1985 could be explained by

the parental care that is quite strongly developed in this species which mainly involves the female (see Chapter 2). However there is no satisfactory explanation for the disproportion of females and males captured during the two springs (1985-86) and, again, the capture method which had to be used is possibly the main source of error.

Analysis of the periodic changes in the reproductive condition of females of Cichlasoma urophthalmus demonstrated a reproductive season which extended from mid April to November. In spring 1985 22% of females were mature with an average ovary weight of 6.43g. The reproductive peak appeared to be summer (June 1985) when 95.31% of females were mature with an average ovary weight of 7.49g. During autumn (October 1985) the ovaries could not be observed because only two immature females (and 59 males) were captured. Again, this was probably due to the behavior of this species during the care of fry (see Chapter2). During winter (late February) large females were captured (140-165mmSL) but none of these had mature ova, the average ova weight being 0.37g. The reproduction season appeared to begin again in the spring (early May-1986), when 7.63% of females were mature, with an average ovary weight of 5.36g (Table 1.3).

Table 1.3.

Percentage total mature females along the seasonal collections in Celestun Lagoon with its respective average ovary weight.

YEAR	1985				1986
	SPRING	SUMMER	AUTUMN	WINTER	SPRING
FEMALE % MATURATION	22	95.31	0.0	0.0	7.63
MEAN OVARY WEIGHT (g)	6.43	7.49	-	0.37	5.36

It appears that reproduction in the Celestun Lagoon began about mid April, in 1985 and finished probably by mid-November, starting again in April 1986. In the laboratory this period can be extended relatively easily by maintaining adequate conditions of isolation, food and appropriate temperature throughout the year (see Chapter 2).

In all mature females the development of both ovaries was always observed and these were yellow to slightly orange when ready for spawning (Plate 1.2). The mean size of the mature ova in mm was measured by eye-piece graticule under the microscope. The mean size of the mature ova was 1.72 mm (n=70, st.dev.= 0.1977) with a maximum diameter of 2.17mm.

Numbers of mature ova from 56 females (48 from summer 1985 and 8 from spring 1986) were estimated by counting the total number of eggs from both ovaries under the microscope. Fecundity ranged from 2085 to 6615 ova/female, for fish in the size range, 113-198 mm Standard Length (SL.) (Fig.1.16), and the correlation ($r=0.576, N=51, P<0.001$) between fecundity (F) and SL was highly significant. On a population basis the relationship must probably takes an exponential form but in this particular sample the best fit was obtained using a linear regression.

Plate 1.2.

Mature female of C.urophthalmus showing the two mature ovaries.

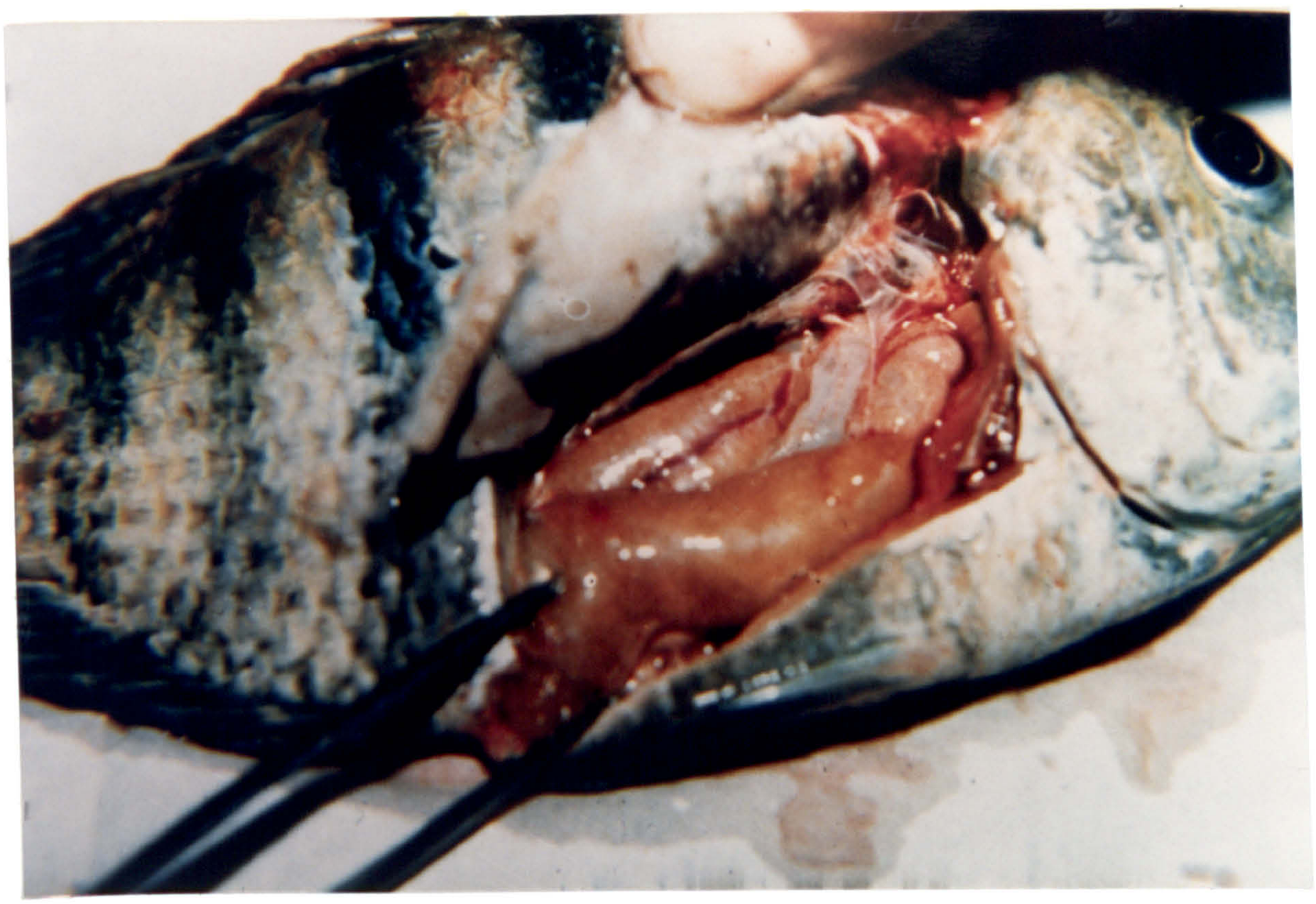
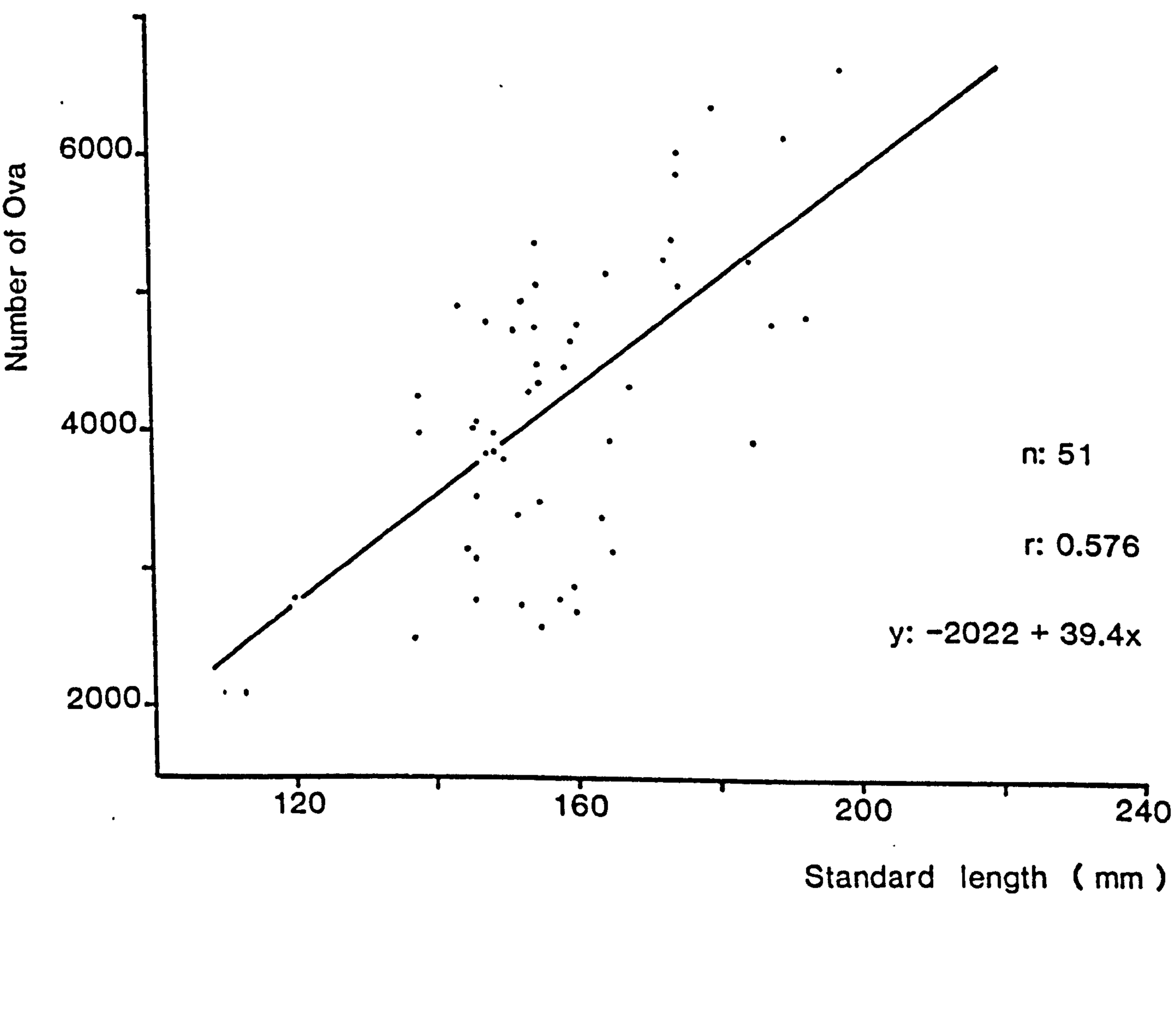


Figure 1.16.

Relationship between number of mature ova and standard length of C.urophthalmus in Celestun, Yucatan.


Number of Ova
6000
4000
2000
120
160
200
240
n: 51
r: 0.576
y: -2022 + 39.4x
Standard length (mm)

The regression equation for this relationship is:

$$F = -2022 + 39.4(SL.). \qquad \text{(Equation 5.)}$$

As standard length and body weight are highly correlated (equation 4) a similar correlation was found between the number of ova (F.) and the total weight (TW.) of the females (Figure 1.17), and this was, again highly significant ($r=0.576, N=56, P < 0.001$). The equation for this relationship was calculated to be:

$$F = 1500.73 + 15.25(TW.). \qquad \text{(Equation 6.)}$$

The gonado-somatic index (ovary weight/somatic weight, as a percentage, GSI) was calculated for all females and was compared with standard length in an attempt to use this index as an estimate of fecundity (F). The data are shown graphically in Figure 1.18. No clear relationship between GSI and SL could be discerned and the calculated correlation was not significant ($r=0.27$, $N=55$).

The weight-specific fecundity (No of eggs/Kg female) was calculated and plotted against body weight of female and there was also no clear relationship.

Figure 1.17.

Relationship between fecundity (number of mature ova) and total weight of C urophthalmus in Celestun, Yucatan.

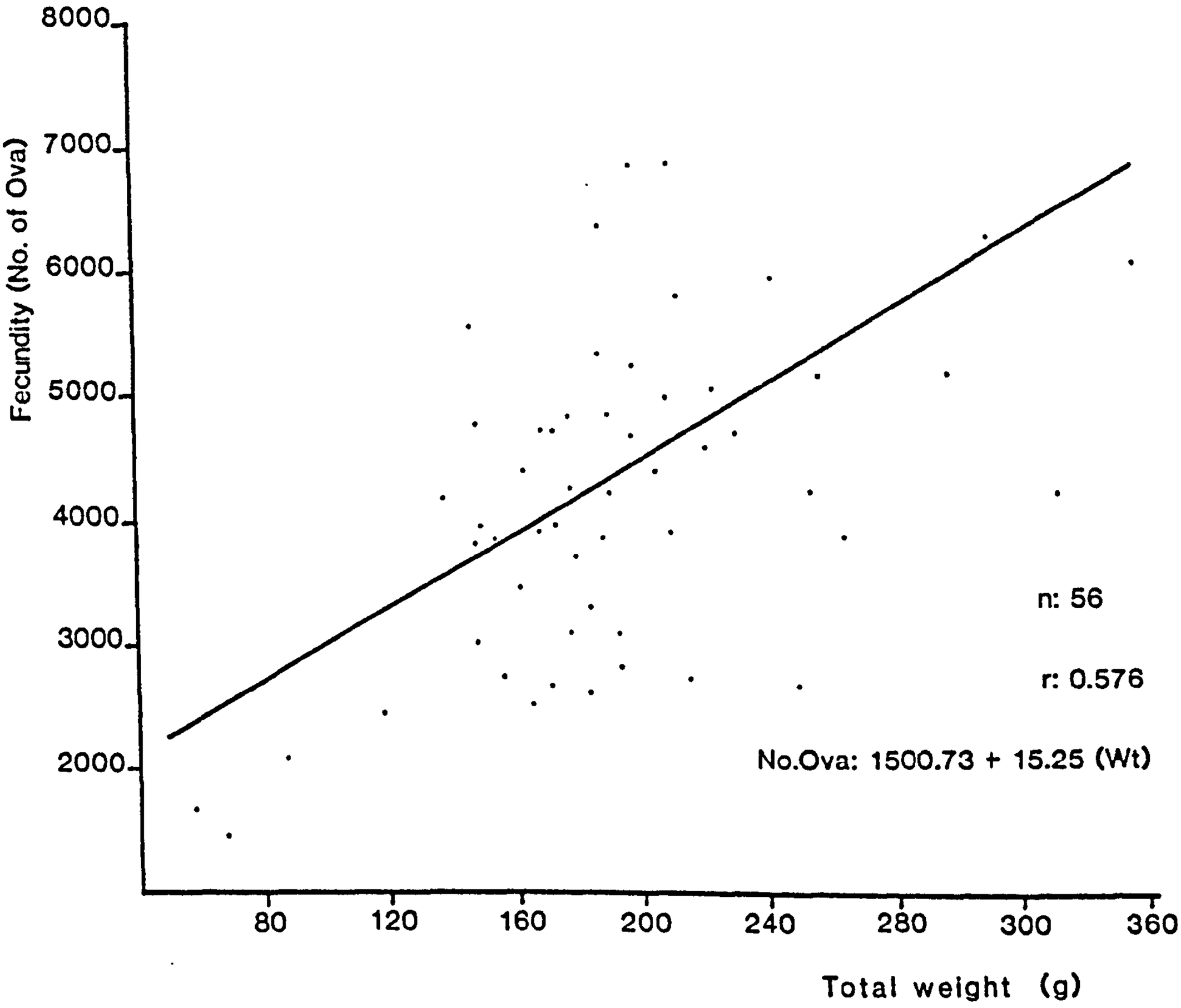
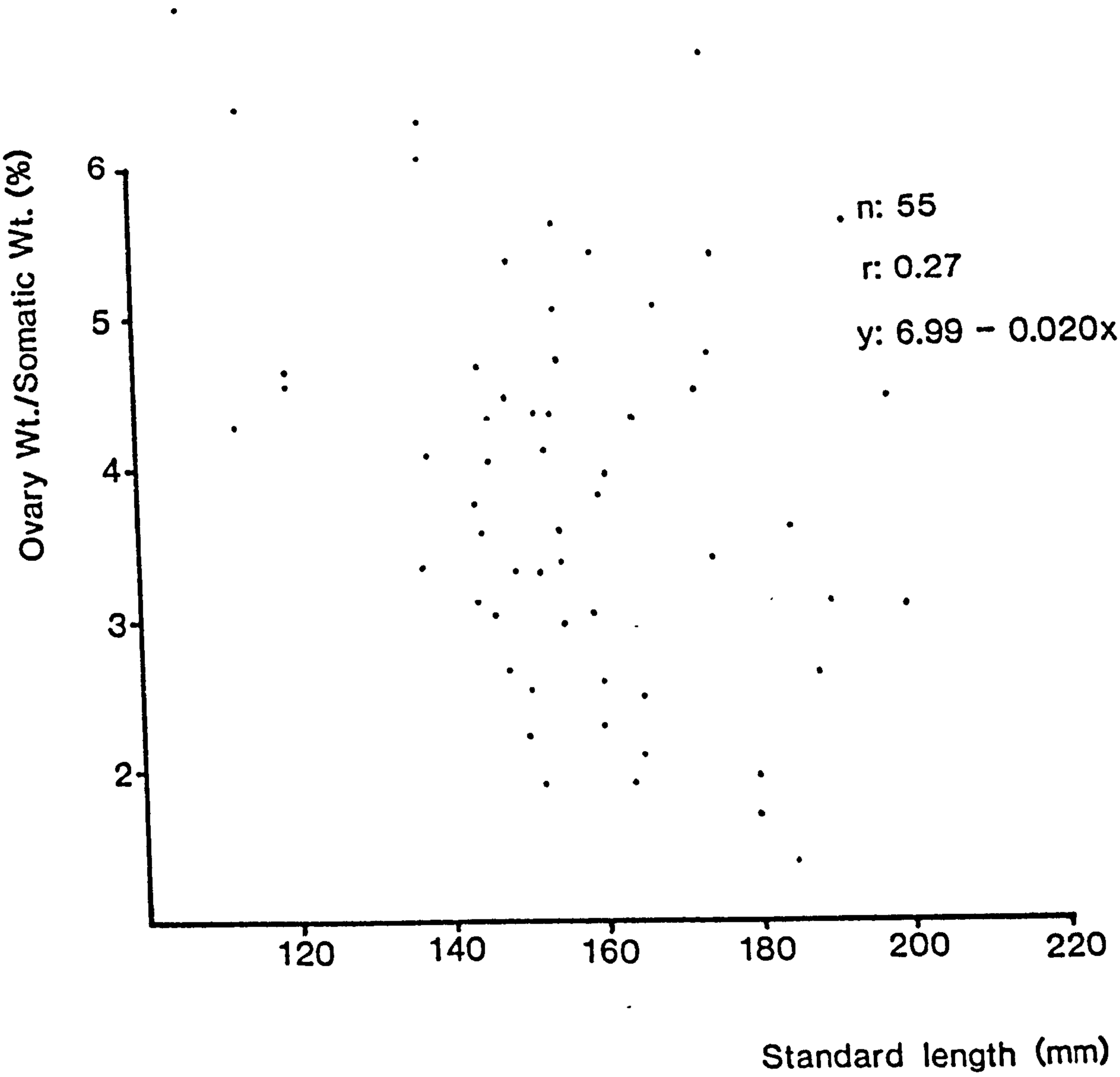

8000
7000
6000
5000
4000
3000
2000
Fecundity (No. of Ova)
n: 56
r: 0.576
No.Ova: 1500.73 + 15.25 (Wt)
80
120
160
200
240
280
300
360
Total weight (g)

Figure 1.18.

Relationship between ovary weight/somatic weight in percentage (GI%) against the standard length (mm).


Ovary Wt./Somatic Wt. (%)
6
5
4
3
2
120
140
160
180
200
220
Standard length (mm)
n: 55
r: 0.27
y: 6.99 - 0.020x

DISCUSSION

It is clear that C.urophthalmus is a euryhaline species. From the field observations it can be seen that the animal successfully survives and reproduces in fresh to brackish waters. Resendez, (1981) collected this species in waters with salinities of up to 21.10‰ and this work has shown that the fish has clear natural preferences for brackish waters between 10-20‰. It is, however, interesting to note that the species survives well in a much wider range of salinities (between 4-40.3‰.) in the Celestun lagoon. C.urophthalmus has also demonstrated its ability to invade and reproduce in fresh waters, as reported by Resendez, (1981) and Chavez et al., (1983). This was confirmed during the field work of the present study and the experimental work realized afterwards (see chapter 2 and 3). Thus Cichlasoma urophthalmus is quite similar to other commercially cultured cichlids such as T.zillii, O.aureus, O.mossambicus, O.niloticus and S.galilaeus which are also euryhaline species (Chervinski, 1982; Watanabe et al., 1985).

C.urophthalmus is well adapted to the high temperatures found in these tropical lagoons. Resendez, (1981) collected this species in waters with a temperature range of 24.2°C to 38.6°C. In this study it

was collected from waters with a temperature range of 25.2°C to 31.1°C with a mean annual temperature of 28.4°C. Resistance to live in ranges of high temperature is, again, a characteristic that C.urophthalmus shares with members of the African cichlids, such as O.mossambicus, O.niloticus, O.macrochir, O.aureus and T.rendalli which are also strongly thermophilic species, having temperature preferenda between 30-36°C. These temperatures are not uncommon in the shallow and marginal waters of tropical and equatorial lakes (Caulton, 1982; Chervinski, 1982)).

Wide temperature ranges and salinity tolerances (see Chapter 3) would permit Cichlasoma urophthalmus to have, in common with the other euryhaline and thermophilic members of the family, a wide range of distribution and good potential for culture in brackish, marine and fresh waters.

The anatomical characteristics observed during the present study strongly suggest that Cichlasoma urophthalmus has mixed adaptations to be a carnivorous fish in first instance, due to its strong dentition with very well represented simple unicuspid teeth and very flat and short gill rakers. On the other hand the pharyngeal teeth are most probably adapted for crushing small invertebrates rather than for dealing with vegetable materials with high cellulose contents.

Furthermore, the short alimentary tract of C.urophthalmus (2.2 times the Standard length) would reduce the efficiency of digestion of large amounts of vegetable material. However, the fish does have the habit of eating some soft algae as was revealed the stomach content analysis. This phenomenon was quite persistant overall during the summer period and in both of the size classes analyzed. However, the fish mainly fed on invertebrates throughout the five seasons studied and the bucco-pharyngeal and gut anatomy support these observations.

In these aspects, C.urophthalmus is quite different to species such as O.niloticus, O.mossambicus, O.aureus and S.galileus, which all have in common very small bicuspid and tricuspid teeth on both jaws and with very small and crowded pharyngeal teeth (Trewavas, 1983). The total intestine length in these species is 8 to 10 times the total length of the fish (Trewavas, 1983). Thus while the African species are only opportunistic carnivores and are mainly vegetarians with proper adaptations to crop, grind and digest plant materials along with mixtures of algae, bacteria and detritus (Jalabert and Zohar, 1982; Trewavas, 1983). C.urophthalmus is by contrast a carnivorous fish, with some sporadic vegetarian habits. It is probably not omnivorous fish with carnivorous

tendency as described by Chavez, et al., (1983).

All these aspects clearly demonstrate the great differences between the African cichlids currently used in culture systems and C.urophthalmus with respect to their food habits.

In the Celestun lagoon it is clear that Cichlasoma urophthalmus is predominantly a young population. The bigger fish scarcely reach two periods of reproduction, and these animals were the oldest in the population. A possible explanation for these results is simply that the adults are overfished. As Gulland, (1974) pointed out, it seems that if the adult population decreases, conditions for the young improve, there is less competition between each other, or between adults and young for food and living space. However, more data would be required to establish the actual status of the species from the point of view of exploitation. Nevertheless, it is clear that the high biological potential of C.urophthalmus in terms of reproduction (number of fry and spawns during the year) and the fast growth displayed, could support a sustained fishery effort. Clearly, more information from the fisheries point of view is neccessary in order to develop a sensible fishery administration of this species in this lagoon.

The highly significant difference in the proportion of males and females found in spring and autumn (1985) could be explained by the strong habits of this species to form cohesive families. This trend is quite common in cichlids (Buchard, 1965), where both parents take care of the fry (see Chapter 2). The parents consume very little food during the incubating time, and thus are not available to be captured with the method of hooks and baits used in the present work.

The relationship between weigth and lenght in males, females, juvenils and in the total population, is not very different from the data previously presented by Chavez et al., (1983), for fresh water in Rio San Pedro, Tabasco. Overall it would appear that this species maintains this relationship in both fresh water and brackish water environments as far apart as Tabasco and Yucatan.

<u>Cichlasoma urophthalmus</u> is a species which reproduces in the wild throughout a substantial part of the year with a reproductive peak in summer, when the maximum temperature is reached in the lagoon. Reproduction stops during winter when the temperature drops below about 24°C as was confirmed later in the laboratory (see Chapter 2.). <u>C.urophthalmus</u> females do have the ability to spawn many times in captivity laying

successful batches of eggs sometimes within a minimum span of 23 days in the experimental trials (Chapter 2). It is quite probable that, in the wild, the fish spawn many times but full confirmation of this must be the subject of future research using labelled fish.

The wide reproductive season exhibited by Cichlasoma urophthalmus during the year in Celestun lagoon is not a surprise for a tropical species and it is quite similar to the data reported by Chavez et al., (1983) for Tabasco, with the only difference that in Celestun lagoon the period of reproduction is extended slightly further to include April and October, with a rest period from November to March. This is probably due to the longer period of high temperatures in the lagoon (Table 1.). It seems that in C.urophthalmus, temperature is probably one of the most important environmental factors in regulating the reproductive cycle (Schwassman, 1971).

There is a clear three to six-fold difference in fecundity between the mouth brooders and the substrate spawners of the family Cichlidae, the mouth brooders being less prolific (less number of eggs per spawn). This is almost certainly due to their specific type of incubation, with bigger eggs than the bottom spawners (Balarin and Haller, 1982; Jalabert and Zohar, 1982).

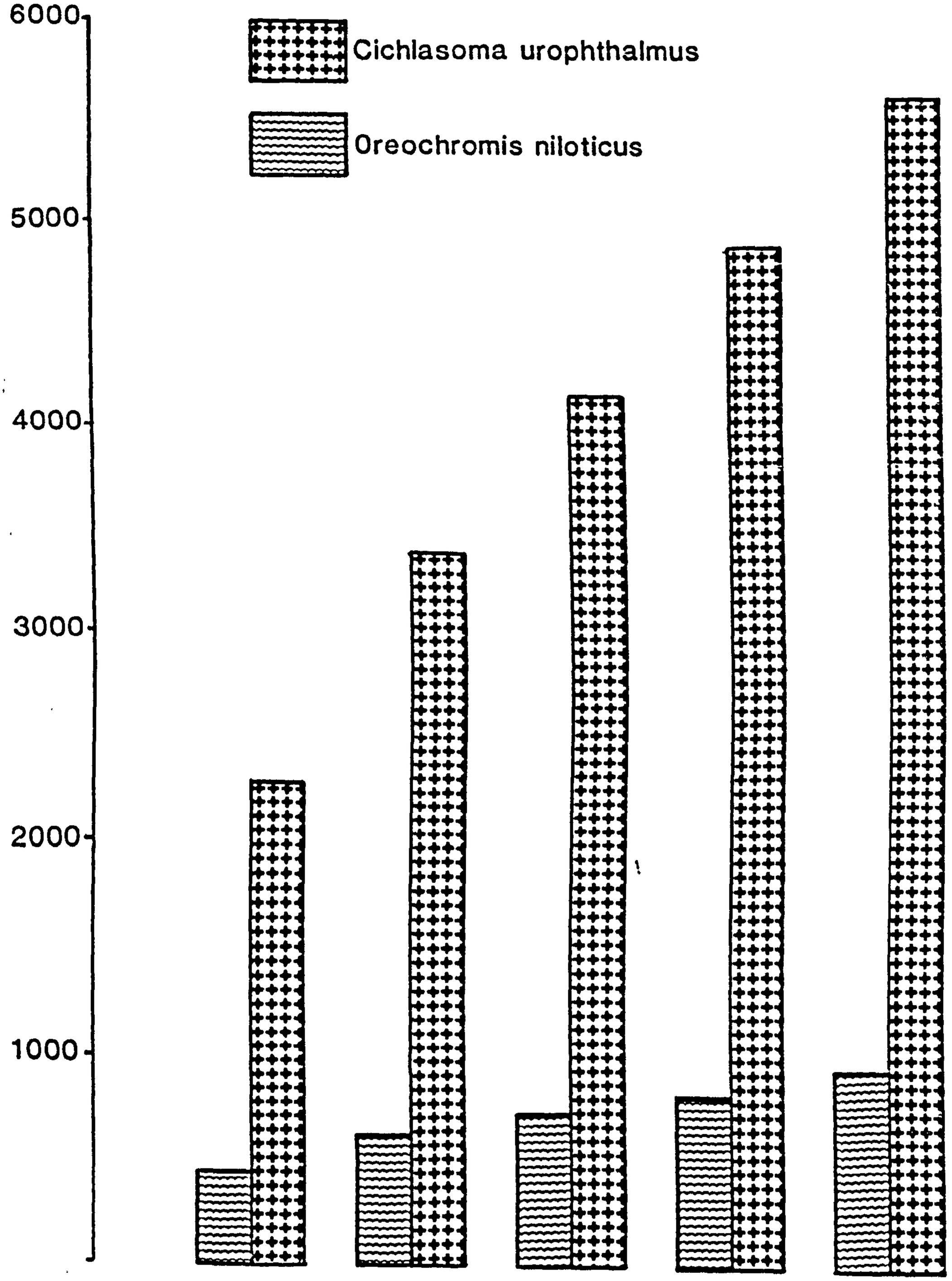
C.urophthalmus is a bottom spawner, and its fecundity is very high per spawn, being approximately twice that of T.tholloni (3009 eggs) and to T. zillii (7000) (Peters, 1987) for fish of a similar weight.

The high fecundity of C.urophthalmus gives some advantages over the mouth brooding cichlids actually utilized in Mexico, because in order to obtain the same number of fry, a smaller number of broodstock is required than in mouth brooders (approximately five times less). In figure 1.19 is shcwn clearly the difference in fecundity between C.urophthalmus and the mouth brooder O.niloticus (data taken from Rana, 1986). It should be noted that the species of African cichlid with similar fecundity such as T.rendalli and T.zilli, are of much less importance in Mexico.

The smallest sexually mature Cichlasoma urophthalmus observed during this study was a fish of 48.65g (102mm SL) and this general observation was reconfirmated later during the laboratory work with fish of 50g. Below this weight the fish never show mature gonads and this is an obvious advantage over the tilapias in which precocious breeding can be a severe management problem (Guerrero, 1982).

Figure 1.19.

Fecundity in number of eggs/spawn against total female weight for different classes for comparison in the mouth brooder O.niloticus and the bottom spawner C.urophthalmus.


Cichlasoma urophthalmus
Oreochromis niloticus
6000
5000
4000
3000
2000
1000
Fecundity (No. eggs/spawn)
50-100
101-150
151-200
201-250
251-300
Total weight of female (g)

CHAPTER 2

REPRODUCTIVE STRATEGY AND BEHAVIOUR

INTRODUCTION.

A knowledge of the reproductive strategy and behaviour of fish can provide invaluable insights which are of relevance in the management of the organisms under controlled culture conditions. This data is of particular importance when the aquaculturist tries to substantially increase the production of a species. The knowledge of fish behaviour thus becomes a fundamental tool in the development of aquaculture, especially in those species in which there is aggressive behaviour or complicated brood care, as for example in the cichlids (Baerends and Baerends Van-Roon, 1950: Barlow, 1974; Barlow, 1979; Keenleyside, 1979; Pitcher, 1986).

The parental behaviour of fish is widely but not uniformly distributed (Keenleyside, 1979), but in the cichlids uniparental or biparental care is a common practice in the whole family with some specific modifications. The Cichlids form a vast group of fish which has developed the most complicated behaviour with respect to caring for the fry (Baerends and Baerends-Van Roon, 1950). Thus cichlids are the only fish capable of forming a "true family" in which the parents take care of the young until they are large

enough to fend for themselves (Keenleyside, 1979). By way of this complicated behaviour some cichlids, for example the substrate spawners Lamprologus brichaldi from Lake Tanganyika, demonstrate communal care of the young. This occurs to the extent of the siblings passing fertilized eggs from mouth to mouth to share the work of incubation whilst the parents aggressively keep other adults away (Keenleyside, 1979).

Cichlids have then a complex social behaviour and are commonly used in behaviour research due to the ease of handling of some species (Baylis, 1974). Thus many ethological studies have been undertaken on members of the group. However, for historical reasons, the majority of these studies have been carried out with old world cichlids and as the behaviour of the new world species is somewhat different they deserve more study (Baylis, 1974).

Recently, some studies have described the behaviour of Central American Cichlids such as Herotilapia multispinosa (Baylis, 1974); Cichlasoma nigrofasciatum (Piron, 1978); C.citrinellum (Barlow, 1974 and 1979), C.managuense (Bleick, 1970; Meyer, 1987). Unfortunately most of this information is on species with limited potential for aquaculture. It is often the case that when an aquaculturist tries to

develop an attractive species from a commercial view point, he usually finds little useful information on their growth in captivity.

Some work on Central American cichlids has been done by Chavez, et al., (1983). They described the colouration and dominance of a number of Cichlasoma species during their reproductive phase in the wild. Although, the observations were limited and not a great deal was done with C.urophthalmus. Because of this lack of information on the behaviour of C.urophthalmus in terms of both parental and reproductive behaviour in general, the present study sets out to describe the behaviour, of this species during both courtship, and parental care. The principal objective was to identify the most relevant details that could be used during its domestication and possible utilization in intensive aquaculture systems.

MATERIALS AND METHODS.

Experimental animals.

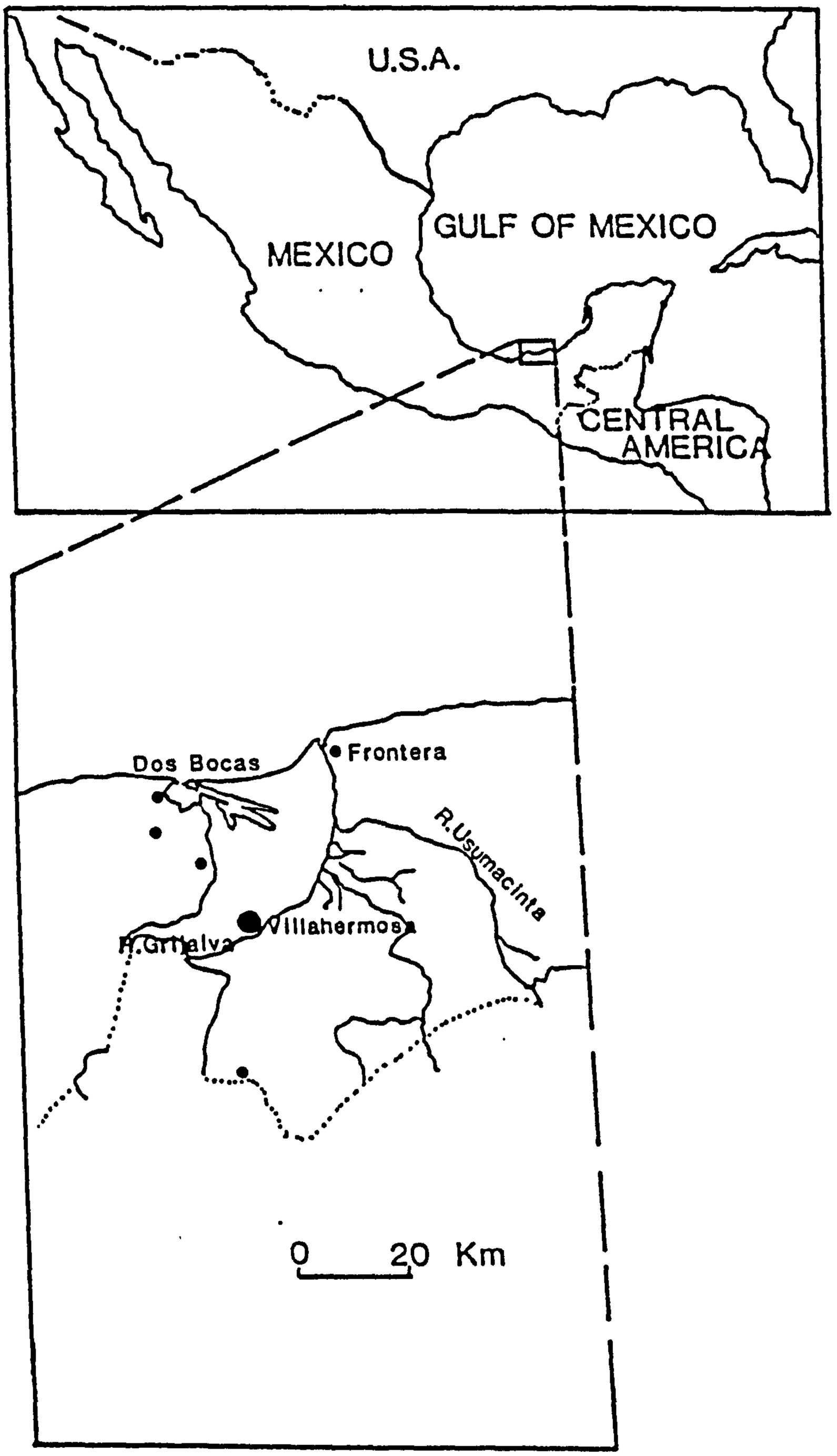
Fish, adults and juveniles of Cichlasoma urophthalmus were obtained in various successive collections from a brackishwater laggon at Dos Bocas, Tabasco, Mexico (Figure 2.1.) (a brackish water lagoon) and transported to the laboratory in Merida, Yucatan. The collections were made by cast net in shallow waters and with a seine net in deeper waters. The animals were placed in oxygen-filled plastic bags, closed with rubber bands and transported on ice to keep the temperature down to about 23°C. On arrival, after 10 hours, the fish were gradually transfered, over two hours, to freshwater and maintained in circular 1m3 fiber glass tank with recirculated water to allow recovery from their capture, transport and salinity changes prior to being fed.

Positive identification of the species was made with the help of Dr.Rush Miller as described in Chapter 1.

Those fish which appeared healthy were selected as broodstock for all the trials. The adult males and

Figure 2.1.

Geographical location of Dos Bocas, Tabasco, showing the collecting area.


U.S.A.
MEXICO
GULF OF MEXICO
CENTRAL AMERICA
Dos Bocas
Frontera
R.Usumacinta
R.Grijalva
Villahermosa
0 20 Km

females were individually sexed with the help of a stereoscopic microscope and indian ink as previously described. Later all males were marked for ease of identification by pan-jetting one to three spots of alcian blue onto the front of the snout.

The observations on behaviour and reproduction were conducted in a wide variety of systems, the principal features of these systems are described here.

Aquarium and tank systems.

One large aquarium with a capacity of 900l (50 x 150cm), was used for the initial observations on couple formation. The bottom was covered with shell gravel (4mm diameter) to a depth of 10cm, and some rocks were placed in the aquarium to give the fish some sheltered areas.

Fibreglass aquaria of 120l, capacity, fitted with one glass wall were used to maintain animals individually for use in later trials.

The majority of observations were made in either cylindrical or square 1m3 and rectangular 0.75 m3 fibreglass tanks. These were either operated as a

recirculated system or as a separate unit with air stones (Plate 2.1). In ten of the cylindrical 1m3 tanks one bucket full(± 10Kg) of shell gravel was placed to produce nesting material and to facilitate spawning. These tanks were observed daily.

A large cement tank of 250001, and 80cm deep operated as recirculated system. Although no substrate material was provided, this tank had an irregular form and sheltered areas provided by aquatic plants in pots and was used for observations on fish in more natural conditions.

Water supply and environmental conditions.

Dechlorinated tap water (aereated and conditionated by ageing) was used for all systems in which fish were maintained. The temperature in the aquaria and fibreglass tanks was maintained at 27-28°C, with the use of a heater. This was close to the natural variation of 26 to 29°C. The pH was 8.5± 0.5, and total hardness 320± 10ppm.

The photoperiod in the controlled environments was maintained at 12 hours light and 12 hours dark, whilst the outdoor pond was subjected to the local natural photoperiod (12 hours light, 12 hours dark).

Plate 2.1.

Fibreglass tanks (1m2) used for maintaining the adults and fry for the major sets of observations.

Food.

The food given to the broodstock was dry pellets (40% protein using brown fish meal as the main protein source) based on the formulation shown in Chapter 4. The fish larvae were fed either with crushed pellets (50% protein) of different sizes, or alternatively recently hatched Artemia salina nauplii were fed Ad.libitum.

Observation methods.

The experiments were carried out over a period of aproximately 20 months and encompassed three reproductive seasons. For the general behavioural observations groups of 2, 3 or 6 fish were placed either in aquaria or 1m 2 tanks and maintained for periods of observation varying between 5 days to 7 months. A further group of 20 fish were maintained in the small pond for a period of 16 months for constant observation.

For the study of aggressive behaviour in crowded situations four circular concrete tanks of 8 m2 and 80cm depth were used. These tanks had a tangential water input and central drainage. Two tanks were stocked at a density of fifteen 100 to 200g fish/m2

and the other two tanks were stocked at fifteen 30 to 60g fish/m2. For observations on aggressiveness where space was limited six fibreglass tanks of 1m2 were used. These tanks were part of an outdoor recycled water system and each held fifteen 50-80g fish of both sexes.

For the experiments on aggressiveness of both separated and mixed sexes, nine fibreglass tanks (0.75m2) in a recycle system were used, each containing eleven fish of between 80-140g. The first three tanks held both males and females. The photoperiod was 12L:12D and the temperature was maintained at 26°C±2.

Observations of the behaviour in the field were conducted in the Celestun Lagoon, were the salinity varied between 7-20‰. and temperatures between 27-30°C.

Experiments were conducted to determine the swimming velocity of the fry, during the period in which the fry has a positive geotaxis. This was carried out in a 1000 ml graduated cylinder of 40cm height, filled with the same water from the tank in which the fry had recently hatched at 28°C. Fry were taken from the nest with a plastic pipette, in groups

of 20 to 25 and dropped one by one onto the water surface in the graduated cylinder. The time taken by fry to swim from the surface to the bottom of the cylinder was recorded with a hand chronometer, and at the end of the daily measurements the fry were returned to their nest and new fry were taken for the next days measurements. This procedure was repeated every 24 hours, starting with recently hatched fish, up to the point at which the fish reabsorbed the vitelline sac and became free swimmers without geotaxis. For comparison, a similar experiment was conducted with fry of T.zillii (kindly supplied by Dr C.Rana) at the Institute of Aquaculture, Stirling. Time averages were calculated and used to calculate the swimming velocity in the following formula:

$$v = d/t$$

where v = velocity, d = distance (cm) and t = time (sec).

In order to asses egg and larval development at different temperatures, the time to hatching and free swimming in degree days, was estimated with respect to temperature (Nikolsky, 1968). Nine hundred recently spawned eggs (within one hour of being laid at 28°C) were carefully separated with a sharp blade frcm the

substrate on which the parents had placed them with a sharp blade. Nine batches of 100 eggs were deposited in different one liter ehrlenmeyer flasks containing water from the same tank in which the fish had hatched (28°C). An airstone was placed in each flask to aereate the eggs and simulate the fanning action of the female. The ehrlenmeyer flasks were kept in a room with constant temperature and were maintained at 25, 28, 30 and 35°C in a water bath. The eggs were allowed to adapt to the new temperatures over 10 to 20 minutes. The eggs were observed each hour until they hatched and then every two hours until the fish totally reabsorbed the vitelline sac and became free swimmers. Progress was followed until 50% of eggs had hatched or the fry were free swimmers.

In order to determine the spawning frequency of C.urophthalmus in intensive culture, five couples were selected and each couple was maintained in a fibreglass tank of 1m2 as described above in an outdoor recirculated water system. The average size of each couple ranged between 130 to 200g. The date of the first spawning was recorded and later the date of the subsequent spawns of each couple. To encourage further spawnings all the fry produced were removed after 8 to 10 days when they became free swimmers.

In order to obtain information on the behaviour of both adults and fry with respect to possible communal care of the young the following experiment was conducted. A shoal of 15 day-old fry was separated from its parents. Two days later they were added one by one to another shoal of fry (with no more than 24 hours age difference). The second shoal was being protected by their original parents and these adults were not removed. Data was obtained via direct observation at the moment of fry introduction.

RESULTS

Prespawning Activity.

From observations made in the field, small pond and laboratory, adult C.urophthalmus always displayed a pale blue colouration with some black bars and spots. During the interspawning period or when the animals were juveniles there were five spots on the proximal part of the body, and some blue and green in the dorsal caudal and ventral fins. However, during prespawning and spawning activity ten well-defined transversal bars became evident on the back (Plate 2.2) and a black spot at the base of the caudal fin. The ventral area and isthmus zone were reddish or sometimes yellow, with tones of blue and green in the fins which only appeared during spawning in fish that had already formed a pair or which were taking care of fry. Paired and pairing fish or those guarding eggs/fry can be distinguished from other fish by these pronounced colours.

During these observations C. urophthalmus showed a behaviour typical of a substrate spawner. The male always takes the initiative and selects a spawning area, he remains close to this all the time and displays, increasing from time to time the intensity of the black

Plate 2.2.

A pair of fish showing the characteristics reproductive colouration. The male is attacking a plastic pipe, which is recognized as an intruder, during the fry protection phase.

barred colouration which is still not totally fixed. Throughout the display period the male fights with other males and females which approach his selected nest site and he intimidates them by opening his mouth and flaring his operculae. With time, one female will approach the area more frequently and by momentarily showing the barred colouration it is attacked less and less aggresively by the male. Shortly afterwards the female also begins to show changes in its body colouration from the normal pale to the intensive black barred condition. At a later stage this female helps the male to defend the previously established area and in this manner a strong link is formed. Occassionally the deep black-barred colouration reverts to the pale condition and then returns. Finally the former is totally fixed in both adults until the eggs are laid in the nest. Once the fry leave and the adult separate, the pale colouration returns until a new courtship starts.

It was established that reproduction is not always achieved, as often one of the pair (usually the female) is not completely mature. In such cases the male attacks and often kills the female. Ocassionally, however, if the female is bigger than the male the latter will be killed.

It was found that the most successful way to

achieve pairing was by placing a selection of males and females together and letting them make a natural selection. Once pairing was established, the remaining unpaired fish were removed as the recently-formed pair could kill the remaining fish.

The behaviour pattern for site selection in the wild was the same as that observed in the fibreglass tanks and aquaria. Strong aggressiveness was shown by both males and females during this period, against any organism that approached the pre-selected nest area, even extending to fish of their own species and to humans (Plate 2.2). This aggressiveness was not reduced when the fish were crowded in a small tank and the sexes were mixed. Here the attacks were quite severe and the first mortalities were found 24 hours later. Again, the dominant fish always have the strong barred colouration. Over a sixty day observation period, however, when fish were held at the same density under identical conditions but with the sexes separated, the aggresive behaviour ceased and the fish assumed a pale colour. Only in those tanks containing a particularly large male or female was aggression evident but this never led to mortalities.

Fish held in the circular concrete tanks with recirculated water over a six month period did not indulge in aggressive behaviour or pairing. These fish

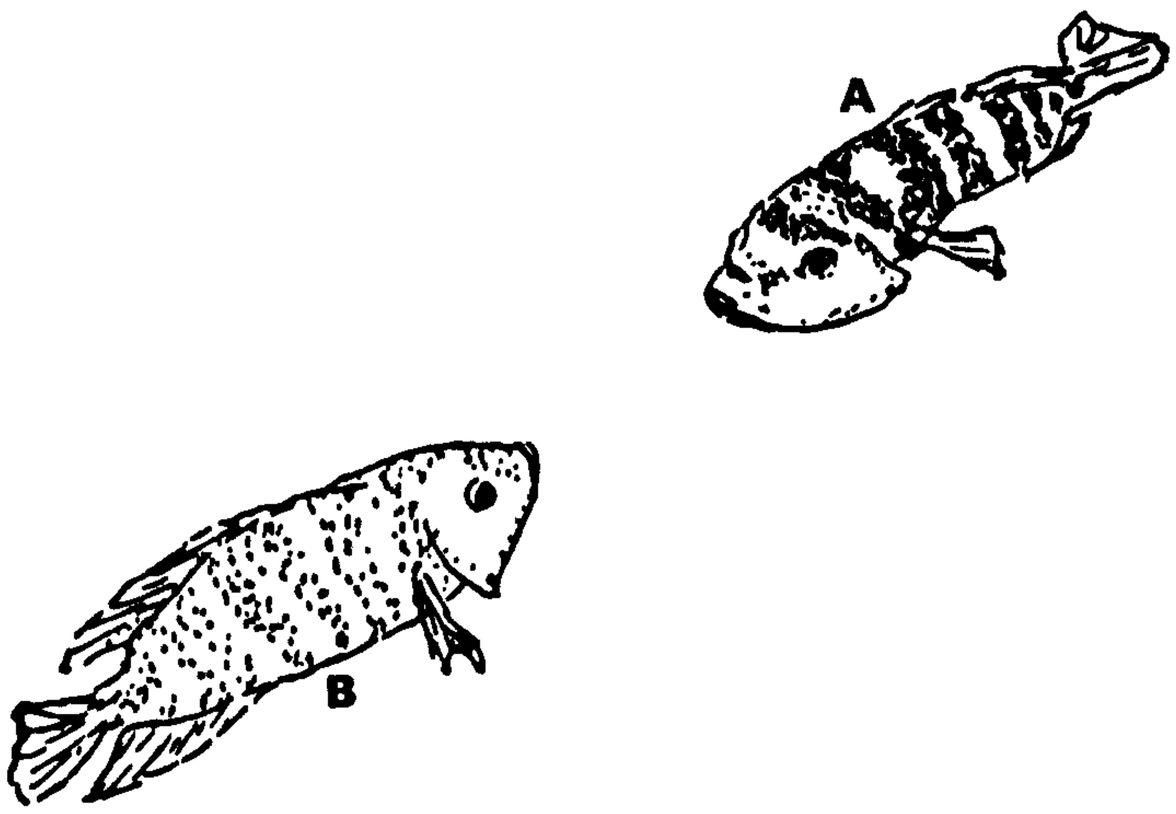
took food pellets readily and grew well. When 100-200g fish were transfered to 1m3 tanks and higher stocking densities they immediately began to attack each other and to form pairs.

In more crowded conditions, (fiften 50 to 80g fish of mixed sexes held in 1m2 tanks) pairing and aggressive behaviour was common. Non-dominant fish were bitten by the dominants and were killed within a few hours (Plate 2.3). The aggressive behaviour by dominant fish and the death of non-dominant fish is similar to that in the wild with the exception that the latter are able to avoid fighting by escaping and thus are not killed.

In both field and laboratory conditions the female appeared to select the males after the latter had displayed. In Plate 2.4 two males (1 and 2) can be seen fighting. This involves biting each other on the mouth, whilst the female (3), with the distinctive reproductive colouration remains in front of them. Other fish in the pond show the pale colouration (4) and do not respond to the fighting males. As can been seen from Plate 2.4 there are two nest areas 1.5 meters apart (N1 and N2). Male number 2 is invading the territory of male number 1 and the female is waiting to select, usually, the victor. In this pond only one couple was observed with fry at any one time, even though other pairs were

Plate 2.3.

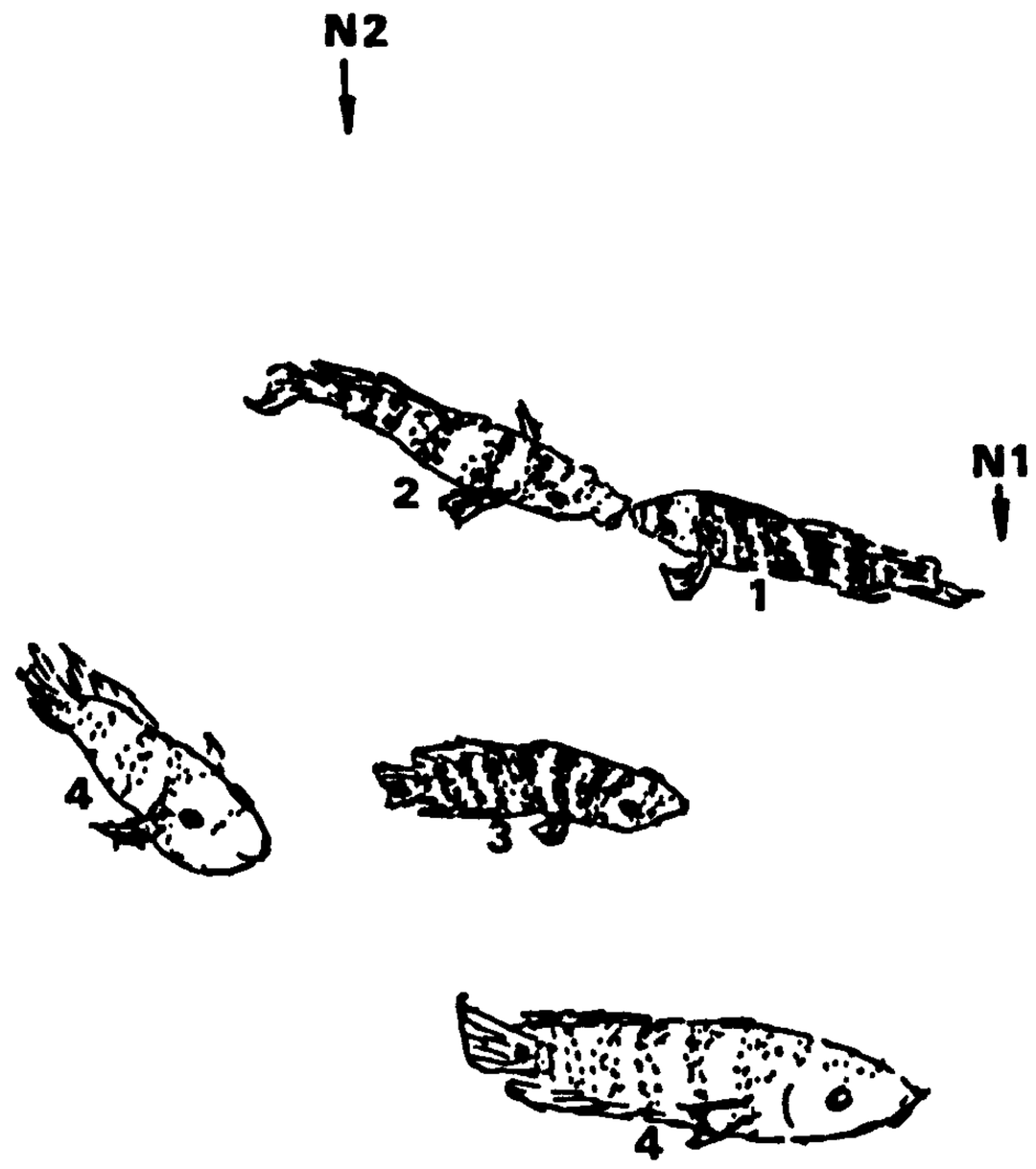
Male with typical aggressive black barred colouration (A) attacking a pale coloured fish (B) which respond the attack and eventually is submitted and escaped.

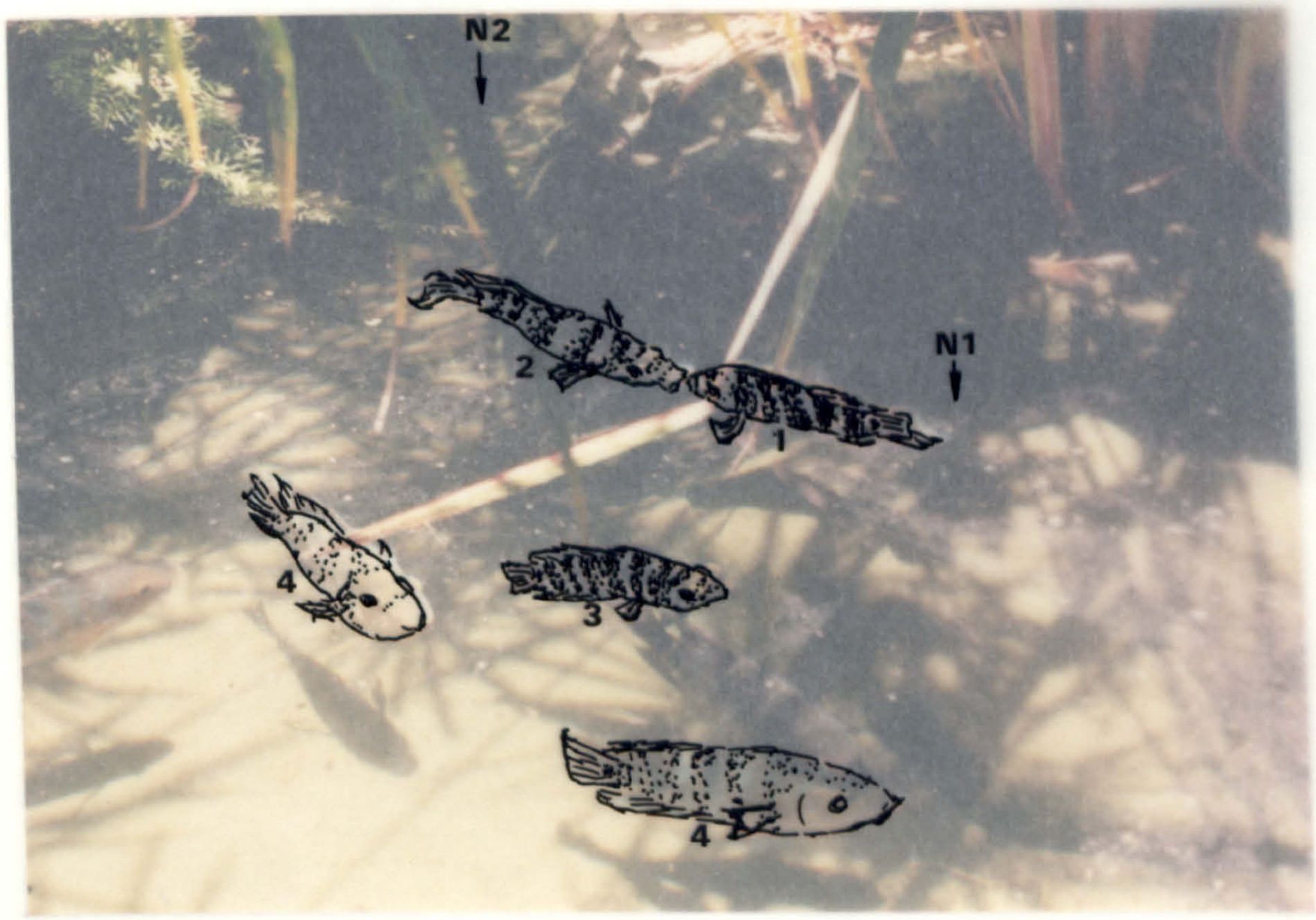

A
B


A
B

Plate 2.4.

The selection of partners. Fish which have formed or are forming pairs show their distinctive barred colouration whilst otherfish are pale and do not have the intensive stripes. 1. Male dominant in its territory;2. Male intruder; 3. Female in reproductive colouration; 4. Other fish showing the pale colouration; N1 nest site of male 1 and N2 nest site of male 2.


N2
N1
2
1
4
3
4


N2
N1
2
1
4
3
4

present. Couples in the field typically weighed 200-250g or more whilst in the laboratory weights of 50-100g were more usual.

Substrate cleaning and nest building.

Both parent fish took part in cleaning behavior, removing debris, algae, mud, silt and gravel by biting and scraping the surface, carrying material in their mouths and spitting it out at the nest edge. The action of excavation can be described as follows; with fine substrata the fish, mouths open, push material forward to the edge of the excavation site and then, snapping the mouth shut, propel the substrate away from the hollow. This action is assisted by strong body movements guided by the pectoral fins. With a coarse substrate, pieces of gravel are carried in the mouth to the excavation edge and spat out. The couples in tanks containing buckets full of gravel cleaned the entire area in just one night, removing from the pail 10Kg of gravel. In the tanks with distributed gravel bottoms, the couples made a burrow in two days big enough to hide both fish. Eggs were layed at the bottom of the burrow and were protected by both parents.

Observations in Celestun Lagoon Yucatan, in Dos Bocas Tabasco, and in the small experimental pond at the laboratory showed that nest shape and size varies but is

normally between 10-15cm wide, occuring over any part of the substrate including the pond corners and in some cases on the sloping margins. In the wild it is possible to see the nests in the bottom of the ponds or in its walls from the edge. Recently excavated nests in ponds with mud and gravel look like white marks, and the distinctively barred parents are usualy close the nest. The more advanced or older nests are less obvious and have the couple fanning eggs or protecting recently hatched fry. In tanks without substrate, the female laid her eggs in a cleaned area on the corners and normally utilized the vertical walls of the tank. This demonstrates that this species can spawn successfully in the absence of a mobile substrate. It was also noted that nest construction can be more complicated and that this complexity depends on the substrate material. Several types of substrate can be used for egg laying but in general, C.urophthalmus prefers smooth surfaces rather than gravel and is also capable of egg laying in fibreglass tanks or in plastic buckets placed in the tanks.

Spawning Behavior.

During spawning, the animals always showed the strong barred colouration and they did not permit any

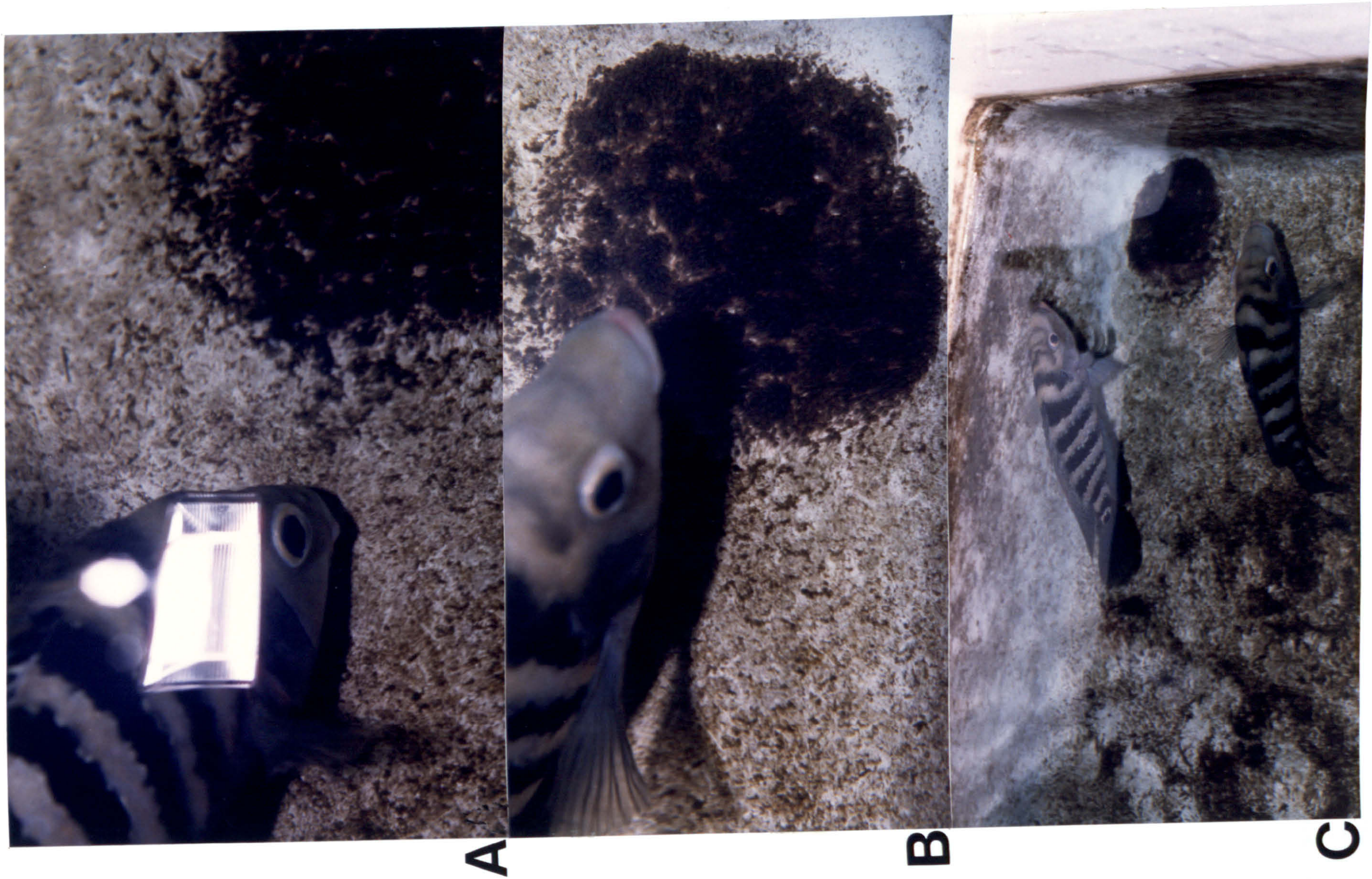
fish to reach the cleaned spawning area (see Plates 2.2, 2.3 and 2.4). Once the fish has established and cleaned the spawning area the female, using the swollen papillae as an ovipositor begins to stick some eggs to the substrate. When the female has deposited a few rows of eggs the male begins to fertilize them, and with the help of the pectoral fins produces a current of water and sperms close to the eggs. This action is repeated several times until the spawning is completed.

The recently-laid eggs are light-yellow, with some pinkish-translucent colouration and are stuck to the substrate in an irregular conformation. Throughout the incubation period the parents take care of the batch alternately (see Plate 2.5). However, it is mainly the female that cleans and fans the eggs by the rhythmic and alternate beating of the pectoral fins.

When other fish invade their territory or even when an observer approaches first the male displays a strong defense behaviour, followed by the female. During this time the aggressive behaviour is using against any intruder regardless of species, size, shape etc. The bites given by the attacking fish can be quite nasty because of their strong dentition (Plate 2.2). This phase lasts until the fry leave the parents between 3 to 5 weeks later.

Plate 2.5.

A and B C.urophthalmus female taking care of the fry and returning strays back to the nest with the mouth. C. Male and female taking care of the recently hatched fry, which are attached to the bottom by their head glands.


A
B
C

Larval development and behaviour at 28°C before vitelline sac absorption.

0-24 hours. Immediately after hatching takes place, the fry show a strong geotactic response swimming quickly from their egg shells to the substrate. If the eggs are suspended on a wall or some part above the bottom, the recently hatched fry will swim with velocities up to 17cm/sec to reach the bottom (Figure 2.2). This geotactic response is reduced as the fry approach the free swimming stage and the swimming velocity also decreases greatly, until it totally disappears, as shown in Figure 2.2. This behaviour was not observed in similar experiments using Tilapia zillii.

Once the fry reach the bottom they immediately adhere to it using the head glands. These consist of three pairs of sticky glands, one on the snout and two on the dorsal part of the head (Plate 2.6 and Figure 2.3a). The mechanism of adhesion using these glands is by the production of sticky mucus.

At this stage the larvae have relatively underdeveloped heads. The eye structures are without pigmentation, but are clearly visible, as are the three well developed three pairs of head glands (Plate 2.6, Figure 2.3a,b).

Figure 2.2.

Swimming velocity of C. urophthalmus fry at 28'C, showing positive geotactism during vitelline sac absorption, and compared with Tilapia zillii fry of the same age at the same temperature. Dotted line is the control velocity range in free fall produced with larvae previously killed by an overdose of benzocaine (vertical bars show the range of velocities found in the fry).

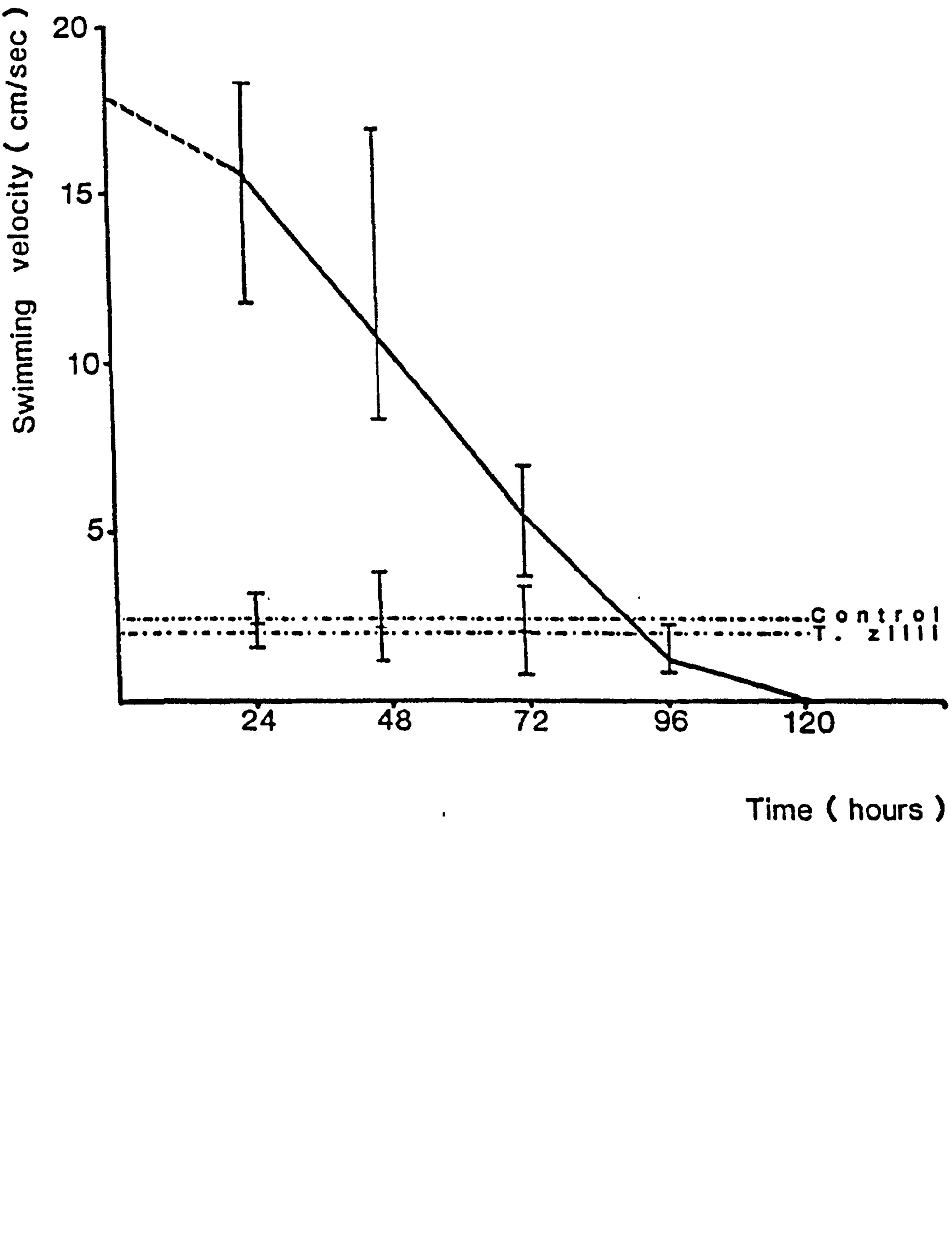
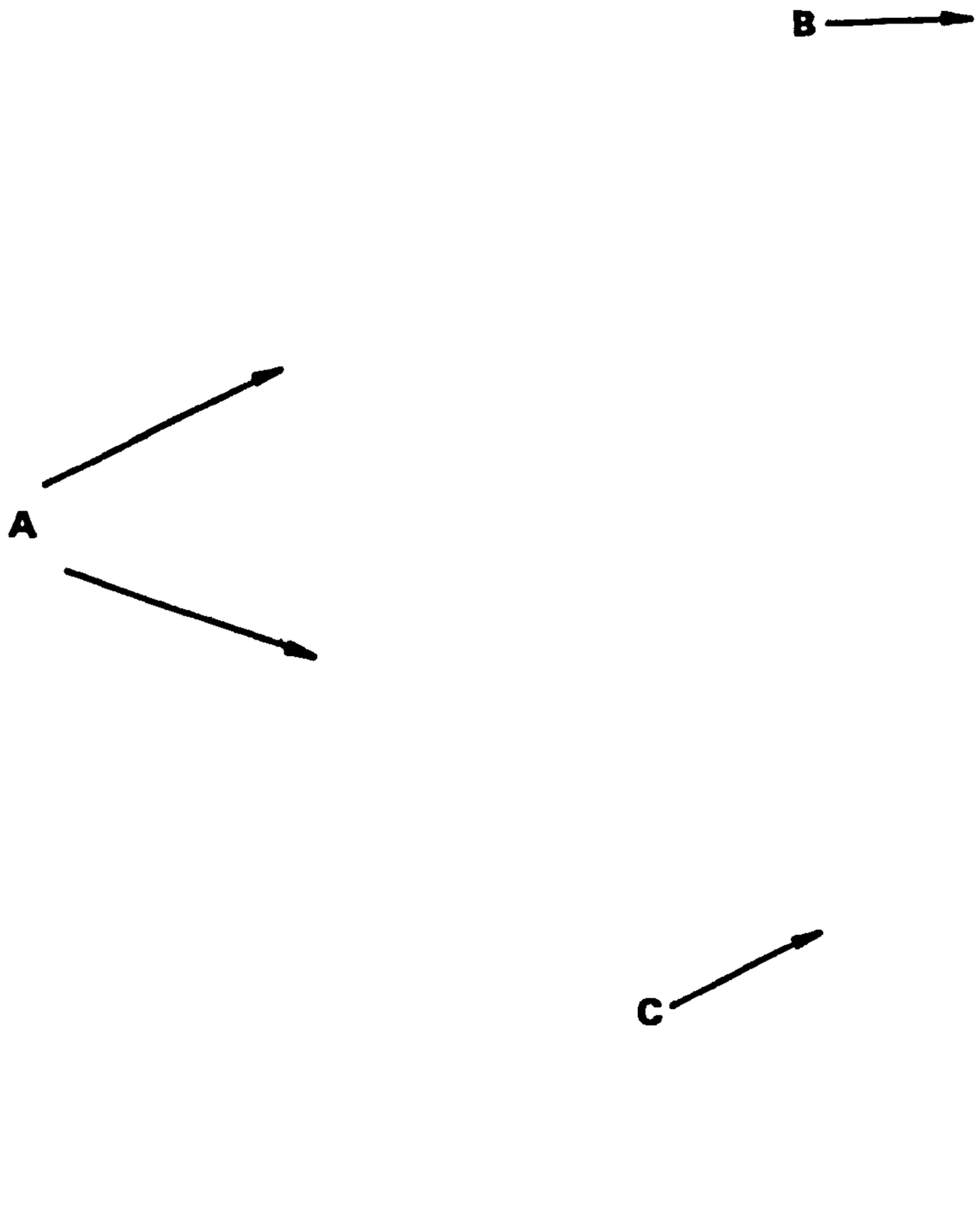
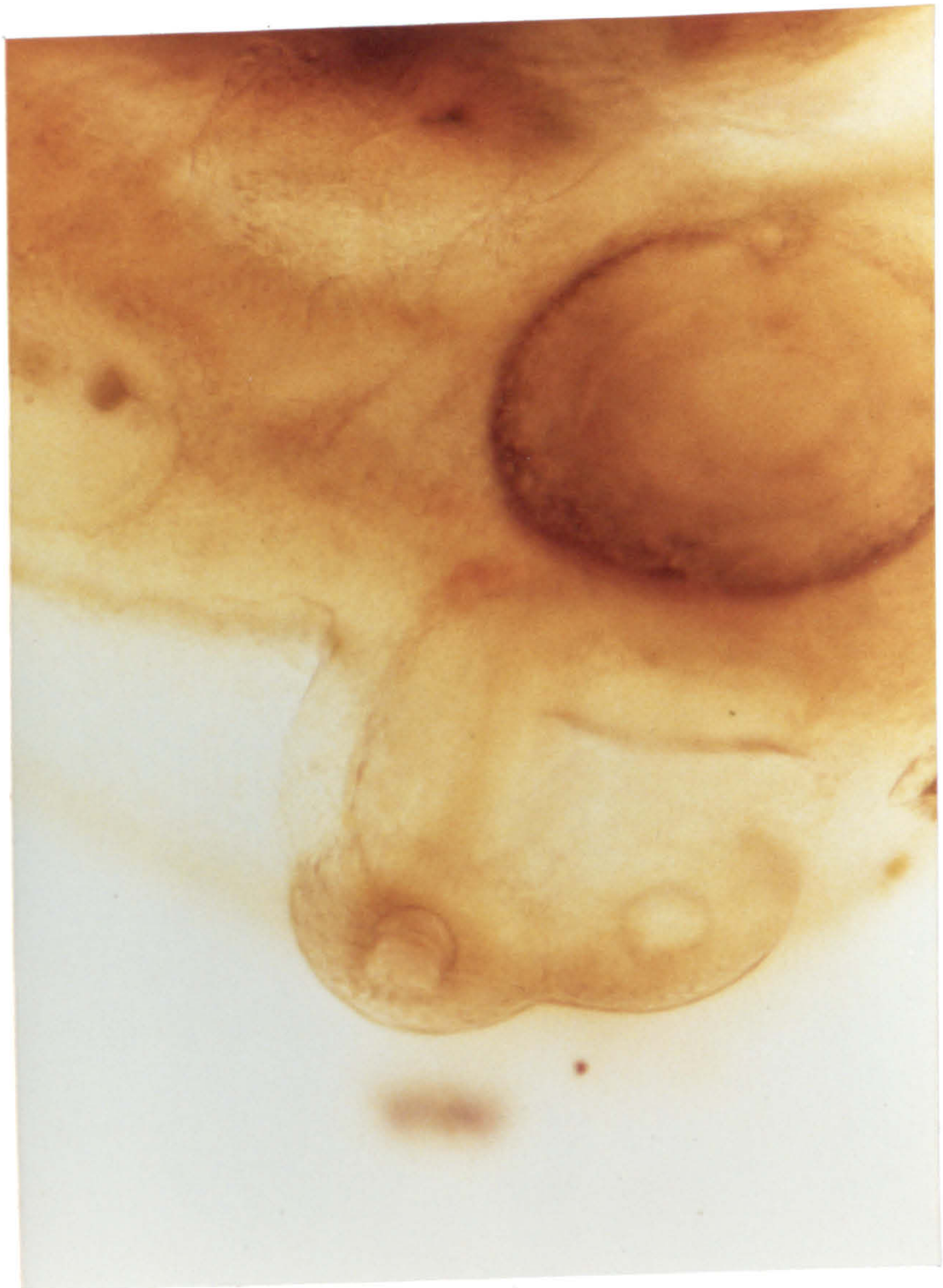

Swimming velocity (cm/sec)
20
15
10
5
24
48
72
96
120
Control
T. zIIII
Time (hours)

Plate 2.6.

The head of C.urophthalmus larvae showing details of the head glands (specimen less than 24 hrs old. A. Dorsal head glands. B. Otolith. C. Eye.


B
A
C



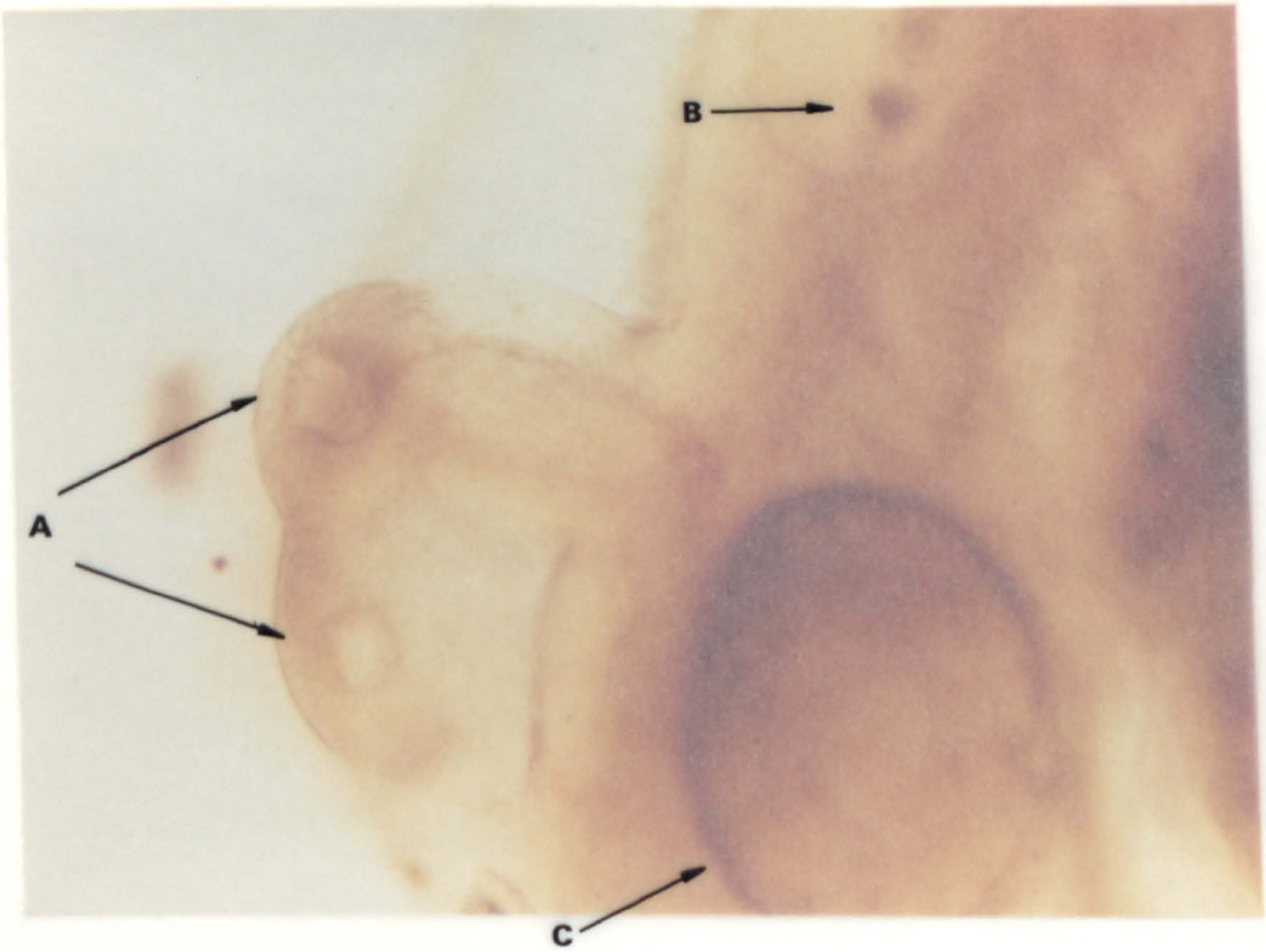
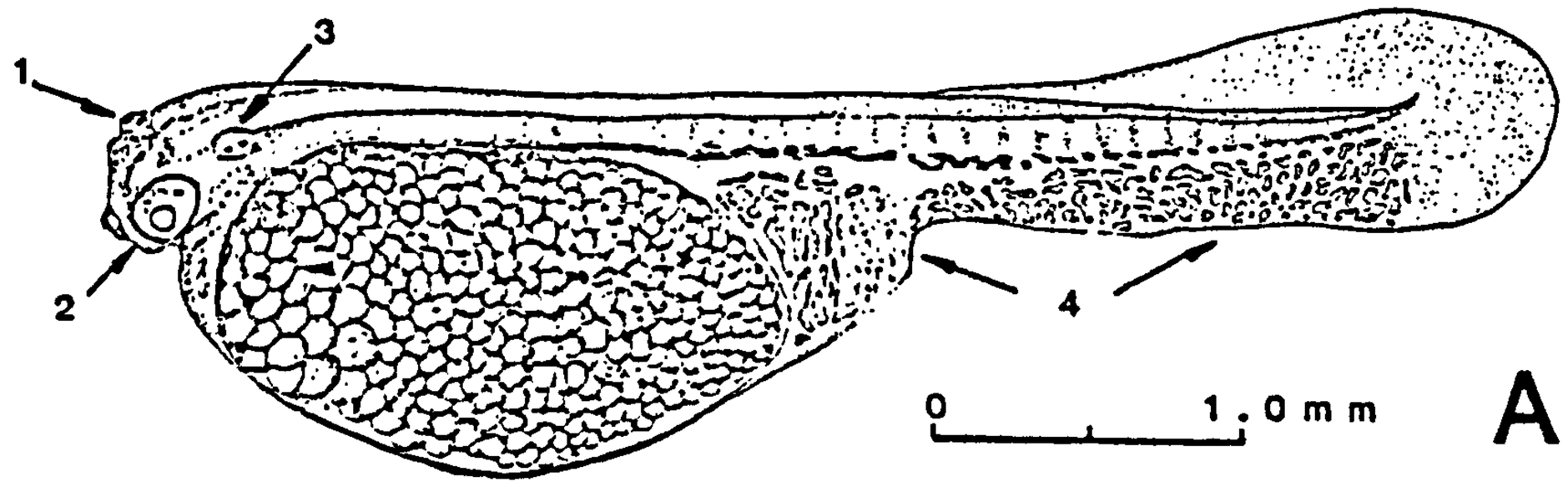
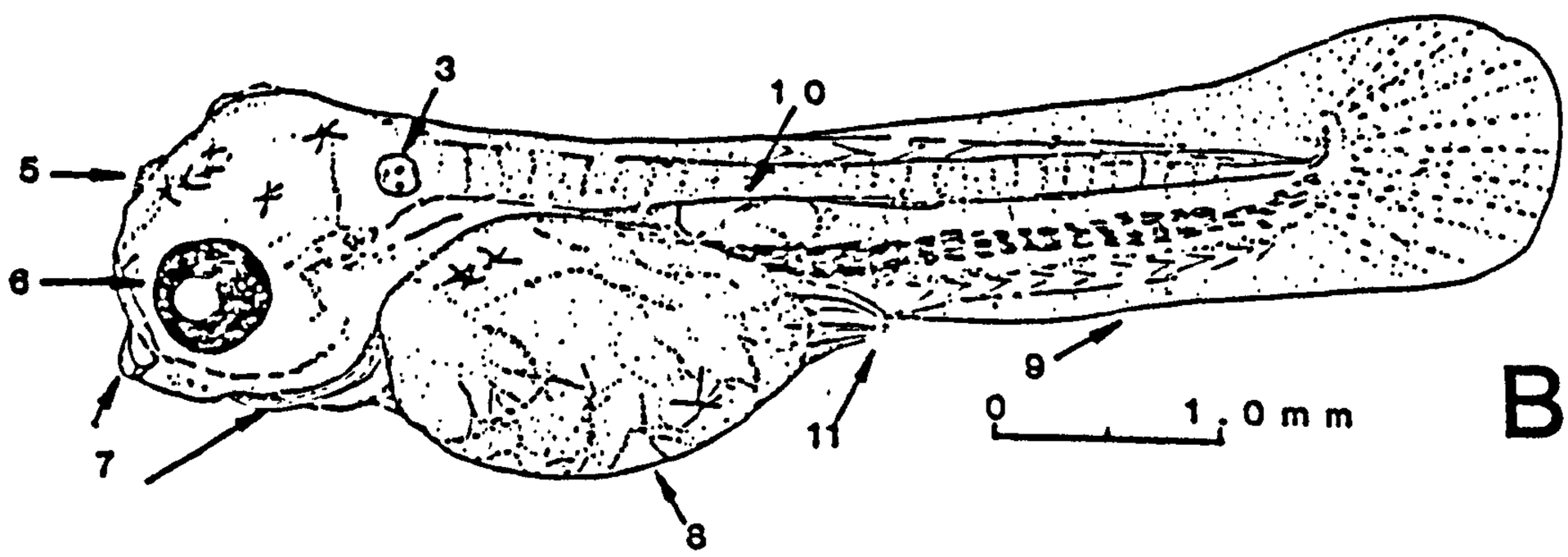

B
A
C

Figure 2.3.

A,B.A)Recently hatched fry showing in the head area: The head glands (1), unpigmented eye (2) and the otoliths (3) plus in the ventral and caudal zone a distinctive well-vascularized respiratory system (4). B)Six day old fry showing reduced head glands (5), well pigmented eyes (6), mouth and gills totally developed (7), the vitelline sac greatly reduced (8) and reduced caudal respiratory (9). It is also possible to observe the gas bladder (10), and anus (11).


1
2
3
4
0
1.0 mm
A


3
5
6
7
8
9
10
11
0
1.0 mm
B

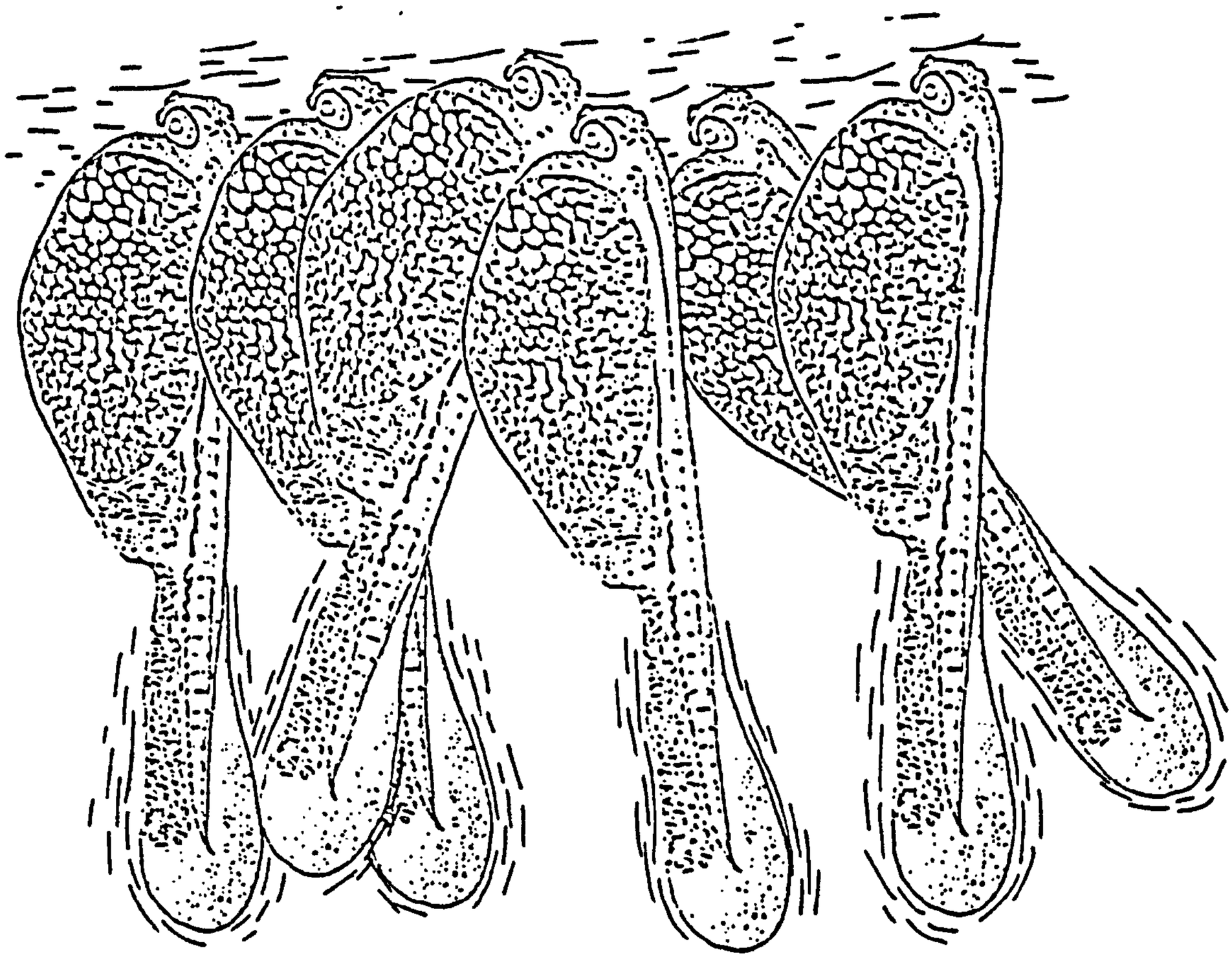
The mouth is not formed and no other structures can been seen in this part of the body, except the pronounced otoliths and in the ventral zone, the conspicuously beating heart. Posterior to the large vitelline sac is a large flat area profusely irrigated by capillaries. This region represents the respiratory area in the larvae and allows the fish to exchange oxygen and carbon dioxide while the gills are still forming. Gas exchange is made more efficient by the larvae continuosly beating their tails whilst the head acts as a point of attachment (Figure 2.4).

After leaving the heart the blood is propelled directly to the well vascularized head gland area and also passes down the fish parallel to the notocord, to the respiratory surfaces (described above) prior returning to the heart.

<u>After 48 hours</u>. After 48 hours no major changes have occurred and the fish continues to respire by means of the ventral capillary area whilst the head glands mantain contact with the substrate.

Figure 2.4.

Recently hatched fry attached to the substrate with the head glands, and moving the tails to facilitate respiration.



After 72 hours. After 72 hours the dorsal aorta becomes interconected with the future gill structures which show capillary loops. In the caudal area it is possible to distinguish a more complicated network of arteries and veins running below the notocord. These loop dorsally and return ventrally in a series of complicated horizontal steps and are probably the developing segmental arteries. From here they run to the end of the tail, where a few capillaries enter the area destined to become the tail fin.

After 96 hours. By 96 hours, the eyes have become pigmented. The mouth is now totaly formed and is occasionally opened by the larva. The area near the gills can be seen to be well irrigated. The caudal respiratory area is somewhat reduced, but that part near the vitelline sac is still profusely irrigated.

After 120 hours. After 120 hours the reduction of the caudal respiratory area is more evident and only a reduced centre near the anus remains where it is possible to observe a few vessels carrying blood. The operculae and mouth make regular ventilation movements, with the result that during this time, both the

remaining caudal respiratory apparatus and the gills supply oxygen to the fish. The head glands are reduced in size with respect to the head, but they are still functional.

After 144 hours. After 144 hours the fish have almost totally lost the vitelline sac and the anus is present. The caudal respiratory system has disappeared along with the geotactic behaviour, and the larvae start to swim with short vertical displacements. The head glands are very reduced but still present. The mouth and opercula have rythmic movements, now supplying all the oxygen required. The gas bladder is developed at this stage.

After 168 hours. After 168 hours the fry swim in a shoal protected by the parents. During the night they return to the substrate and possibly at this stage the head glands, although quite reduced, are still used. The gas bladder is quite obvious and the ventilatory movements are rythmic (Plate 2.3b).

After 240 hours. After 240 hours at 28°C the head glands had totally dissapeared. While the fry are reabsorbing

the vitelline sac, and until they are capable of free swimming, they remain attached to the substrate. However from time to time, some of them swim in rapid bursts far away from the nest. Throughout this period the female spends the day taking care of them by picking up those that stray away from the main batch and spitting them back into the group. When the fry are near the free swimming stage, their activity is greatly increased and they wander near the bottom far from the main group and the female works very hard to keep them in one place.

During all this time the male stays near the female ready to repel any intruder (Plate 2-2). It is important to note that the male and female are recognized by the fry with the result that no other adult approaches during this period of their lives. During the night the couple rest together on the bottom with the fry, forming a compact group. Even when they are free swimmers they rest during the night in a close batch near the parents. This behaviour becomes less strong as the fry grow, and 10 days later when they become free swimmers they are only around the resting parents at night but no longer form the compact batch shown earlier. It was noted, both in the field and the laboratory that sometimes when the parents are disturbed the recently hatched fry are removed in the mouth of the female from the original nest to another secure place. However, the male was never seen doing this.

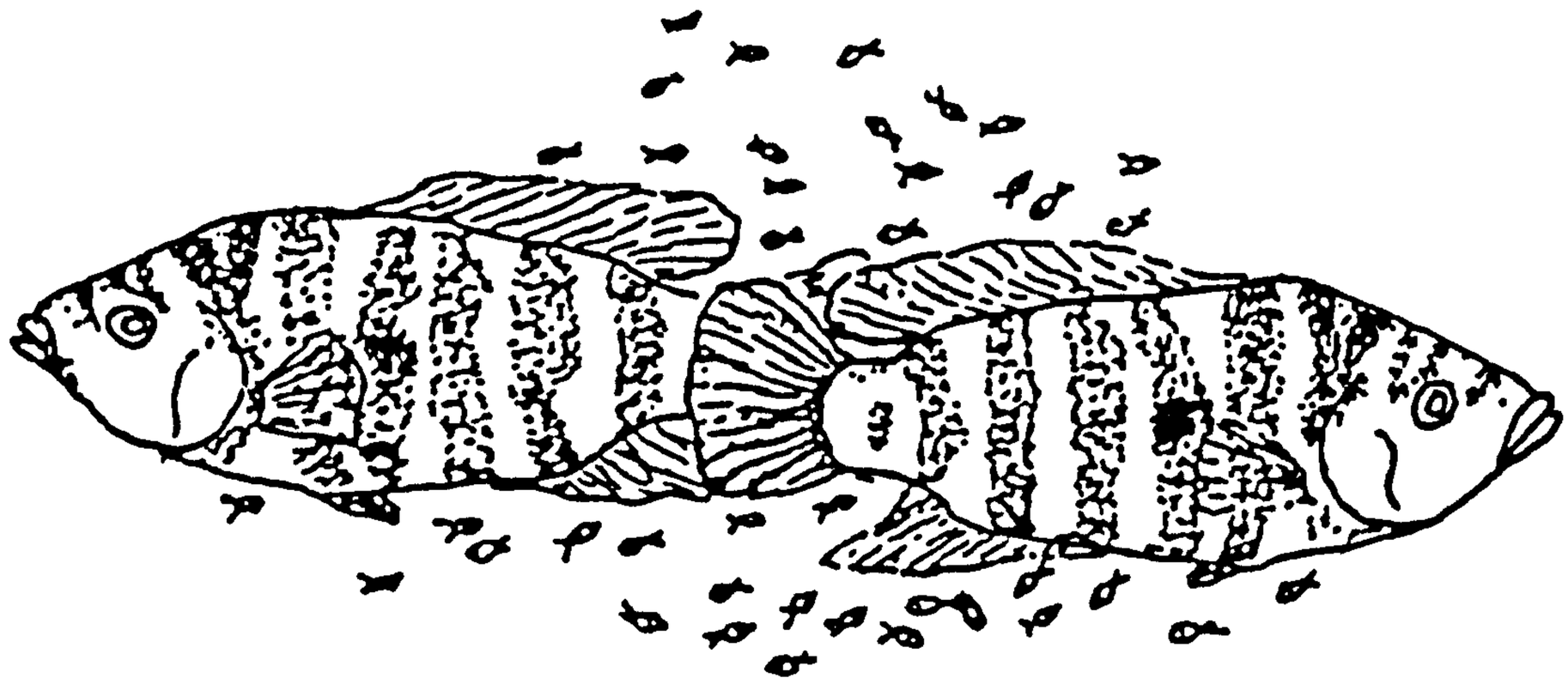
The behaviour of parents and fry during the protective shoal stage.

The male develops a strong behavioural response to repel intruders that approach its established territory. This response can be so strong that the male makes threatening gestures towards human observers, (often by jumping 30-40 cm out of the water in mock attacks) after which he returns to the shoal of fry and prepares for a new attack. Whilst this is happening the female attracts the fry by short contractions of her body and moves the shoal to the bottom. If a predator is detected the shoal moves very close to the parents and remains near the substrate. Sudden water movements typically made by predators, also trigger this response and the fry only leave their grouped position when the parents behaviour returns to normal.

In open areas where the fish can be attacked from any direction by predators both male and female can recall the fry and they assume a back to back position tending to touch their tails or posterior flanks of their bodies (Plate 2.5). The fry respond rapidly by moving close to their parents and remain close under them near the bottom. During this display, rapid attacks against the intruders by both male and female take place, with forward and backward movements so as not to lose the fry. This position enables each parent to have a 180°

Figure 2.5.

Male and female fish showing black barred aggressive colouration and the back to back stance designed to protect the fry from possible predator attack.



area of vision, so that all the possible attack angles are covered. These positions are mantained until the danger has passed whereupon the parents move the shoal of fry to a new feeding area.

Parents attitude towards strange fry.

When a couple with their own fry is disturbed the defensive behaviour described previously occurs. However, when this disturbances is produced by other fry of the same species from another shoal, first the male and then the female try to incorporate the new fry to their own group by strong recalling movements (" jerks" and vibrations of the head and body as described above. The introduced fry have three main behavioural responses:

a) When the fry are removed from their own parents they always show a reflex defensive behaviour, running away from any adult fish near them.

b) A few of the new fry become incorporated into the new group when they are very close to it, and this process takes only a few minutes.

c) Some of the fry occasionaly refuse to join the new group. On newline these three responses produce different parental reactions: the male tries to gather all the fry by recalling movements or jerks, he then

appears "disturbed" when the fry, instead of coming to him, run away in a frightened manner. This triggers the male's response of eating the scared fry. If, on the other hand, the fry are quickly incorporated into the new group the parents defensive behaviour soon calms down.

Fry behaviour before leaving parental care.

During field and tank observations both males and females were always observed near and around the fry when the latter where swimming and looking for food. During this time, parents and fry have daily movements that depend on the fry size, and this may be related to the swimming capabilities of the fry. Thus, it was found that couples with fry of 0.3g show movements of 20-30 metres/hour along the edges of the observation ponds. During these movements both fry and parents have the opportunity of seeking some protection in sheltered areas when a predator is seen. The territory is noticeably increased as the fry grow, probably due to their increased food requirements. During this time when danger approached the fry were frequently recalled by both female and male, and fry in the field as well as in the tanks were observed responding to these signals by swimming and holding station as close to the parents as possible.

The effect of temperature on egg and fry development.

The time taken for the development of C. urophthalmus eggs decreased with increasing temperature (Table 2.1). A highly significant correlation ($r=0.955$, $P < 0.05$) exists between the temperature and the hatching time (Figure 2.6) and the linear equation for this expression is:

$$t = 53.0306 - 0.4581T \qquad \text{(Equation 7)}$$

were t = time in hours, and T = temperature in degree Celcius.

The number of degree days was calculated from egg-laying to hatching (60.6 to 61.3), and this is also shown in Table 2.1. The number of degree days from hatching to free swimming (total absorbtion of vitelline sac) were 196 and 195 at 28°C and 30°C respectively) where no mortality was recorded. The data for 25°C and 35°C were not taken into account due to the high mortalities recorded at both temperatures.

Figure 2.6.

The relationship between hatching time and temperature. Batches of 100 eggs were incubated in three replicates at each temperature.

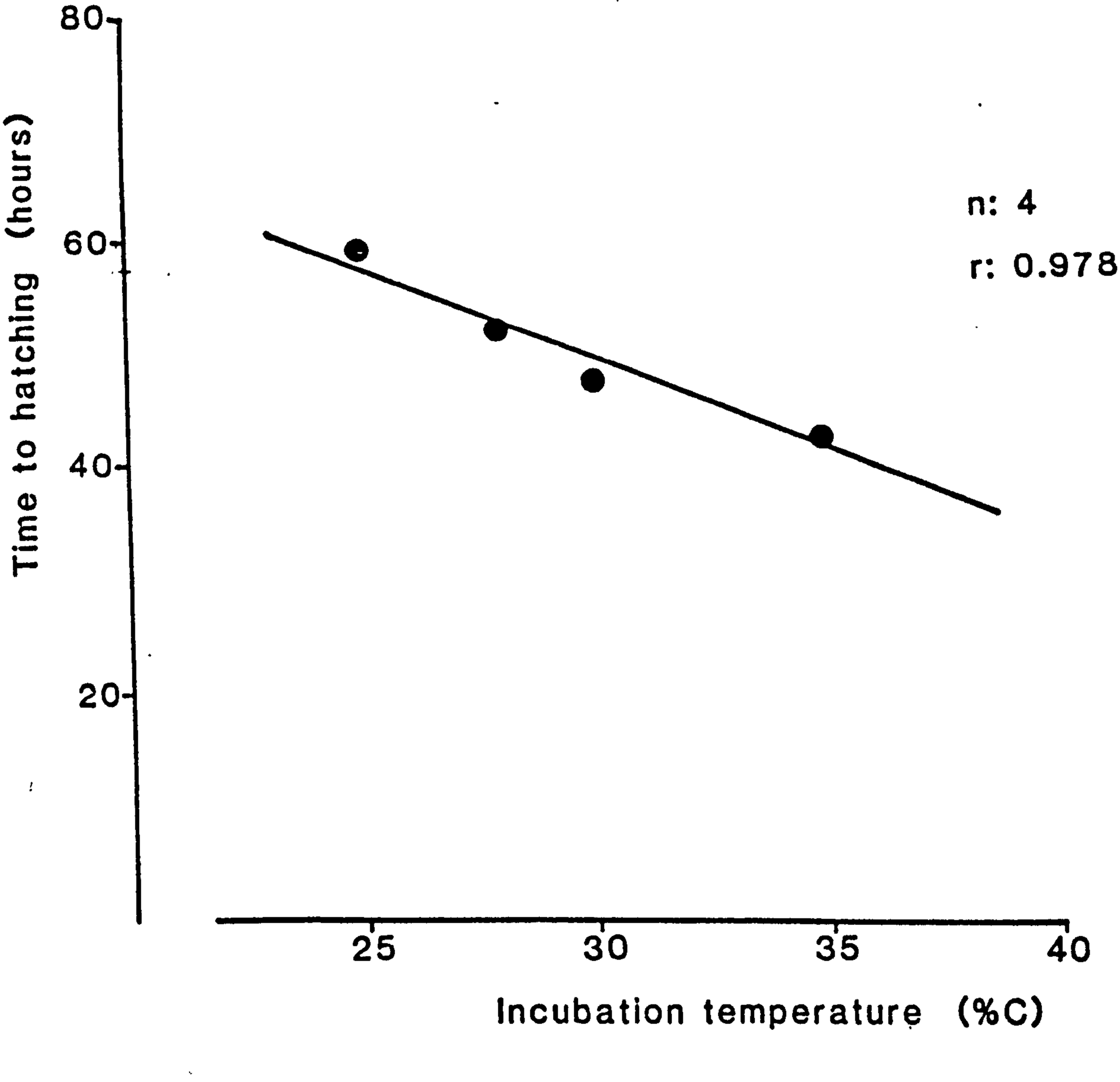

80
60
40
20
Time to hatching (hours)
n: 4
r: 0.978
25
30
35
40
Incubation temperature (%C)

Table 2.1.
Time in days and hours to hatching and free swimming of Cichlasoma urophthalmus fry at four different temperatures, compared with two bottom spawners and two mouth spawners tilapias. (The data refers to the time taken for 50% of the eggs to hatch or the appearance of free swimming).

SPECIES	T°C	HATCHING TIME days	hours	DEGREE DAYS to hatching	FREE SWIMMING days	hours	NO DEGREE DAYS to swimming
C. urophthalmus	25	2.45	58.8	61.25	—	—	—
C. urophthalmus	28	2.17	52	60.76	7.0	168	196
C. urophthalmus	30	2.02	48.5	60.6	6.5	156	195
C. urophthalmus	35	1.75	42	61.3	—	—	—
T. rendalli	28	2-2.17	48-52	56-60.76	6-6.6	144-160	182-187[2]
T. zillii	28	2-2.17	48-52	56-60.76	6-6.6	144-160	182-187[2]
O. niloticus	28	4	9	112	9-11	216-264	252-308[1]
O. mossambicus	28	4	96	112	9-11	216-264	252-308[1]

1 Rana, 1986

2 Rana, Personal Communication

Reproductivity periodicity.

During the trials conducted in the outdoor recirculated water system, all five couples had at least two spawnings. The maximum number of spawns recorded was in couple number 1 with five continuous spawns (Table 2.2). The minimum time between spawns was 23 days and the maximum was 44 days, with and average of 29 days (n = 9, s = 6.9920).

Table 2.2.

Spawning frequency in days for five pairs of C.urophthalmus maintained outdoors at temperatures of between 28-31'C in fibreglass tanks (1m2) with a natural photoperiod and recycled water. Dates of spawn in brackets.

Couple No.	Time 0 (first spawn)	1	2	3	4
1	(10^{th} sep.)	23 (3^{rd} oct)	25 (28^{th}oct)	23 (20^{th}nov)	44 (3^{rd}jan)
2	(10^{th} sep.)	33 (13^{th}oct)			
3	(8^{th} sep)	24 (2^{nd}oct)			
4	(15^{th} oct.)	36 (20^{th}nov)			
5	(25^{th} sep.)	23 (18^{th}oct)	30 (17^{th}nov)		

DISCUSSION

From these studies it is clear that Cichlasoma urophthalmus is a typical substrate spawner, monogamous, with bi-parental care, where both parents collaborate in brood protection. The species also has a similar antipredator behaviour to the rest of the fish from this genus described to date (Baerends and Baerends-Van Roon, 1950; Barlow, 1979; Keenleyside et al., 1979; Keenleyside, 1985; Makie and Itzkowitz, 1985; Barlow, 1986).

The intensification of the black barring and the red, orange, green and olive colours in the ventral zone observed during couple formation is not exclusive to C.urophthalmus. Many adult cichlids become intensively coloured during the breeding cycle with variations in number, size and colour of bars depending on the species (Barlow, 1974; Keenleyside, 1979). In other cichlids the males may be further distinguished by a nuchal hump, as in C.citrinellum and C.nigrofasciatum, with strong black bars on the body (Barlow, 1973 and Barlow, 1974). In some species the red, orange and yellow colours are also commonly used in communication particularly when aggression and reproduction are involved (Barlow, 1974). Regan, (1905) described C.urophthalmus as having 6 to 7

blackish cross-bars, and a similar coloured ocellated spot. This description was probably made on fish during the interspawning period as Chavez et al., (1983) describes nine black bars in the reproductive fish, while in the present work seven distinctive black bars and three less conspicuous bars in the head were observed in animals during the reproductive cycle.

The black ocellated spot at the base of the caudal fin, made a total of eleven distinctive marks. These, together with the strong orange-red colour in the isthmus and distal part of the abdominal area in the reproductive C.urophthalmus, clearly permit the visual identification of a reproductive pair who also show a dominant position against other members of the species in the area.

In C.urophthalmus the high degree of aggression observed in males towards females when the latter are not totally mature is similar to the results obtained by Baerens and Baerends Van-Roon, (1950) with Hemichromis maculatus where the male bites those females which are not in reproductive condition and continues to do so until the female leaves its territory. The aggression between males and females of C.nigrofasciatum and C.spilurum is reduced when the males have a gold patch on their sides (Barlow, 1979), while C.citrinellum gold morphs also appear to inhibit aggression in rivals by

virtue of this colour (Barlow, 1979). Herotilapia multispinosa changes its normal colour to yellow with black marks when it is in reproductive state (Baylis, 1974). In other species such as Haplochromis burtoni an orange patch on the side of the reproductive male inhibits the aggression of other males (Leong, 1969).

In the same way the aggressiveness of C.urophthalmus males towards the female ceases when both develop an intense colouration and this immediately accords the pair a dominant rank among other fish in the area. Vision in these cichlids thus seems to be the first and most important method for recognizing each other as a potential couple. It is possible that other senses such olfaction and hearing become important at a later stage (Myrberg et al, 1965 and Myrberg, 1975). These colour changes, together with the behaviour of digging and cleaning the nest area by both parents may, however, be of practical importance in C.urophthalmus hatchery management as the putative couple is easily recognized.

The agressive behaviour of the male cichlid can be reduced via displacement activities. For example Pelmatochromis subocellatus often vents his aggression by biting the substrate and plants, or by nest excavation (Baerens and Baerens Van-Roon, 1950; Keenleyside, 1979; Heilingenberg, 1965). The same applies to a certain extent in C.urophthalmus in that

there is a reduction in aggression once they start nest building . However it is more likely than the combined effects of colour change, nest building and maybe even chemical stimuli help to form a stable bond between the couple which eventually leads to succesfull reproduction. The aggressive activity also appears to be an important aspect of courtship behaviour, particularly during pair formation in C.citrinellum (Barlow, 1979).

By contrast, the aggression shown by the newly formed couple towards other fish increases with time, this being one of the notable aspects of parental care demonstrated by this species. Parental care is further demonstrated by the protection of the spawning/brood rearing site by both parents who remain close to each other and work as a team. There are interspecific differences in this behaviour in that, sometimes it is the female which is seen close to the fry as in C.citrinellum (Barlow, 1979) and C.nigrofasciatum. The male is in the area but not close to the fry because the female is aggressive to the male when he aproaches the fry (Piron, 1978; Personal observation). The main difference between these two species and C.urophthalmus is that in the latter the male always remains close to the female and there is no aggression between the couple, making parental care more of a combined effort.

The aggressive behaviour found in animals in crowded situations (1m2 tanks and aquaria), was totally different to the results given by Baerends and Baerends Van-Roon (1950) in which they found that the cichlids reduced their aggression when overcrowded in tanks. However, these authors did not specify which species were involved in this behaviour. In the present study it was observed that there was no aggressive behaviour when only one sex was established in the tanks in crowded conditions. The results clearly demonstrated that the males only make displays and exhibit aggression if the females are within visual distance. Non-aggressive behaviour was also found when juveniles and adults were maintained at high densities in large tanks, however when the adults were moved to a 1m2 tank they immediately became aggressive. The behavioural observations made in Celestun lagoon, and those made by Chavez et al., (1983), suggest that the couple requires a minimum territorial area of 1m2 in order to reproduce and take care of their young successfully. Should the available space be less than the 1m2 minimum then high fish mortalities occur. The couple must be able to maintain their territory and keep other fish at a distance without constantly triggering the aggressive response. It was realized that in the circular tanks the water flow was too great as the fish were unable to settle down to nest building. Overall, these results

suggest that this species has some prospect as alternative for aquaculture in that, with good management, the typical aggressive behaviour can be kept to a minimum. Should more intensive techniques be required then monosex culture may provide an alternative.

It was noted that behaviouraly, the size of fish is important in successful breeding. When the larger individuals (200-250g) observed in the field were compared with the smaller (50-150g) fish used in laboratory experiments it became evident that the former were better able to establish and defend a territory. This has also been demonstrated in other Cichlasoma species that mating with the largest available partner should generally be advantageous to both sexes, since the success of a pair is dependant on its ability to take and defend territory (Perrone, 1978; McKaye, 1986), and also in C.nigrofasciatum where larger fish were seen to intimidate others more easily and thus ensure higher fry survival rates (Keenleyside et al., (1985). The widely described sexual precocity common in some cichlid fish, such as tilapia (Oduleye, 1982), is not found to the same degree in C.urophthalmus. Throughout all the experimental work and in the field sampling (Chapter 1) the minimun size at maturity was 45-50g (102cm) even under crowded conditions. By contrast, Oreochromis

mossambicus, O. niloticus and Tilapia zillii of 6 to 10cm (10g) can be sexually mature (Trewavas, 1983; Guerrero, 1982; Rana personal communication). This is a further important factor in favour of C.urophthalmus for use in intensive aquaculture systems.

The two most common habits of cleaning and moving materials with the mouth,called "nipping" and "nibbling" by Baerends and Baerends Van-Roon (1950) and Keenleyside, (1979) respectively, were found during the cleaning and preparation of the substrate in nest building by C.urophthalmus. The high capacity of this fish to remove large amounts of material in a short period during the digging of nests affords an explanation of the rapid changes noted in wild pond areas with soft substrates. Such ponds could, of course, be used as a method of semi-intensive fry production with low attendant labour costs. In considering a nursery pond system it must be born in mind that whilst taking care of the fry, the grazing area and consequently the territory defended, depends on the size of the parents and number and size of the fry. This is probably directly related to the food requirements of the fry during this critical period in the life cycle.

It was also noted that C.urophthalmus does not actually need a substrate with gravel or plants for

spawning, as described for other species of cichlids (Barends and Baerends Van-Roon, 1950; Keenleyside, 1979). Oreochromis.sp. are also capable of cleaning an area of glassfibre tank or aquarium without gravel or other substrates and in all these species succesful reproduction can be achieved. Thus when C.urophthalmus is maintained in tanks with gravel this is immediately removed as a conditioned response. When in a tank without a substrate, however, the fish start to clean a selected site in the same manner that they did with a gravel bottom, biting at the surface in order to prepare the area as a future nest. This behaviour allows the brood stock to spawn in a clean and quite controlled area and this reduces possible contaminations. The use of complex substrates for intensive fry production in a hatchery can thus be avoided, saving investment and handling in commercial systems. On the other hand it is probable that the use of cages for intensive culture will determine any reproductive behaviour and its attendant production losses.

The positive geotaxis displayed in recently hatched C.urophthalmus larvae is probably directly associated with the otoliths and is probably an adaptation to a lotic environment. It is notable that this behaviour is so well developed at this early stage. It would seem that previous work on cichlid larvae has not reported

this behaviour (Baerends and Baerends Van-Roon, 1950; Keenleyside, 1979; Baylis, 1974; Barlow, 1979), although comments have been made suggesting that the heavy yolk sac acts as a weight drawing the larvae to the bottom,(Rana, personal communication). The larvae of Tilapia zilii, another bottom spawning cichlid, attach themselves to their egg shells with their head glands and fall to the substrate under the weight of the vitelline sac. It is clear, however, that this fish simply free-falls without the active swimming behaviour of C.urophthalmus, Figure 2.2.

This strong difference in behaviour between these two bottom spawners is most probably due to the lotic adaptations of C.urophthalmus which require the larvae to settle as fast as possible to the bottom, avoiding displacement by river currents. By contrast, the African bottom spawner Tilapia zillii, is totally adapted to lentic environments where the larvae are not in danger of dispersal by currents. The geotactic behaviour has an important implication in aquaculture, because by installing simple equipment such as removable tiles in the base of the tanks it would be possible to remove the batches of fry into hatching tanks, thus avoiding excessive fry handling.

The anatomical structures in larvae such as head glands and caudal respiratory area, are quite similar in function to those of other cichlid substrate spawners reported by Baerends and Baerends Van-Roon (1950), Keenleyside (1979), and Trewavas (1983). Further studies of these anatomical differences would be useful, however and may help with the understanding of the complicated taxonomy of the Cichlasoma genus which requires a full taxonomic revision, as mentioned by Goldstein (1973).

C.urophthalmus fry doubtlessly recognize both male and female by their distinctive colouration, in a similar manner to that described previously by Noble and Curtis (1939) and Baerends and Baerends Van-Roon (1950) for C.bimaculatum. In the latter case the fry respond more easily to black and blue parent models. It was later demonstrated that cichlids can also respond to sound or to chemical cues, (Myrberg, 1975).

C.urophthalmus parents always call their fry by a display of body jerking and this behaviour was noted in both the male and female. The fry respond to this signal by swimming as close as possible to their parents. In general the fry recall behaviour and the proximity of the fry to both parents and the bottom has already been described for most of the substrate spawning cichlids. Baerends and Baerends Van-Roon (1950) found that the fry

identify their own parents by colours and by some movements of the adults which they refer to as "calling", later denominated "jerking" by Baylis (1974). This behaviour was described by Keenleyside, (1979) and Barlow, (1979) for C.citrinellum and has been further substantiated in C.urophthalmus in this study.

Without doubt C.urophthalmus is unable to distinguish other fry from their own when two groups of similarly aged juveniles are mixed. This behaviour was similar to that found in C.citrinellum, where the couple can accept other fry of 2, 10, 13 and 35 days younger and 2 days older than their own, but not fry which were 10 or 13 days older (Noakes and Barlow, 1973). The fact that C.urophthalmus is unable to recognize other fish from the same species and age could be an advantage for aquaculture as several batches of similarly aged juveniles could be combined and looked after by one adult pair, those allowing the other parents to come back into reproductive condition more quickly. This would only be of importance should parental care be considered necessary in culture at an early stage of development.

In terms of reproductivity periodicity, the delay in spawning recorded between November and January in couple number 2 was possibly due to the drop in

temperature from 28 to 24 during December and January. The results of these experimental trials suggest that, with proper conditions and temperature artificially maintained during the low temperature months at around 28°C, it is possible that out-of season spawning could be promoted. This creates the opportunity of carefully scheduling fry production, but more experimental work must be conducted in terms of hatchery management in this species.

The effect of temperature on egg and fry development showed that C.urophthalmus has the ability to successfuly hatch between 25 and 35°C. High mortality was recorded from hatching to free swimming at temperatures of 25°C and 35°C while no mortality was recorded at 28°C or 30°C. In comparison with C.urophthalmus, O.niloticus has demonstrated similar narrow tolerances for successfull hatching between 25 and 30°C, at it has been shown that at 20°C and 34.5°C. substantial mortalities will occur in the latter species (Rana, 1986). At 35°C, hatching successes of C.urophthalmus is clearly limited, as was found in O.niloticus. However, O.niloticus does not hatch at all at this temperature, whereas C.urophthalmus does hatch but sufferes high mortalities. However, no explanation can be provided for the high mortality observed at 25°C, because for personal observations, C.urophthalmus are

able to hatch and survive without problems at this temperature. It is probably that the temperature and time for which the eggs were maintained before incubation at the different experimental temperatures had an influence on the development of the fish, as was found for O.niloticus (Rana, 1986) and Essox lucius (Hokanson et al., 1973; Hassler, 1982).

In terms of degree days to hatching and to be free-swimming C.urophthalmus has a similar performance to that observed in other bottom spawners such as Tilapia rendalli and T.zillii (Rana, Personal communication) (Table 2.1). The small differences in the values between these species are probably principally due to natural and experimental variations. It can be clearly seen, however, from Table 2.1 that the bottom spawners take in fewer degree days to achieve hatching and free swimming than the mouth brooders, and this characteristic, will confer some advantages in terms of hatchery management to the bottom spawners.

Overall, the work reported here provides a basis for preliminary design of hatcheries or nursery pond systems for reproduction of C.urophthalmus although there is clearly a need for more research before finalising hatchery techniques for full scale commercial ventures.

CHAPTER 3

ENVIRONMENTAL PHYSIOLOGY

of

Cichlasoma urophthalmus

INTRODUCTION.

Among vertebrates fish are the richest and most widspread group of species, their abundance and distribution are the product of interactions between them and their chemical, physical, and biological surroundings (Nikolsky, 1968; Lagler et.al. 1977; Love, 1980). The most important environmental factors influencing the growth, reproduction and behaviour of fish and which limits their distribution are temperature, salinity and dissolved oxygen (Nikolsky, 1968; Randall, 1970; Lagler et al., 1977; Hawkins, 1981; Stickney, 1986).

In aquaculture management environmental parameters are critical factors mainly in intensive systems, where the objective is to maximize the rates of growth to obtain the maximum production of a species within the minimum space. Aquaculture is an economic technology in which implementation is often restricted by the quality and availability of the water in a particular geographical region, where the temperature and salinity are normally the most important constraints. This has forced the investigation of the resistance and tolerance to these factors of different species, (Coutant, 1977;; McCauley and Casselman, 1981; Stickney and Winfree

1983).

From the wide range of fish studies perfomed there is a distinct lack of information on temperature and salinity requirements of tropical species (Macintosh and DeSilva, 1984; Sylvester et al., 1974) and more so in those species with high aquaculture potential. Intensive aquaculture presupposes a high stocking density and because of this dissolved oxygen is probably the most critical water quality variable. The culturist does, however, have some measure of control over dissolved oxygen concentration; its value can, for example, be increased by mechanical processes. In contrast temperature and salinity are variables that are more under natural control (Stickney, 1986) and are generally more difficult and more costly to manipulate. For these reasons the investigation of the general requirements and limits of these environmental factors on a species with high aquaculture potential is most desirable at an early stage in development. In general, fish with high tolerances and wide resistances to the main factors; temperature (eurytherms), salinity (euryhalines) and oxygen; have great potential for culture in a wider range of conditions.

This chapter describes the results of a series of experiments conducted to determine temperature, salinity and oxygen requirements of C.urophthalmus juveniles as a contribution to optimization of the environment for both experimental work and commercial culture of this cichlid.

THE EFFECTS OF WATER TEMPERATURE ON FOOD INTAKE, GROWTH AND BODY COMPOSITION OF *Cichlasoma urophthalmus* FRY.

INTRODUCTION.

Temperature is one of the most important factors governing aquatic poikilotherms and influencing the distribution or success of fish species (Nikolsky, 1968; Lagler et. al., 1977). Repeatedly, water temperature has also been shown to be one of the most influential environmental factors affecting both the growth of the fish and its body composition (Paloheimo et al., 1966; Fry, 1971; Elliot, 1976a; Caulton and Bursell, 1977; Brett et al., 1969; Wootton et al., 1980; Love, 1980; Cinncotta and Stauffer, 1984; Weatherley and Gill, 1983; Gill and Weatherley, 1984; Herzig and Winkler, 1986).

Environmental temperature, and change in temperature, often acts as a natural stimulus, determining the onset of spawning, migrations, growth, food intake, and a wide variety of different interactions between these factors (Andrews and Stickney, 1972; Lagler et al., 1977; McCauley and Casselman, 1981). Because of this, most species of fish have evolved and adapted to be able to tolerate particular environments, which may have widely different mean temperatures, temperature ranges and patterns of seasonal variation.

Thus, species of antarctic icefish such as Trematomus borchgrevinki have optimum temperatures of between -1.9 to -1.7'C, its activity declines at -0.8 and the animals die at +5'C. In complete contrast one of the highest temperatures selected by a fish species seems to be 40'C by Cyprinodon macularis (Love, 1980).

The great importance of temperature in the life of fish, has stimulated much research most of which has been related to species of importance in aquaculture. In all this previous work the salmonids have received great attention, for example the effects of temperature and ration size on the growth in Onchorhynchus nerka have been studied by Brett et al., (1969); Shelbourn et al., (1973); Brett and Shelbourn, (1975); in Salmo trutta by Elliot, (1976a,b), Salmo gairdneri by Wurtsbaugh and Davis, (1977), and Salvelinus alpinus by Jobling, (1983). Salmonid temperature tolerances and lethal temperatures have been studied by McCormick et al.,(1971); Symons et al., (1976) and Jobling, (1981, 1983). The requirements of energy for maintanence at different temperatures have been studied by Smith et al., (1978) and Thomas et al., (1986). The effects of temperature and photoperiod on smoltification has been described by Pereira and Adelman, (1985).

Detailed, but less extensive work, has been carried out on other temperate and subtropical species. Thus the effects of ration size and temperature on the growth of Cyprinus carpio have been studied by Goolish and Aldeman, (1984); while the effects of temperature on cyprinid embrionic development has been described by Herzig and Winkler, (1986). Acclimation temperature and temperature selection has been evaluated in 13 species by Cherry et al., (1975) and temperature preferenda of six fresh water species by Cincotta and Stauffer., (1984). The effects of temperature on body composition have been evaluated in the English sole Parophrys vetulus at five temperatures (William and Caldwell, 1978), while in Perca fluviatilis the effects of temperature have been studied by Craig, (1977). In a number of other species the effects of body weight and feeding ratio at different temperatures have been described (Wootton et al., 1980; McCauley and Casselman, 1981; Allen and Wootton, 1982; Gill and Weatherley, 1984).

Limited work has been carried out on tropical species with importance for aquaculture. Thus, in terms of selected temperature and temperature optimum for growth some work has been done in Oreochromis mossambicus (Bandehuizen, 1967), Orechromis niloticus (Beamish, 1970); Mugil cephalus (Sylvester et al.,

1974); Ctenopharyngodon idella and Hypophthalmichthys molitrix (Bettoly et al., 1985) and in Brachidanio rerio (Craig and Fletcher, 1984). The effects of temperature on body composition has been studied in Ictalurus punctatus (Andrews and Stickney, 1972); and Clarias lazera (Hogendoorn et al., 1983). An the influence of temperature on metabolism has been investigated in Tilapia rendalli (Caulton, 1977); Orechromis mossambicus (Caulton, 1978); and Oreochomis (=Tilapia) aureus (Kindle and Whitmore, 1986). The behaviour of Tilapia zilli at different temperatures has been described by Saclauso, (1985).

Thus, although many species of cichlids with aquaculture potential require more investigation, some useful research has already been carried out. A wide thermal range has been demonstrated in Oreochromis niloticus from 7°C to 42°C (Avault and Shell, 1966; Denzer, 1966), and this species has a preferred temperature between 25-30°C (Beamish, 1970). In Oreochromis mossambicus a final median temperature preferendum of 28.5°C and a selected temperature range from 27 to 33.5°C was demonstrated (Bandehuizen, 1967). Tilapia rendalli commonly experiences temperature ranges varying from 20°C at night to 31-35°C during the day (Donnelly, 1969), but very few survive acclimation to 40°C (Caulton, 1977). Tilapia zillii similarly

experiences a wide range of temperatures in normal life from 16 to 32°C with optimun growth a 31°C (Cridland, 1962; Hauser, 1977). Thus the African cichlids are generally thermophilic. Further work is however, needed on the effect of temperature on tropical and subtropical species, particularly in terms of growth and energetics (Bandehuizen, 1967; Beamish, 1970; Fry, 1971; Sylvester et al., 1974; Caulton and Bursell, 1977; Chua and Teng, 1980; Mcintosh and DeSilva, 1984; Casselman, 1978; Bettoli et al., 1985).

Temperature has a strong influence over food intake (Brett et al., 1969; Love, 1980), thus with increasing temperature, the efficiency of food conversion to body tissue is reduced, and when food becomes limiting, the temperature for optimum growth is progressively lowered (Brett et al., 1969). Further increase in temperature has a definite limiting effect on the growth of small fish, while the growth rates of larger fish are generally much less affected (Shelbourn et al., 1973).

Thus temperature is a very important factor in fish growth and production, and in the investigation of a new species for its potential in aquaculture systems, temperature is one of the first factors to consider. The methodology used to date in the determination of the temperature is one of the first factors fish in culture

systems, has concentrated on the concept of optimun temperature, which can be defined as "The temperature at which the maximum growth of somatic tissue, expressed as wet weight, occurs when fish are fed ad libitum while reared at constant temperatures within the temperature range tolerated by the species", (Hokanson et al., 1977). This type of determination requires long term experimentation with precise temperature control (Jobling, 1981). In contrast to this, final temperature preferendum of fish, is a concept which has been used by many authors to simplify the determination of the optimum temperature. This approaches requires much less labour, shorter experimental trials and gives faster results than optimun growth temperature experiments (McCauley and Casselman, 1981; Goolish and Adelman, 1984; Beamish, 1970; Jobling, 1981; Bettoli et al., 1985; McCauley and Casselman, 1981). It has, however, been shown that the preferred temperature is somewhat higher than the optimum temperature (McCauley and Casselman, 1981; Love, 1980), and some authors have further demonstrated that the preferred temperature is highly correlated with the previous thermal acclimation of the fish prior to the experiment (Beamish, 1970; Fry, 1971; Shelbourn et al., 1973; Crawshaw, 1977; Beitinger and Fitzpatrick, 1979; Love, 1980). McCauley and Huggins, (1979) and Love, (1980) have also suggested that final preferenda of rainbow trout may be dependent

upon age and younger rainbow trout have a higher temperature preferendum than older fish. Some authors suggest that the concept of preferred temperature requires more study before it can be used as a practical determination of optimal temperatures in fish (Crawshaw, 1977; Love, 1980). For these reasons it seems that the optimal temperature as classically defined by Hokanson et al., (1977) is the best way to obtain reliable information on fish temperature relationships. With the knowledge of the effects of temperature some insight can be given into the optimal frequency of feeding, amount of feed and to some extent composition of the diets. In addition to such biological and husbandry considerations it is also necessary to consider the economic feasibility of controlling temperatures at different levels of intensity of aquaculture, with the objective of increasing fish production.

The objective of the present study was to determine the temperature of optimum growth, food intake, food conversion efficiency, body composition and mortality of Cichlasoma urophthalmus juveniles at different temperatures ranging from 22.5°C to 36.3°C.

MATERIALS AND METHODS.

Experimental animals..

Juvenile C.urophthalmus, in the weight range 0.08 to 0.09g were reared in the CINVESTAV laboratory from wild brood stock captured at Dos Bocas, Tabasco, as previously described.

Experimental system.

Six independent experimental tank systems were constructed using 20l polypropylene buckets and PVC plastic pipes. Each system consisted of two experimental 20l circular tanks with independent water inflow of 1.5 to 2 l/min regulated with a rubber water tap (Martinez et al., 1986) and one bottom drain with central stand pipe and collar. The tanks were arranged to be self-cleaning and were protected with a plastic net to avoid fish losses. The water to the system was pumped by a submersible water pump (Little Giant, model 8a) from a 50l tank placed below the two experimental tanks. This reservoir tank received the direct drain from the two 20l experimental tanks, and contained two gravel aquarium filters, two 200-watt thermostatically

controlled heaters to maintain the preselected temperature and two air stones to aerate the water, maintaining the oxygen levels near saturation. During water changes, water removal was from this section of the system in order to avoid any fish disturbance to the fish in the experimental tanks (Figure 3.).

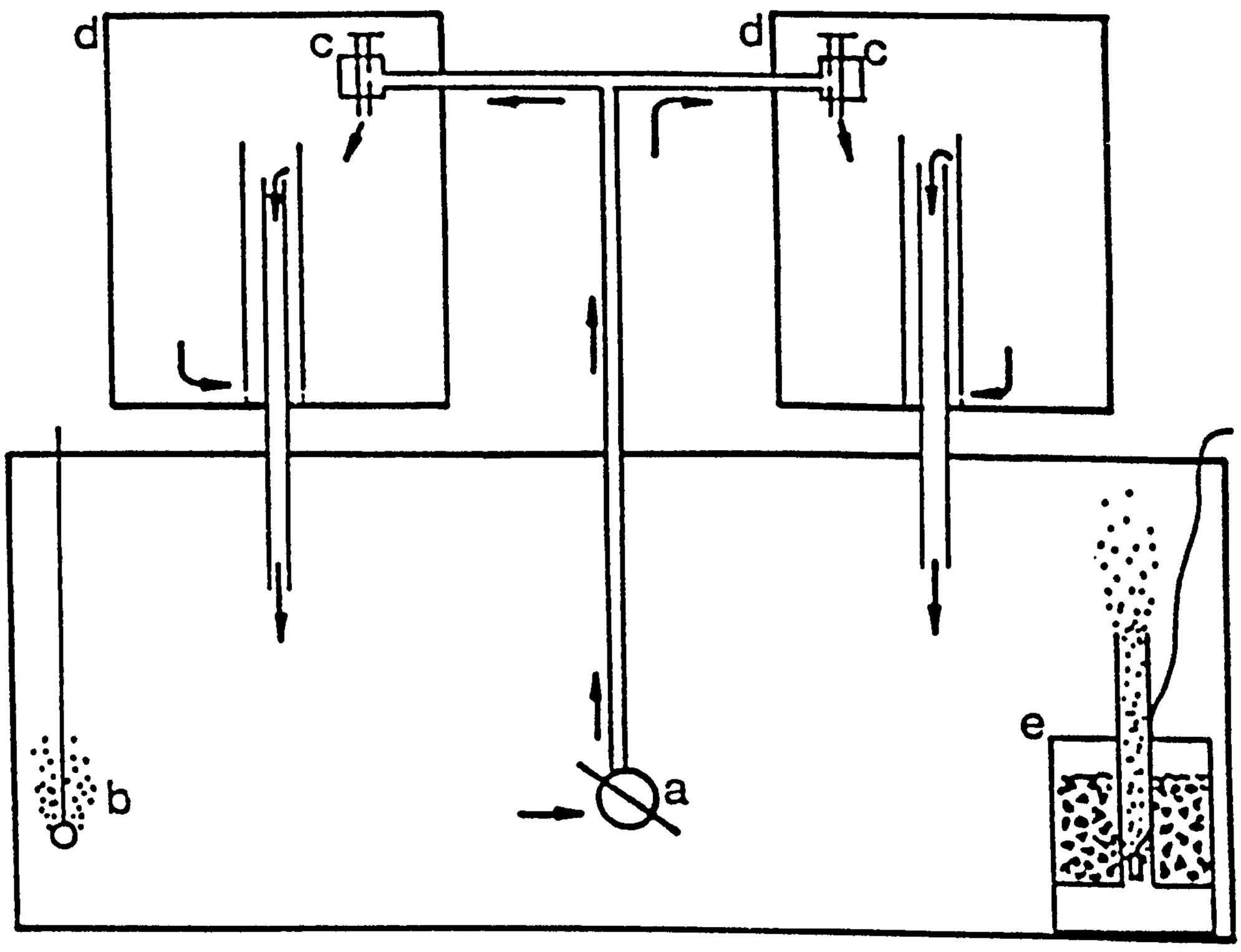
City water was aerated for at least three days before introduction to the system, to remove chlorine. A 1000 l tank was also maintained and this water was used as a partial replacement of the water in the experimental systems, where 40% of the total water was replaced every two days. Care was taken to match the temperature of the new water to that of the experiment. The whole system was maintained in a controled temperature room at 21+1°C, with a photoperiod of 12 hours light 12 hours dark.

Monitoring of environmental parameters.

Six systems as already described were maintained at the following temperature: 22.5, 25.5, 27.0, 30.0, 33, and 36.0°C. The individual system temperature was controlled by the thermostats in each submersible heater, and was recorded twice a day with a mercury thermometer and with the thermistor probe of a Y.S.I. model 57 Oxygen Meter. Oxygen concentration was measured daily in the tanks and pH was measured weekly in each

Figure 3.

Diagram showing the experimental system used to maintain the fish at six different temperatures. a) pump. b)airstone. c)water valve. d)experimental tank e)gravel filter. The arrows show the water flow.


d
c
d
c
b
a
e

system using a Pye-unicam pH meter (Model 9409). Total Ammonia was measured fortnightly from each different system using the method of Lind, (1979). Table 3.1 shows the mean values of each parameter monitored during the present experiment, with its respective variation.

Experimental diet.

The experimental diet was formulated, using brown fish meal as the main source of protein, with solvent extracted soybean meal and wheat meal. The composition of the experimental diet is shown in Table 3.2. Experimental diets were prepared by first mixing all the dietary ingredients thoroughly in a Hobart A200 food mixer. The feed mixture was then pelleted through a 2mm die plate using the extrusion facility of the same apparatus, and the resultant pellets were air dried by convection at 35°C. The experimental diet was stored in air-tight containers at -15'C, until required.

Experimental protocol and analysis.

The experimental fish were stocked at 20 per tank, with two replicated tanks per temperature treatment. The fry were acclimated to the tanks for two weeks prior to the start of the experiment, feeding the experimental diet at 3% body weight/day. At the start of the

Table 3.1.

Mean temperature, pH, dissolved oxygen (mg/l), and total ammonia NH3-NH4 (mg/l) in the experimental controled tanks.

TEMPERATURE (°C)	pH	Oxygen (mg/l)	Total Ammonia ($NH_3 - NH_4$) (mg/l)
22.5 ± 0.02	8.61 ± 0.03	7.39 ± 0.3	0.043 ± 0.01
25.7 ± 0.02	8.62 ± 0.04	6.91 ± 0.3	0.045 ± 0.01
27.1 ± 0.01	8.65 ± 0.02	6.85 ± 0.3	0.049 ± 0.01
29.7 ± 0.02	8.68 ± 0.02	6.13 ± 0.3	0.051 ± 0.01
33.1 ± 0.09	8.71 ± 0.05	5.51 ± 0.3	0.053 ± 0.01
36.3 ± 0.02	8.77 ± 0.01	5.15 ± 0.3	0.072 ± 0.01

Table 3.2.

Formulation and proximate analysis of the experimental diet.

Ingredients (%)	
Brown fish meal	37.88
Soybean meal	24.00
Wheat meal	24.00
Starch	0.78
Fish liver oil	2.89
Soybean oil	4.95
Binder	0.5
Vitamin Premix	3.0
Mineral Premix	1.5
Indicator (Cr_2O_3)	0.5
Nutrient Content (%)	
Moisture	5.57
Crude protein (Nx6.25	43.79
Lipid	11.27
Crude fiber	2.46
ASH	10.00
Energy Value	4944.059 Kcal/Kg

experiment 150 fish were killed by an overdose of benzocaine (1:300) and stored at -15°C for subsequent analysis of moisture content and gross chemical constituents. The feeding regime was to satiation, which was considered as persistent refusal of further food, within a limit time of 15 minutes, after which no more food was supplied. Satiation feeding was maintained throughout the experimental period and the food was weighed before and after feeding to determine the precise amount of food taken daily. The frequency of feeding was maintained at 5 times/day.

At the start of the experiment and at subsequent weekly intervals, fish were batch weighed to the nearest two decimal points (0.01g) on a Mettler top-pan balance (model. PE-3600) in a dish containing preweighed water. At the end of the experiment, after batch weighing, the individual total lenghts were taken to the nearest 1.0mm. Fish mortality was recorded daily as required and the weight of the deadfish was discounted from the total to adjust feeding rates.

As Fish were weighed, at weekly intervals, the rates of growth were calculated for each week period according with the following formula:

$$SGR = \frac{\text{Loge Wt} - \text{Loge Wo}}{T} \times 100$$

Where SGR is Specific Growth rate or instantaneous rate

of weight increase, expresed in percentage/day, Wo is fish weight at time 0, Wt is the average fish weight at the end of the growth interval and T is the total time in days of the interval (In this case T=7) (Ricker, 1975).

The crude protein content of the experimental diet and fish carcass was determined using the Kjeldhal technique (AOAC, 1984) with a Kjeltec Tecator System 1030, distilling unit. Ash content was determined by heating a preweighed sample in a silica crucible in a muffle furnace at 450°C for 12 hours (AOAC, 1984). Crude Fibre content was determined by the digestion method with dilute H2SO4 (0.22 5N) and NaOH (0.313N) (AOAC, 1984). Lipid analysis was determined using a Soxhlet extraction apparatus and microsoxhlet when required, with petroleum ether as the solvent (AOAC, 1984). Moisture content was determined by drying in an oven at 105°C for 24 hours.

The formulae utilized for the nutritional calculations is shown in appendix 1.

Data was analysed by analysis of variance (Parker, 1980) and mean values were compared using Duncan's multiple range test (Duncan, 1955). Arithmetical calculations were made using the "Swift" spreadsheet, in

a Commodore 64 personal computer and full regressions and multiple regressions were performed using Minitab (Ryan et al., 1985) on a DEC-VAX 11/750 main frame computer.

RESULTS.

Acceptance of the experimental diets.

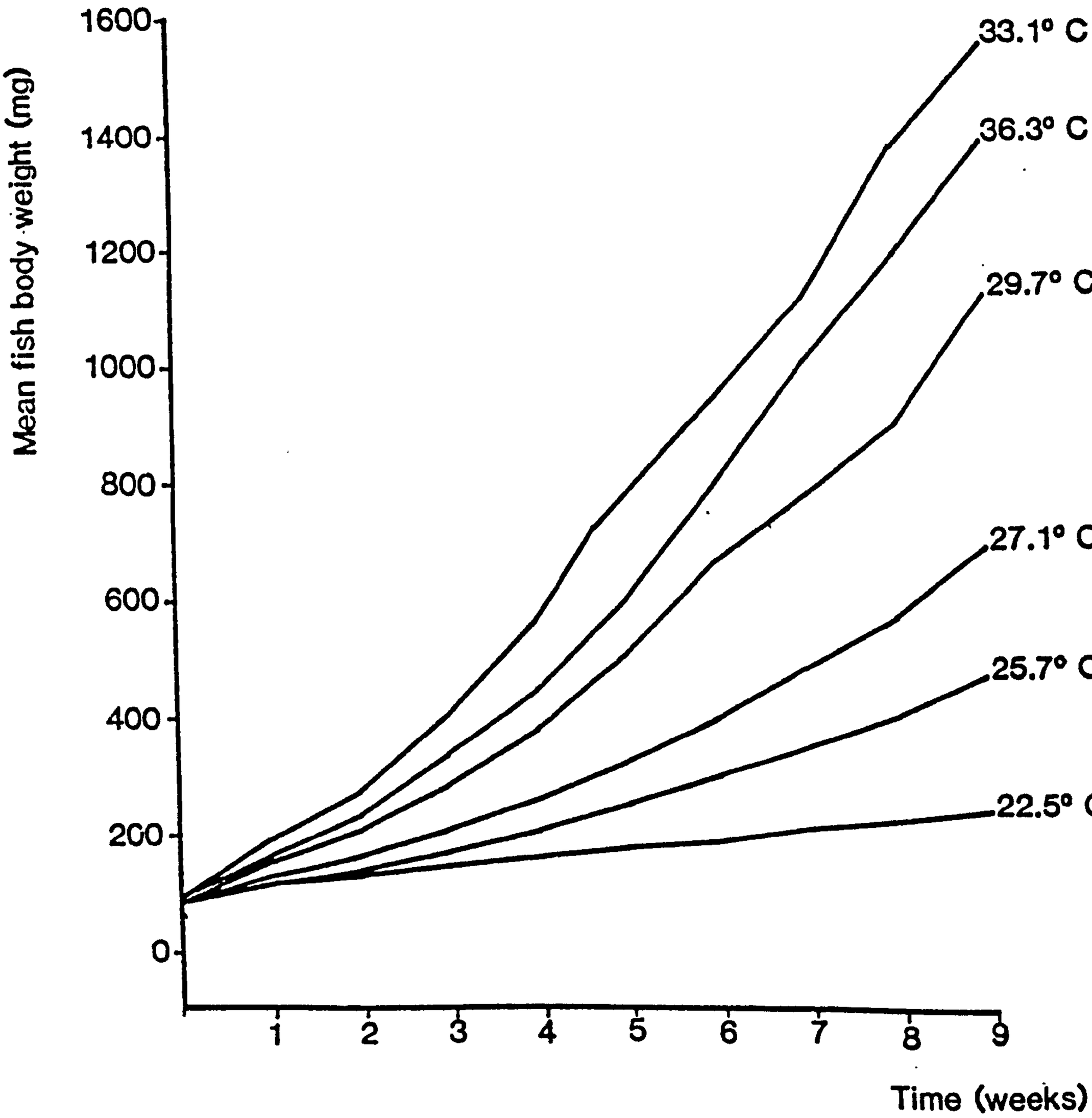
The acceptance response for the diets in the fish varied considerably with the experimental temperature. The fish at 22.5°C fed slowly, compared with higher temperatures where the fish ate agressively, eating the food before it reached the bottom of the tank. This behaviour was maintained throughout the whole experimental period.

Growth.

The growth response and weekly weight increase of C. urophthalmus over the experimental time at different temperatures is shown in Figure 3.1. The best growth response was observed in the fish at 33.1±0.09°C although there was no significant difference between this temperature, 29.7 and 36.3°C ($P<0.01$). Similarly, there was no significant difference ($P\leq0.01$) between

Figure 3.1.

Overall mean growth response of Cichlasoma urophthalmus at successive weekly intervals over the experimental period at 6 temperatures. Temperatures with same subscript were not significantly different (P=0.01).


Mean fish body weight (mg)
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time (weeks)
33.1° C b
36.3° C b
29.7° C b
27.1° C a
25.7° C a
22.5° C a

final weights in the fish at 22.5, 25.7, and 27.1°C but these results were significantly different ($P<0.01$) from those at the higher temperaturos (Table 3.3). In terms of specific growth rate the highest value was observed at 33.1°C but no significant difference was found between the latter and 29.7 with 36.3°C. The lowest SGR was found and the lowest temperature (22.5°C) but this was not significantly different from those at temperatures of 25.7 and 27.1°C. The highest weight gain (mg/day) was found at 33.1°C and this was significantly different ($P<0.01$) from all others temperatures. The next best weight gain was at temperature of 29.7 and 36.3°C then 25.7 and 27.1 and the lowest value was found at 22.5°C. These groups were signifficantly different from each other (Table 3.3).

When specific growth rate (SGR) was plotted against experimental temperature a trend of rapid increase in the specific growth rate was observed until near 33.1°C, Figure 3.2. At 36.3°C, however, SGR decreased and this trend is probably continued at even higher temperatures. a model for this relationship was obtained and is given by:

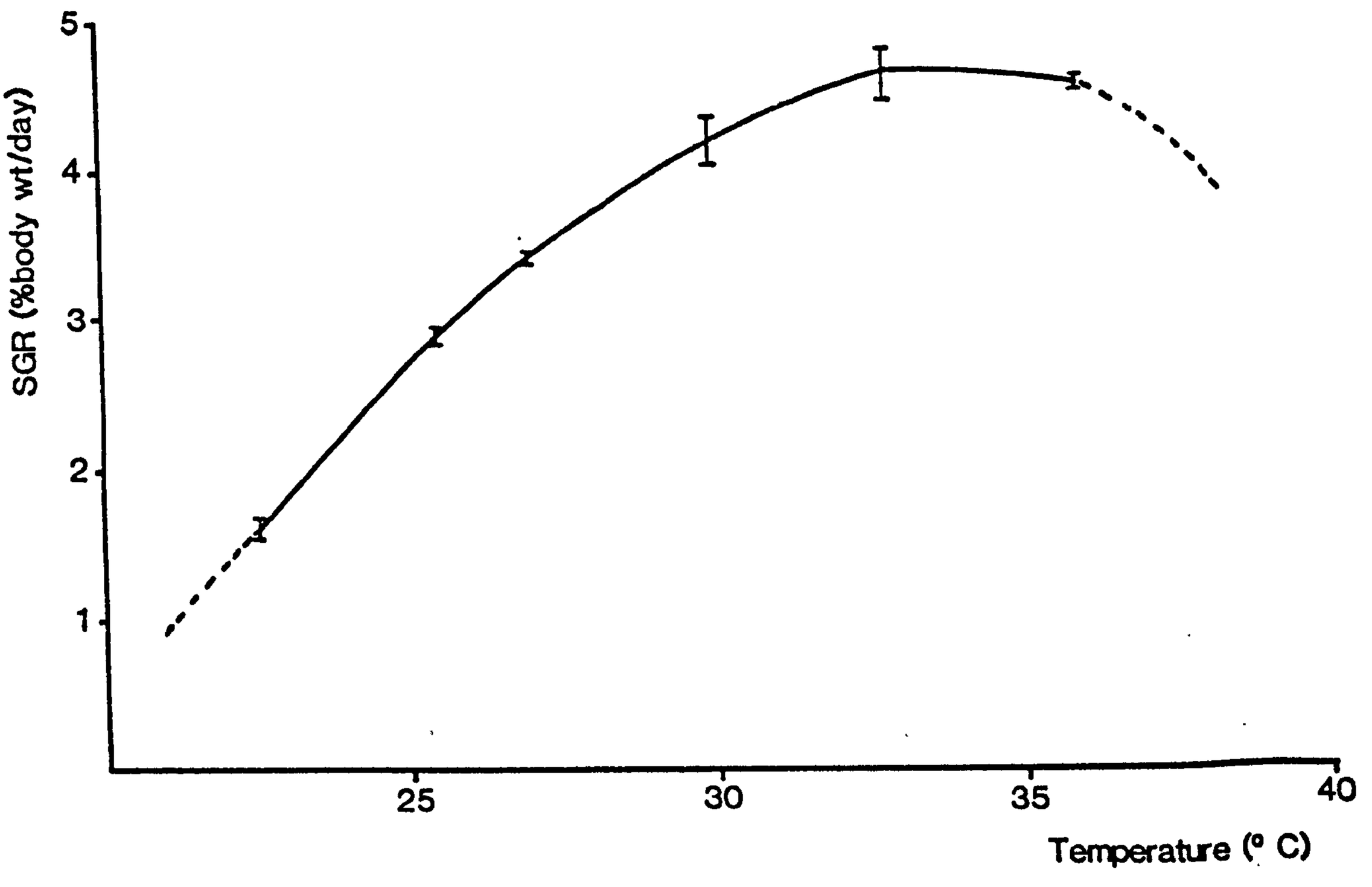
$$\text{LogeSGR} = -3.23 + 0.698(\text{LogeWt.}) \qquad \text{(Equation 8.)}$$

Table 3.3.
Mean growth performance, feed utilization defficiency and carcass composition of Cichlasoma urophthalmus, at the six different experimental temperatures for nine weeks.

MEAN VALUES	TEMPERATURES					
	22.5	25.7	27.1	29.7	33.1	36.3
INITIAL BODY WT (MG)	83.250a	75.000a	80.20a	82.00a	84.85a	78.30a
FINAL BODY WT (MG)	233.500[a]	465.00[a]	681.50[a]	1,122.00[b]	1,542.90[b]	1,385.00[b]
WEIGHT GAIN (%)	190.214a	520.60ab	749.54b	1,296.57c	1,720.19c	1,671.99c
SPECIFIC GROWTH RATE (%/DAY)	1.635a	2.90a	3.40a	4.17b	4.60b	4.56b
FOOD INTAKE (MG/DAY)	6.000a	8.50a	12.50b	21.50c	30.00c	28.50c
WEIGHT GAIN (MG/DAY)	2.423a	6.29ab	9.70b	16.77c	27.26d	21.05c
FOOD CONVERSION RATIO	2.49a	1.35b	1.29b	1.28b	1.11b	1.35b
PROTEIN EFFICIENCY RATIO	0.92a	1.70a	1.77a	1.78a	2.09a	1.69a
NITROGEN INTAKE (MG/DAY)	0.420a	0.60a	0.88ab	1.51bc	2.10cd	2.00d
CARCASS NITROGEN DEPOSITION (MG/DAY)	0.070a	0.17ab	0.24b	0.42c	0.62d	0.55cd
APPARENT NITROGEN UTILIZATION (%)	16.667a	29.30b	27.63b	27.76b	29.54b	27. 81b
MORTALITY (%)	0	2.5	10	40	32.5	45
CARCASS COMPOSITION (% WET WEIGHT)						
	INITIAL					
MOISTURE	83.01	73.64	72.23	73.10.	73.36	71.64	72.13
CRUDE PROTEIN	12.00	16.07	16.69	15.40	15.53	16.53	16.42
LIPID	1.48	7.37	8.12	8.56	8.11	8.65	8.29
ASH	3.50	2.85.	2.94	2.84	3.00	3.17	3.16.

Figure 3.2.

Specific Growth Rate of C.urophthalmus (%/day) against temperature. Bars show the range of variation.


SGR (%body wt/day)
5
4
3
2
1
25
30
35
40
Temperature (° C)

Were LogeSGR, is the natural logarithm of the specific growth rate, LogeWt. is the natural logarithm of the initial body weight. This model show a highly significative correlation (r=0.88, n=108, P<0.001). Using the above equation predictions on SGR at any initial body weights could be obtained.

The specific growth rates calculated weekly at each temperature, were used to derive six different regression equations shown in Table 3.4. From this data it is clear that the slope of each regression reduces as the temperature increases until the optimun temperature of 33.1°C is reached. Beyond that point, the slope increases again. This linear modelling of growth data is valid, for comparative purposes, within this limited weight range of these experiments.

As the growth was highly correlated with both temperature and initial weight (Figures 3.1, 3.2) a multiple regression model was used to explain the highest proportion of the variance in the independent variable, (SGR) taking into account initial weight and temperature. The relationship is given by:

LogeSGR = -5.18 + 0.632(LogeWt.)+ 0.692(LogeTC.) (Eq.9)

Were LogeSGR, is the natural logarithm of the specific growth rate LogeWT, is the natural logarithm of the

Table 3.4.
Regression equations of Loge Specific Growth Rate (SGR) on Loge initial body weight(W) for each temperature based on the average wet weight of fish measured weekly.

Temperature	Regresion equations	n	r(sq. adj.)	Significance of r.
22.5± 0.02 SE	$SGR = W^{1.36}$ Loge SGR= −6.77+1.36 Loge W	18	.956	P < 0.001
25.7± 0.02 SE	$SGR= W^{0.932}$ Loge SGR= −4.41=0.932 Loge W	19	.960	P < 0.001
27.1± 0.01 SE	$SGR= W^{0.910}$ Loge SGR= −3.91=0.910 Loge W	18	.961	P < 0.001
29.7± 0.012 SE	$SGR= W^{0.598}$ Loge SGR= −2.53+0.598 Loge W	18	.914	P < 0.001
33.1 ±0.09 SE	$SGR= W^{0.488}$ Loge SGR= −1.91+0.488 Loge W	18	.979	P < 0.001
36.3 ±0.02 SE	$SGR= W^{0.511}$ Loge SGR= −2.02+0.511 Loge W	18	.949	P < 0.001

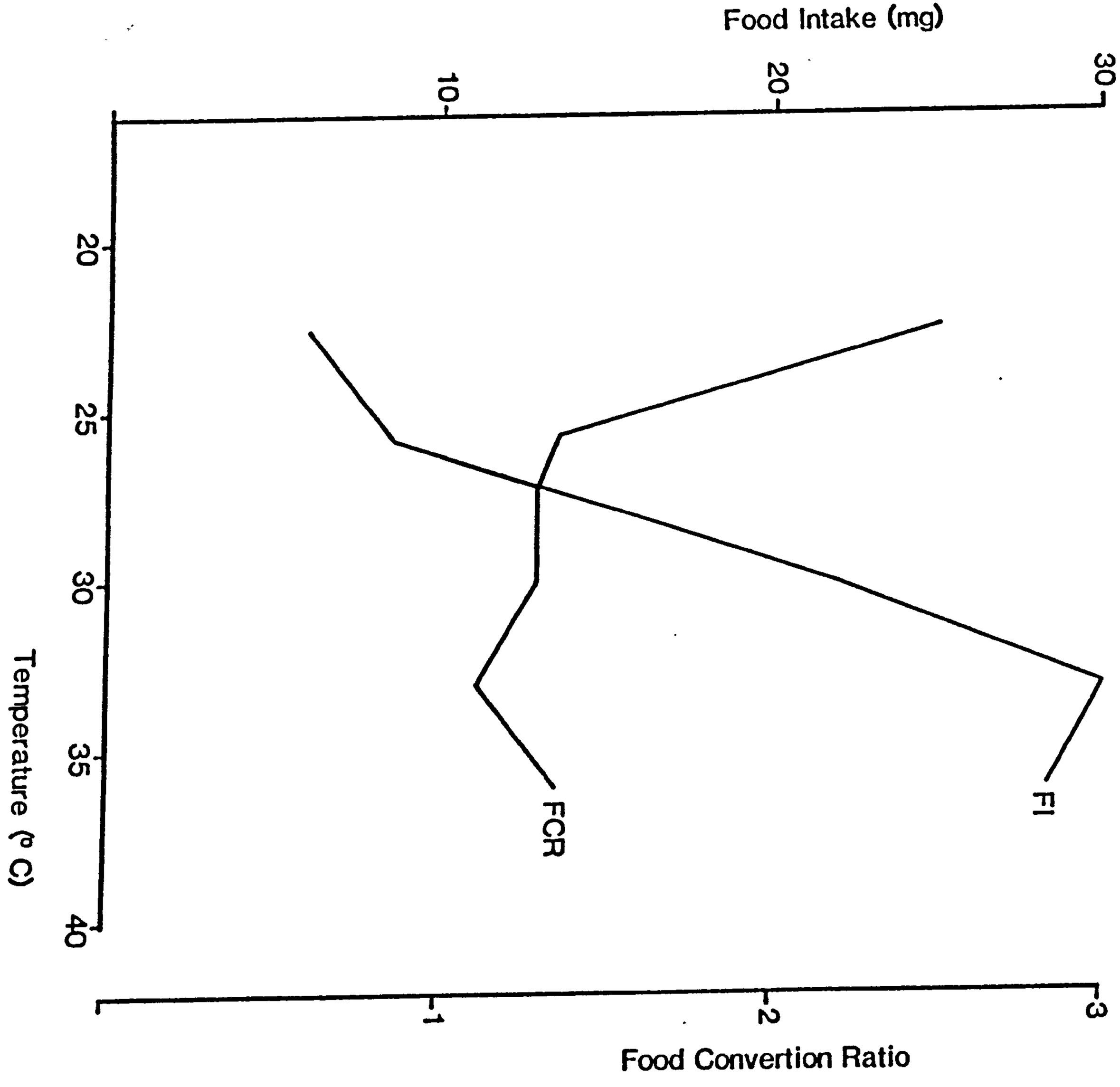
initial mean weight of each replicate taken weekly during the nine weeks and the LogeTC, is the natural logarithm of temperature. This model showed a highly significant correlation ($r=0.903$, $n=108$, $P<0.001$). Giving the food ad libitum and with similar food formulation in terms of energy and protein used as in this experiment, the above equation could be used for prediction of SGR at any temperature, giving the initial body weight.

Food conversion efficiency.

The food intake and mean final food conversion ratio (FCR) of the fish at the different experimental temperatures is shown in Table 3.3 and graphically in Figure 3.3. Within the range of experimental temperatures FCR decreased from a value of 2.49 at the lowest temperature (22.5°C) to 1.11 at 33.1°C at the end of the experiment. Only the FCR at 22.5°C is significantly different ($P<0.01$) from the rest of the treatments (25.7, 27.1, 29.7, 33.1, 36.3°C). Food intake shows a dramatic increase with increasing temperature. The data can be distinguished into three significantly different ($P<0.01$) groups with the lowest food intake at 22.5 and 25.7°C, the second level at 27.1°C and the higher intake at the higher temperatures (29.7, 33.1 and 36.3°C). Figure 3.3 shows the relationship between FCR, food intake and temperature, and it can be seen that maximun food intake and minimun FCR is reached at

Figure 3.3.

The relationship between environmental temperature and food conversion ratio FCR)(g Food fed/g Wt. Gain) and food intake in grams (FI) for Cichlasoma urophthalmus fed ad libitum. Each point represents combined data from duplicated tanks, each containing twenty fish.


Food Intake (mg)
10
20
30
20
25
30
35
40
Temperature (° C)
FCR
FI
1
2
3
Food Convertion Ratio

33.1'C, showing a clear optimum. It is clear that the growth is directly related to the food intake, and a linear model describing the relationship between the maximum daily rate of food consumption (FI) and body weight (Wt) was:

$$\text{LogeFI} = 0.023 + 0.815(\text{LogeWt.}) \qquad \text{(Eq.10)}$$

Where LogeFI, is the natural logarithm of the average daily food intake and LogeWT is the natural logarithm of the initial mean weight of the fish. This model assumes that loge food intake is linearly related to logeI body weight and is quite significant ($r=0.8$, $n=108$, $P<0.001$).

As food intake is influenced directly by temperature, a more comprehensive model of food intake can be derived using multiple regression based on logeFI, logeWt and the natural logarithm of the experimental temperature (LogeTC), resulting in the following equation:

$$\text{LogeFI} = -5.60 + 0.626(\text{LogeWt.}) + 1.99(\text{LogeTC.}) \qquad \text{(Eq.11)}$$

This model is highly significant ($r = 0.945$, $n = 108$, $P < 0.001$). Thus, for Cichlasoma urophthalmus, this model describes 94.5% of the variance in the dependent

variable.

Protein efficiency ratio

The efficiency with which fish were able to convert dietary protein into new fish tissue (Protein efficiency ratio, PER) is shown in Table 3.3. Two significantly different groups ($P < 0.01$) can be distinguished with the lowest value obtained at 22.5°C while the data between 25.7 to 36.3°C are not significantly different from each other ($P < 0.01$), but are different from that at 22.5°C.

Apparent nitrogen utilization.

The values of apparent nitrogen utilization obtained for fish maintained at different temperatures are shown in Table 3.3, and show the same pattern observed in PER.

Carcass composition.

The proximate composition of the whole fish carcass at the start and end of the experiment is shown in the

Table 3.3. With respect to protein, moisture, lipid and ash content there were no significant differences ($P < 0.01$) during the whole experiment.

Fish production and mortality.

Total production of fish (final weight in grams) was plotted against temperature and is shown in Figure 3.4. The survival of the fish during the experimental period is shown in Figure 3.5. The highest value of fish production was obtained at 33.1°C even though there was a high mortality (32.5%, Figure 3.5). A second, reduced, level of production was obtained at 27.1, 29.7 and 36.3°C, where the slightly reduced growth rate at these temperatures was compensated by differences in survival rate. The lowest gross productivity was obtained at 22.5°C temperature even though survival was 100%.

Behaviour.

Those fish in the trials at lower temperature (22.5, 25.7 and 27.1°C) always showed a normal social behaviour, with no aggression throughout the experimental period and with a very low mortality (0.0%, 2.5% and 10% respectively) as shown in Figure 3.5., Table 3.3.

Figure 3.4.

Net production of <u>Cichlasoma urophthalmus</u> maintained at six different temperatures at the end of 9 weeks of experimental time.

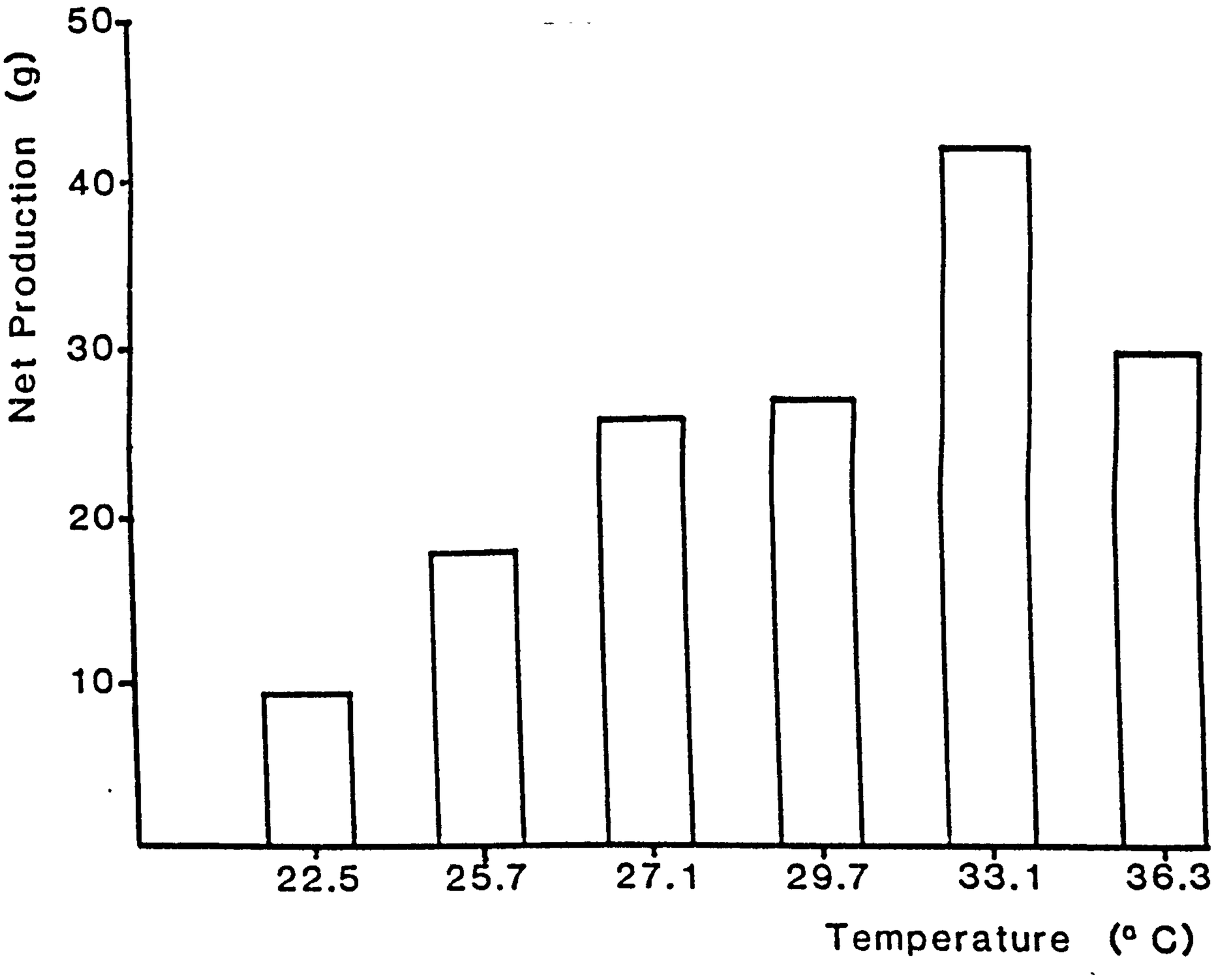
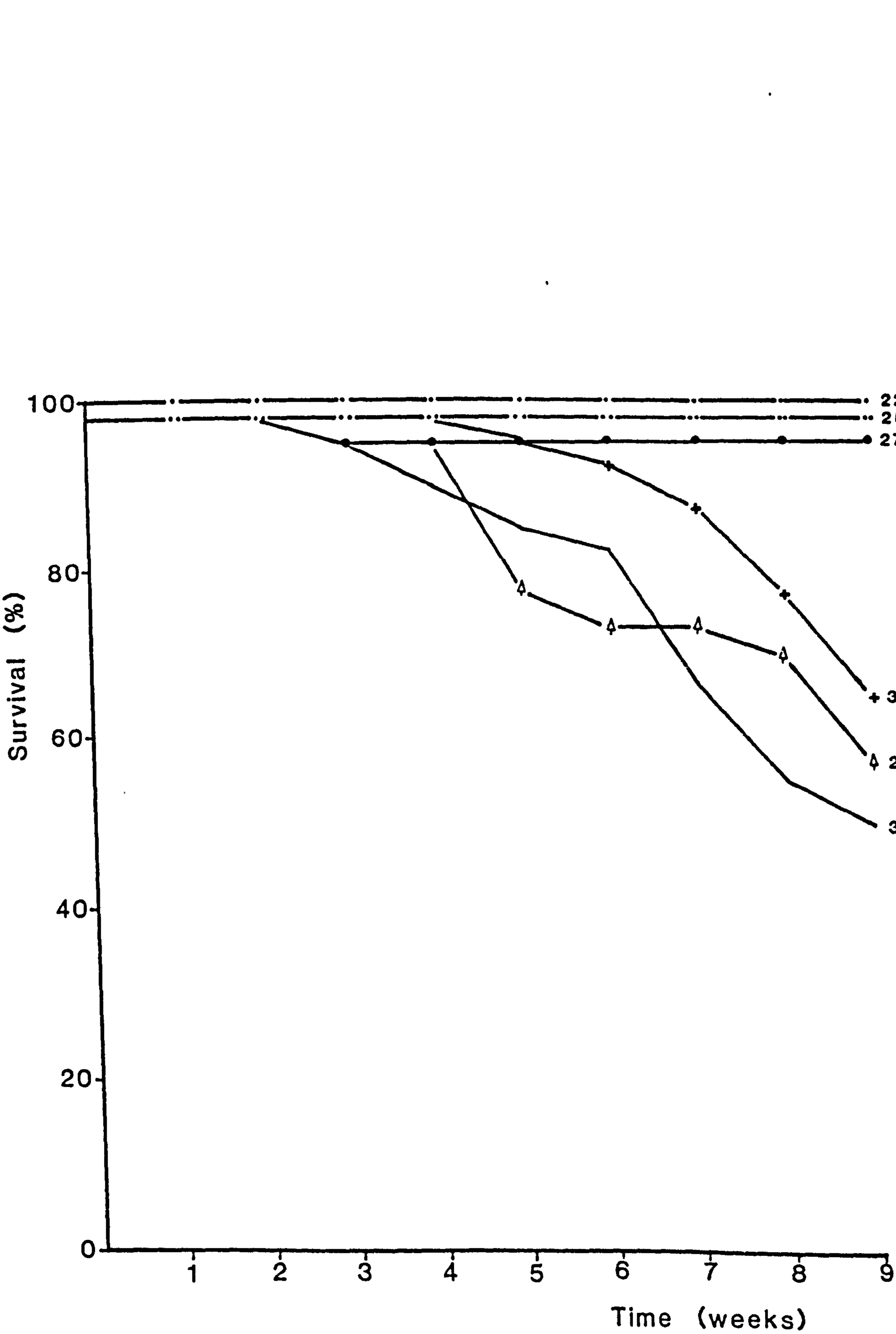

Net Production (g)
50
40
30
20
10
22.5
25.7
27.1
29.7
33.1
36.3
Temperature (° C)

Figure 3.5

% Survival of Cichlasoma urophthalmus during 9 weeks at different temperatures experiment.


100
80
60
40
20
0
Survival (%)
22.5° C
25.7° C
27.1° C
33.1° C
29.7° C
36.3° C
1
2
3
4
5
6
7
8
9
Time (weeks)

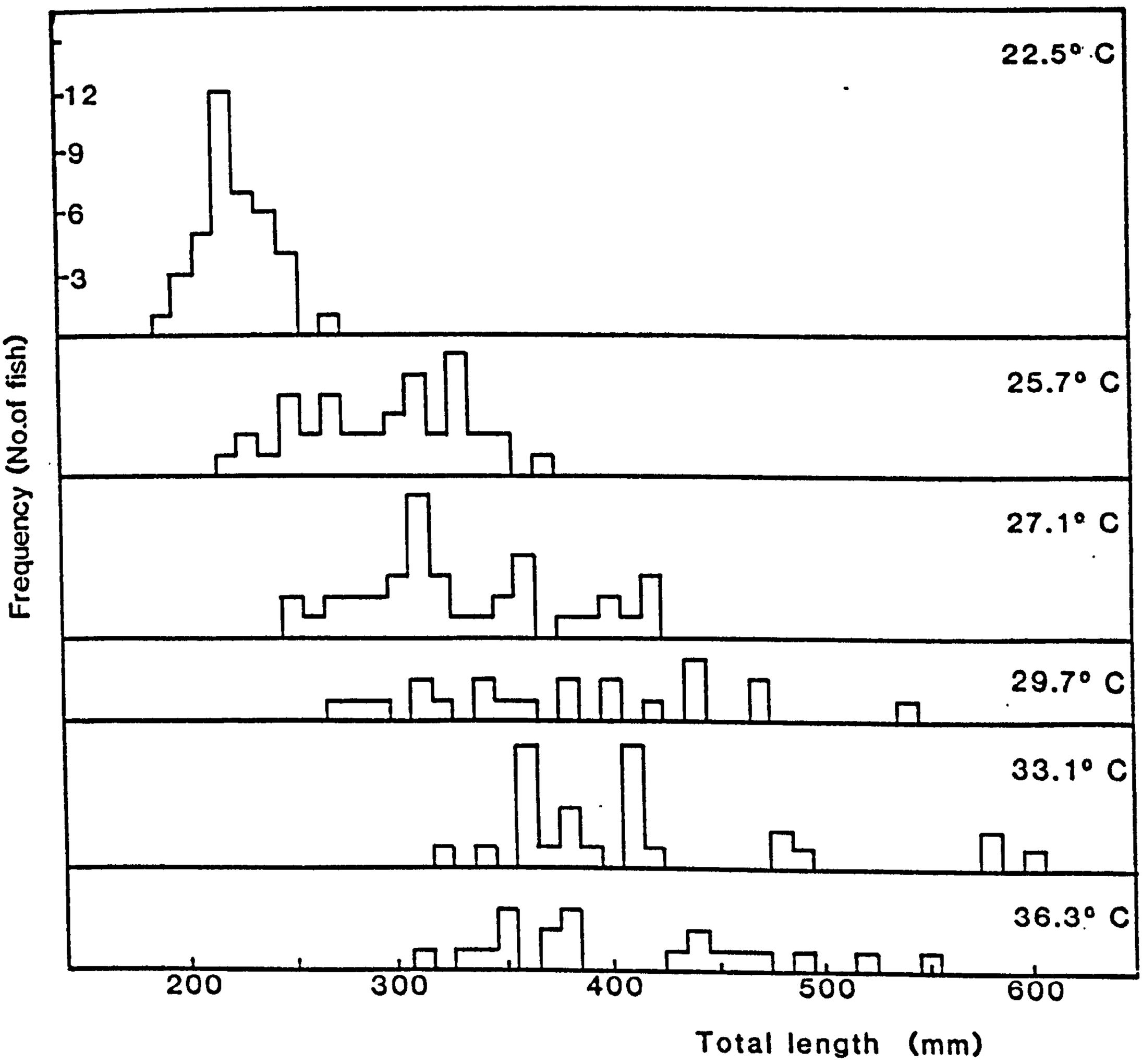
At the higher temperatures (29.7, 33.1 and 33.6°C) it was notable that the bigger fish were very nervous swimming rapidly around the experimental tanks and chasing the smaller ones even when fed. There was a great deal on biting and fighting, particularly triggered by the addition of food. Some of the smaller fish, and less frequently the bigger ones, were found with strong bite wounds on their bodies. The large dominant fish prevent the smaller ones from eating adequately and this situation was often the main cause of their death. Further analysis of the length-frequency distribution of all fish at the end of the experiment showed a much narrower spread at the three low temperatures, when compared with the wide dispersion at the three highest temperatures (29.7, 33.1 and 36.3°C respectively); Figure 3.6. It thus seems probable that the high mortality at these three temperatures (40.0%, 32.5% and 45% respectively, Figure 3.5) was caused mainly by the greater spread in size distribution, and its consequences as the fish grew.

Figura 3.6.

Size-frequency distribution of

Cichlasoma urophthalmus after 9 weeks

at 6 different temperatures.


22.5° C
12
9
6
3
25.7° C
27.1° C
29.7° C
33.1° C
36.3° C
Frequency (No.of fish)
200
300
400
500
600
Total length (mm)

DISCUSSION

In this experiment small fish were utilized in order to demonstrate the effect of temperature on growth as clearly as possible, without the interference of growth reduction with age (Needham, 1964; Brett et al., 1969; Love, 1980).

It can be seen that food intake and specific growth rate in Cichlasoma urophthalmus increase, while food conversion ratio decreases when temperature increases, rising to an apparent optimum. Similar results were observed by Brett et al., (1969) and Brett (1979), Brett and Shelburne (1975), Brett and Higgs (1970) for Onchorhynchus nerka; Elliot, (1975b and 1979) for Salmo trutta; Andrews and Stickney (1972) for Ictalurus punctatus; Wurtsbaugh and Davis (1977), for Salmo gairdneri; William and Cadwell (1978) for Parophrys vetulus; Allen and Wootton (1982) for Gasterosteus aculeatus; Jobling, (1983) for Salvelinus alpinus. These authors suggest that, at temperatures less than the optimum acceptance of food is reduced even when presented to the fish ad libitum. At temperatures above the optimum, fish drastically reduced their growth because, despite the presence of excess of food the animals did not eat when the temperature is near lethal.

Thus the maximum growth was observed in the present experiment at a temperature of 33.1°C. Similar temperature optima have been noted in other members of the cichlidae, for example, O.mossambicus has an optimum temperature for growth at 30'C, and T.zilli has an optimum feeding temperature between 28.8°C to 31.4°C (Stickney, 1986).

Below the optimum temperature the growth rate of Cichlasoma urophthalmus of known weight can be predicted using equation 8. A better prediction of specific growth rate, based on temperature and initial body weight can be made using equation 9 and this equation is probably more useful for aquaculture purposes. It should be noted that these linear equations are reliable within the narrow weight ranges examined in these experiments and where only a small section of the Von Bertallanffy growth curve is being considered.

Many authors have demonstrated that small fish consume more food in proportion to their body weight than larger fish (Brett, 1971; Elliot, 1975a; Wootton et al., 1980) and this is confirmed by the fact that the slope (b) in equation 10 is usually less than one (Allen and Wootton, 1982). The value of b for Cichlasoma urophthalmus is 0.815 and is similar to other exponents observed in other fish, such as rainbow trout (0.84) and brown trout (0.76) (Elliot, 1975 a,c and Sperber et al., 1977).

Equation 10 can be used for food intake predictions for C urophthalmus when the initial weight is known.

A multiple regression model was proposed by Wootton et al., (1980) as means of describing the food intake when the initial weight and the temperature is known. In the present work this model gave a good description of that relationship, and equation 11 can be used for C. urophthalmus for food intake predictions when temperature and initial weight are known. Thus it seems that this model is not restricted to temperate-water fish and that it is appropriate for use with tropical fish.

A low food conversion ratio of 2.49, was shown in Cichlasoma urophthalmus when the temperature was 22.5°C, and this was significantly different ($P<0.01$) from the FCR's measured at other experimental temperatures. As discussed by Goolish and Adelman (1984) it is still not totally clear why reduced food intake occurs at lower temperatures. However there appears to be a limitation on some metabolic processes, established for each individual species; for instance, it is possible to see a reduction of digestion in rainbow trout when maintained at low temperatures (Brett and Higgs, 1970). These observations permit the conclusion that 22.5°C is a limiting temperature for C.urophthalmus. Despite its

high survival at this temperature the growth is so slow and the conversion ratios are so high that production costs would be very high should commercial aquaculture of these species be carried out at this temperature.

Many authors have published analyses of body composition of fish (Love, 1980), but there have been few investigations of the changes in body composition in relation to body weight, temperature and ration size (Brett et al., 1969; Andrews and Stickney, 1972; Weatherley, 1976; Elliot, 1976a; Caulton and Bursell, 1977; Reinitz, 1980; Wandsvik and Jobling, 1982a; Gill and Weatherley, 1984). There also appears to have been no work carried out on the effects of temperature on protein and nitrogen efficiencies. The present work has shown that the protein efficiency ratio (PER) was affected by an increase in temperature. At 22.5°C the lowest PER was observed and this was highly significantly different from PER at the other temperatures ($P<0.01$). Above 22.5°C slight increases in PER were noted but these were not significantly different. A similar trent was noted in the case of nitrogen utilization. In terms of nitrogen carcass deposition, the incorporation of protein into body tissue is more efficient at higher temperatures, with an optimum in this work again at 33.1°C.

Most studies to date show that in a healthy fish total protein as a percentage of body weight tends to be relatively constant for a given species (Wandsvik and Jobling, 1982a; Elliot, 1976b). Other authors have found that at low temperatures the fish tends to have a slightly higher protein content than those at or near their optimum temperature (Gill and Weatherley, 1984). In the case of C.urophthalmus the carcass crude protein slightly increases with temperature, until the optimum temperature (33.1) but this trend was not significant ($P<0.01$), and may be assumed to be generally constant as found by Elliot (1976b), for Salmo trutta. Similarly there was no significant difference between the optimal and the sub-optimal groups in respect of moisture, lipid and ash.

In the present experiment aggressive behaviour, mortality and net fish production could be correlated with high temperatures (29.7, 33.1 and 36.3°C). This aggressive behaviour has also been observed in other species when maintained at their optimum temperature as for example Pharophrys vetulus the English sole (William and Caldwell, 1978), Coregonus artedii (McCormick et al., 1971), Salvelinus alpinus (Jobling, 1983) and Tilapia zilli (Saclauso, 1985).

High mortalities caused by the aggressive behaviour of fast-growing individuals at high temperatures, have been noted in T. zilli, (Saclauso, 1985), and Lepomis gibbosus, (Power and Todd, 1976). A similar differential growth and behaviour pattern was clear in C.urophthalmus in the present study and it seems that in these three fish (T.zilli, L.gibbosus, and C.urophthalmus) this reported behaviour is quite similar. This is probably due to the fact that they have a strong social territorial behaviour linked to defence of substrate areas for nest-building and bottom-spawning. In contrast to the findings of Saclauso (1985), however, the present study shows that, even where mortality is high and the final fish size distribution is great, the total production observed was always higher at higher temperature.

Overall it is possible to conclude that a temperature near 33°C is the optimum for growth of C.urophthalmus. High mortalities are associated with the aggressive behaviour displayed at high temperatures due to a wider size distribution and concommitant social behaviour. Temperatures around 28°C will give a lower mortality and a smaller size distribution and are more appropiate for long-period experimentation or culture in which these effects will not cause extra mortality. Even lower temperatures, such as 22.5°C result in slow growth

but with very low attendant mortality and could be useful in terms of hatchery management where fry can be held ready for the market. In intensive aquaculture of this species especially when cultured at high temperature, an adequate management procedure must be adopted to avoid the mortalities caused by dominant fish. Where a reduced level of stock management is required the fish can be maintained at temperatures around 27°C, with slower growth but with lower food intakes and good food conversion ration.

THE EFFECTS OF SALINITY ON THE SURVIVAL AND GROWTH OF *Cichlasoma urophthalmus*

INTRODUCTION.

Salinity, in a similar way to temperature, has a large influence on the survival and distribution of many fish (Holliday, 1969) and is of great significance in understanding fish distributions and their impact on ecosystems (Chervinsky, 1984a,b). This important factor in the aquatic environment, has forced fish to develop different osmoregulatory adaptations. Thus in the aquatic environment changes in salinity can be a major barrier to those fish which are adapted to tolerate a small salinity range (stenohalines), whereas a much wider ecological range is available to those that can tolerate wide salinity variations (euryhalines).

In recent years many studies of the effects of change in salinity have concentrated on the physiological processes of osmoregulation in fish (Kessel and Beams, 1962; Potts et al., 1967; Farmer and Beamish, 1970; Holliday, 1969; Potts et al., 1973; Depeche and Schoffeniels, 1975; Smith and Thorpe, 1977; Fishelson, 1980; Love, 1980; Leray et al., 1981; Suresh and Jayaranam, 1983; Suresh et al., 1983; Chervinski, 1983; Potts, 1984; Febry and Lutz, 1987). By contrast, much work has been conducted on a wide range of species with importance for aquaculture, in an

effort to fully understand their tolerances and to explore the possibility of culturing fresh water species in brackish water as, for example, Ictalurus punctatus, I.furcatus and I.catus (Guthrie and Avault, 1968 and Allen and Avault, 1969); Tilapia hybrids (Fishelson and Loya, 1969); Cyprinus carpio (Al-Hamed, 1971); Oncorhynchus kisutch, (Otto, 1971); Tilapia zillii, (Chervinski and Hering, 1973); Clarias lazera (Clay, 1977; Chervinski, 1984a,b); Colossoma macropomum, (Pereira and Lubin, 1980) and Oreochromis spilurus and O.mossambicus, (Payne, 1987). Attempts have also been made to adapt freshwater species to marine environments, for example Oreochromis aureus (Chervinski and Yashouv, 1971; Watanabe et al., 1985); Tilapia zillii (Chervinski and Hering, 1973); O.niloticus (Al-Asgah, 1984; Alava et al., 1987); O.mossambicus, (Kader et al., 1981 and Juers et al., 1984). Finally some marine species have been adapted to brackish waters and even fresh water, including Mugil cephalus and M.capito (Mires, 1970); Anguilla japonica (Usui, 1974); Sparus aurata and Dicentrarchus labrax (Chervinski, 1979) and Liza abu (Ahmad et al., 1983).

In many cases these investigations have been prompted by the lack of suitable environments for culture of a particular species. Good examples of this are rainbow trout and brown trout (Salmo gairdneri and S.trutta respectively.) which are cultured traditionally in fresh

water. After demonstrating their tolerance of, and good growth in, brackish and marine waters, it is now possible to obtain good commercial production of these species in these environments, particularly with rainbow trout which have a high market demand (Bardach., et al 1972; Stevenson, 1980).

Adaptations of other fish such as carps, to low salinities, permit the alternative of culturing these stenohaline species at salinities up to a maximum of 17‰ as is neccessary in south Iraq (Al-Hamed,1971). The milk fish (Chanos chanos) have similarly been introduced into, and cultured successfully in fresh, brackish and marine environments (Qainitio and Juario, 1980).

Cichlids, in general, have been widely reported as being euryhaline species, often living in brackish water areas. For example Tilapia melanopleura is found in brackish water lagoons in New Guinea (Chimits, 1955); O.mossambicus reproduces at high salinities up to 49‰, in seawater ponds (Popper and Lichatowich, 1975); and O.niloticus grows and reproduces well in brackish waters with no signifficant differences observed at 0, 7.5 and 17.5‰, although, survival of the fry was reduced at the higher salinity (Chervinski, 1961a). Tilapia hybrids have been succesfully cultured in brackish water and this has proved useful in terms of aquaculture in

Israel (Fishelson and Loya, 1969; Pruginin and Fishelson, 1987). Sarotherodon galilaeus showed no differences in growth at salinities from 0 to 15.5‰ (Chervinski, 1961b) and O.aureus has shown a good growth in sea water ponds (Chervinski and Yashou, 1971). Tilapia zillii and O.aureus have also been cultured in sea water and the greater ability of T.zillii to acclimate and grow in sea water than O.aureus was shown by Chervinski and Zorn, (1974). Hybrids of O.hornorum x O.mossambicus cultured in high salinities show less variation in growth and reduction of territorial aggression, and growth was better in brackish and sea water than in fresh water (Watanabe et al., 1987). Payne, (1987) showed that O.spilurus has a similar growth rate from 0 to 20‰, but at 24 and 28‰ growth depression occurred. In the same study, the growth of O.mossambicus was not limited between 6 and 14‰ and increased temperature increase the growth in the high salinities. On the other hand red tilapia reduced their growth when the salinity was raised to 16‰. He concluded that the threshold for growth inhibition in the estuarine O.spilurus is considerably higher than that shown from early work on the freshwater O.niloticus x O.aureus hybrids.

Many authors have shown that euryhaline species have better growth in an isotonic environment. Salmo

gairdneri improved its growth at 10‰, (Zeitoun et al, 1973) and O.mossambicus at 17.5‰, (Canagaratnam, 1966 and Job, 1969). By contrast, Ictalurus punctatus showed similar growth at 11-12‰ to that in fresh water, (Allen and Avault, 1969). The physiological explanation for this better growth response of the fish in these different environments is that in an isotonic environment much less energy is expended in maintaining ionic and osmotic homeostasis and this energy saving can be diverted into other important metabolic functions, such as growth (Farmer and Beamish, 1969; Holliday, 1969; Love, 1980).

In terms of aquaculture, euryhaline fish have greater potential than stenohaline species as they can be adapted to, and introduced to, a wide range of culture environments. Coastal and brackish water lagoons are characteristic ecosystems on both the Pacific and Atlantic Coasts of Mexico, and these areas are one of the most important natural resources available for aquaculture in this country. It is thus important and necessary to identify the specific salinity tolerances for any species which is to be cultured in these environments because of the dynamic nature of salinity changes often found in these areas.

Based on all these considerations, two experiments were designed, firstly to assess the effect of rapid change from freshwater to salinities of 5, 10, 20, 30 and 40‰ on the survival of fry C.urophthalmus. A second, longer term experiment was designed to observe the effects of salinities of 5, 10, 20, 30 and 35‰ in the growth of fry C.urophthalmus.

MATERIALS AND METHODS.

A.-Tolerance of an abrupt change in salinity.

Experimental animals.

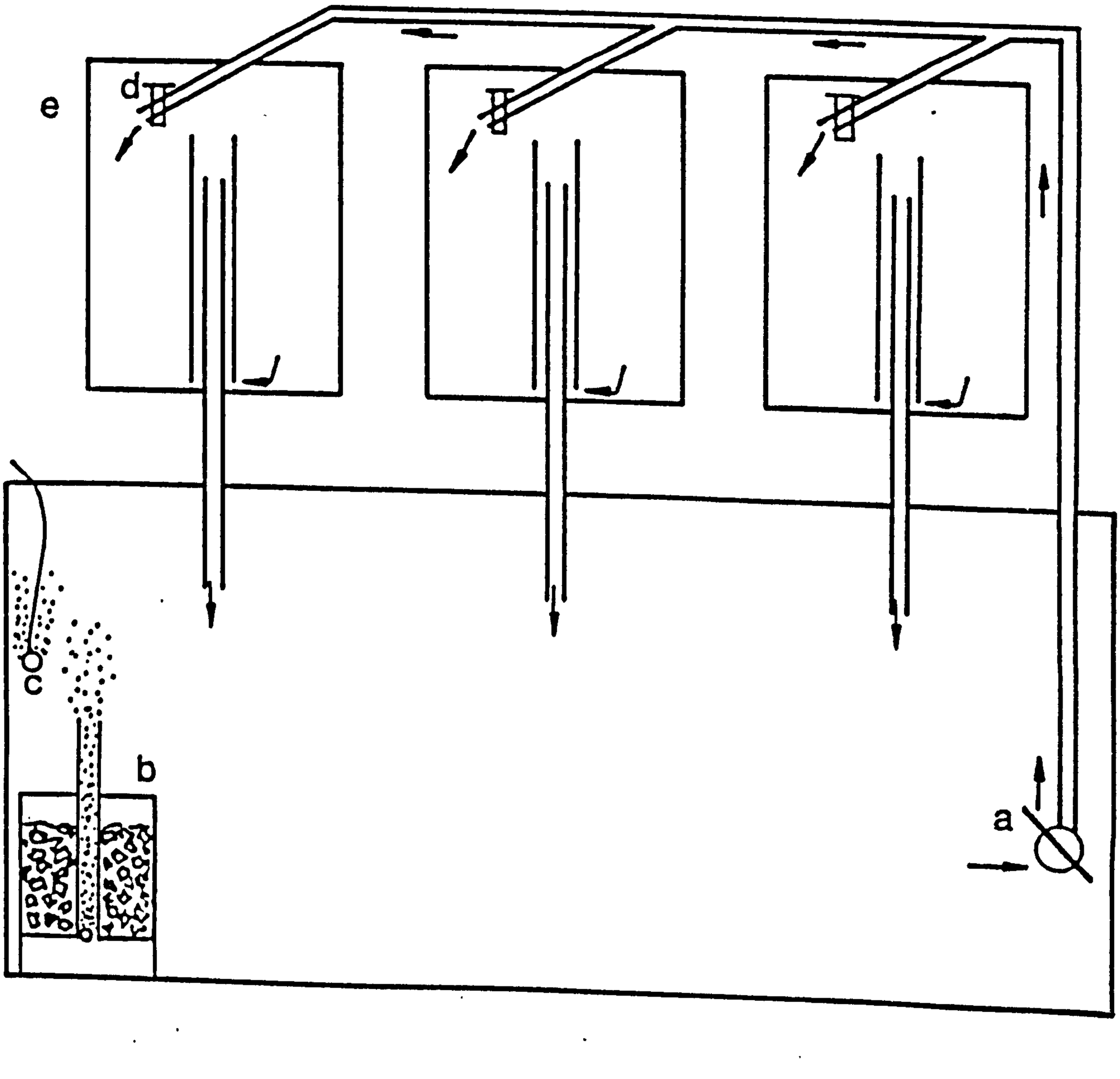
C.urophthalmus fry with a weight range of between 450.85 to 475.2mg were reared in the CINVESTAV laboratory from wild brood stock, as previously described.

Experimental system.

Six independent recirculation systems, each having three polypropylene tanks of 20l capacity where constructed (Figure 3.7). The experimental tanks had independent water inflows of 1.5 l/min regulated by a

Figure 3.7.

Diagram showing one of the experimental systems used to maintain the fish at six different salinities. a)water pump. b)bottom filter. c)air-stone. d)flow valve. e)experimental tanks. The arrows show the direction of the water flow.


e
d
c
b
a

rubber tap (Martinez et al., 1986) and were equipped with a bottom drain with central stand-pipe and self-cleaning collar protected by plastic nets to avoid fish losses. The water in each system was pumped by a submersible water pump (Little Giant, model 8A) from a 100l tank placed below the three experimental tanks. This tank received the direct drain from the 20 l experimental tanks and it was also equipped with two gravel aquarium filters and two air stones to maintain the oxygen levels near saturation.

Marine water (34‰) was obtained from the sea in the area near Progreso, Yucatan, transported to the laboratory as required and stored in plastic and fibre glass containers. This water was aereated and filtered prior to use. Fresh water was obtained from the city supply and was aereated for at least five days before use to avoid any problem with chlorine. Low salinities were obtained by diluting seawater with freshwater. High salinities (35-40‰) were obtained by heating and aereating seawater to increase the salinity to 60‰. This concentrated stock was then diluted with sea water to produce the required salinities. The six recirculating systems were operated at salinities of 0, 5, 10, 20, 30 and 40‰.

Forty percent of the water in each system, was

replaced every 2 days, siphoning from the sump tank and taking care that temperature and salinity were maintained. Salinity was measured with an induction salinometer (Kalsico), and daily increments were corroborated with a manual refractometer. Temperature was recorded daily with a mercury thermometer, and dissolved oxygen was measured with an oxygen meter (Y.S.I. Model 57). The experimental system were maintained in a controlled temperature room at 28 °C+0.5 and artificial illumination without interruption during the first 24 hours; later a photoperiod of 12 hours light and 12 hours dark was maintained until the end of the experiment.

Experimental diet.

The experimental diet for both experiments was formulated, containing brown fish meal as a source of protein. The composition and proximate analysis is shown in Table 3.5. and the diet was prepared and chemically analysed following tha methods described earlier. The experimental diet was stored in air-tight containers at -15°C, until required.

Table 3.5.

Formulation and proximate analysis of the experimental diet in the salinity trials.

INGREDIENTS (%)	
Brown fish meal	54.68
Corn starch	21.11
Dextrin	10.56
Cod Liver oil	2.15
Corn oil	5.0
Carboxy methyl Cellulose (Binder)	3.0
Vitamin premix	2.0
Mineral premix	1.0
Indicator (Cr_2O_3)	0.5

NUTRIENT CONTENT (%)	
Moisture	5.20
Crude Protein (N X 6.25)	40.55
Lipid	9.10
Crude fibre	2.36
ASH	12.62

Experimental protocol.

Twenty fry C.urophthalmus were stocked into each of the three replicate tanks in the 6 systems at different salinities. Initial transfer of the fish was by hand net and this was carried out rapidly to ensure a simultaneous start to the experiment. The experimental period was 144 hours, and the mortality was recorded at 2, 4, 6, 9, 12, 18, 24, 48, 72, 96, 120 and 144 hours. Food was offered to the survivors three times a day after the first 24 hours at a rate of 3% body weight. Progressive mortality was recorded and plotted on a probit scale against the logarithm of time to death in minutes (Bliss, 1937). The median resistance time of the fish, measured as the period of time in which 50% of the fish died at each salinity, was obtained from the line fitted by eye to the probit plot and used for comparison between tests. The median lethal salinity (50%) at 144 hours was obtained by plotting the survival in percentage against the experimental salinities in ‰.

B.-Assesment of growth rate at different salinities.

Experimental animals.

C.urophthalmus fry, with initial weights of between 507.33 to 531.67mg were obtained from the same

source as in the previous experiment. The fish from the first experiment that were totally adapted to salinities of 5, 10 and 20‰ were acclimatized to the higher salinities of this experiment by gradual changes of 5‰ every 48 hours. The fish did not experience mortalities during these changes and were allowed to stay at the new salinity for seven days before the growth experiment started. For the lower salinities 5, 10, and 20‰ fish were preadapted in the same way.

Experimental system.

The recirculated water system and procedures were the same as in the first experiment but with three experimental tanks per system. The individual salinity was controled daily with a manual refractometer and induction refractometer. Small daily increments in salinity due to evaporation were corrected by appropriate dilutions with fresh water. Temperature and oxygen was recorded daily, pH was measured weekly from each system with a Pye-Unicam pH meter (Model 9409). Table 3.6 shows the mean values of these environmental parameters in the experimental systems.

Table 3.6.
Environmental parameters of the water and its respective variation along the experimental period.

SALINITY	TEMPERATURE	OXYGEN	PH
0 ‰ ± 0.3	28.2 ± 0.5	6.95 ± 0.5	8.4 ± 0.2
5 ‰ ± 0.5	28.1 ± 0.5	6.93 ± 0.5	8.4 ± 0.2
10 ‰ ± 0.5	28.05 ± 0.5	6.70 ± 0.5	8.5 ± 0.2
20 ‰ ± 0.5	28.3 ± 0.5	6.75 ± 0.5	8.6 ± 0.2
30 ‰ ± 0.5	28.15 ± 0.5	6.6 ± 0.5	8.58 ± 0.2
40 ‰ ± 0.5	28.35 ± 0.5	6.5 ± 0.5	8.7 ± 0.2

Experimental protocol.

The preadapted fry, stocked at 20 fish per tank, three replicas per treatment were fed to satiation, defined as persisten refusal of further food within a time limit of 15 minutes. This feeding regime was offered three times per day for the twelve week of the trial and the food taken was measured daily.

At the start of the experiment 50 fish were killed by an overdose of benzocaine (1:300) and stored at -15°C for subsequent carcass analysis. At weekly intervals, fish were batch weighed to the nearest two decimal points (0.01) on a Mettler top pan balance (Model PE-3600)in a dish containing preweighed water. Fish mortality was recorded daily as required. The rates of growth were calculated at the end of experimental period with the formulae of Ricker, (1975) shown in Appendix 1.

Statistical methods.

Data was analysed by the analysis of variance (Parker, 1980) and mean values were compared using Duncan's multiple range test (Duncan,1955).

RESULTS.

A.-Tolerance of an abrupt changes in salinity.

Cichlasoma urophthalmus juveniles that were subjected to a sudden change from freshwater to 5 and 10‰. had no mortalities, within the whole experimental period (Table 3.7). A sudden change to 20‰, however resulted in 20% mortality during the first 18 hours, but there were no subsequent deaths up to the full 144 hours. Following a sudden change from fresh water to 30‰. there were high mortalities during the first six hours, reaching 96.6% after 18 hours, and exposure to 40‰. resulted in 100% mortality within in the first two hours of the experiment (Table 3.7). After the sudden changes to 20, 30 and 40‰, the fish always showed irritation, dark colouration and convulsive swimming, finally becoming paralysed at the bottom of the tank until death. Only those fish at 20‰ or lower salinities were able to show rapid recovery within a short time. Fish from 0, 5, 10‰ and survivors (80%) at 20‰ salinity, fed actively during the whole experimental period.

Table 3.7.
Mortality of C.urophthalmus in percentage (%) after direct transfer from fresh water to various saline concentrations.

TIME FROM START OF EXPERIMENT	CONCENTRATION OF SEA WATER (‰ ,ppt)					
(HOURS)	0	5	10	20	30	40
2	0	0	0	0	11.7	100
3	0	0	0	5	33.3	
4	0	0	0	5	75.	
6	0	0	0	11.5	90	
9	0	0	0	13.3	93.3	
12	0	0	0	13.3	95.0	
18	0	0	0	20	96.6	
24	0	0	0	20	96.6	
48	0	0	0	20	96.6	
72	0	0	0	20	96.6	
96	0	0	0	20	96.6	
120	0	0	0	20	96.6	
144	0	0	0	20	96.6	

Figure 3.8 shows the cumulative mortality, plotted in probit units, against the time to death in minutes and shows that the median resistance time, or time required to reach 50% mortality was obtained for 40‰ at 55 minutes. However at 20‰ there was only a maximum of 20% mortality within the 144 hrs of the test. When survival was plotted against the experimental salinities at 144 hours, the median lethal salinity (50% survival)was 24‰ as shown in Figure 3. 9.

During field observations, high mortality was found when fish were transferred from 20‰ to freshwater, but no mortality was observed when the transference was made gradually in two or three changes reducing the salinity from 5-7‰ daily down to fresh water.

As reported in Chapter 1, this fish has been found reproducing in salinities between 0-20‰, and this data further demonstrates the fact that this species has the flexibility to reproduce and grow in these salinity ranges.

Figure 3.8.

Log-Probit distribution of time to death for fresh water juveniles of Cichlasoma urophthalmus exposed to salinities from 0‰ to 40‰ at 28°C, distribution lines were fitted by eye.

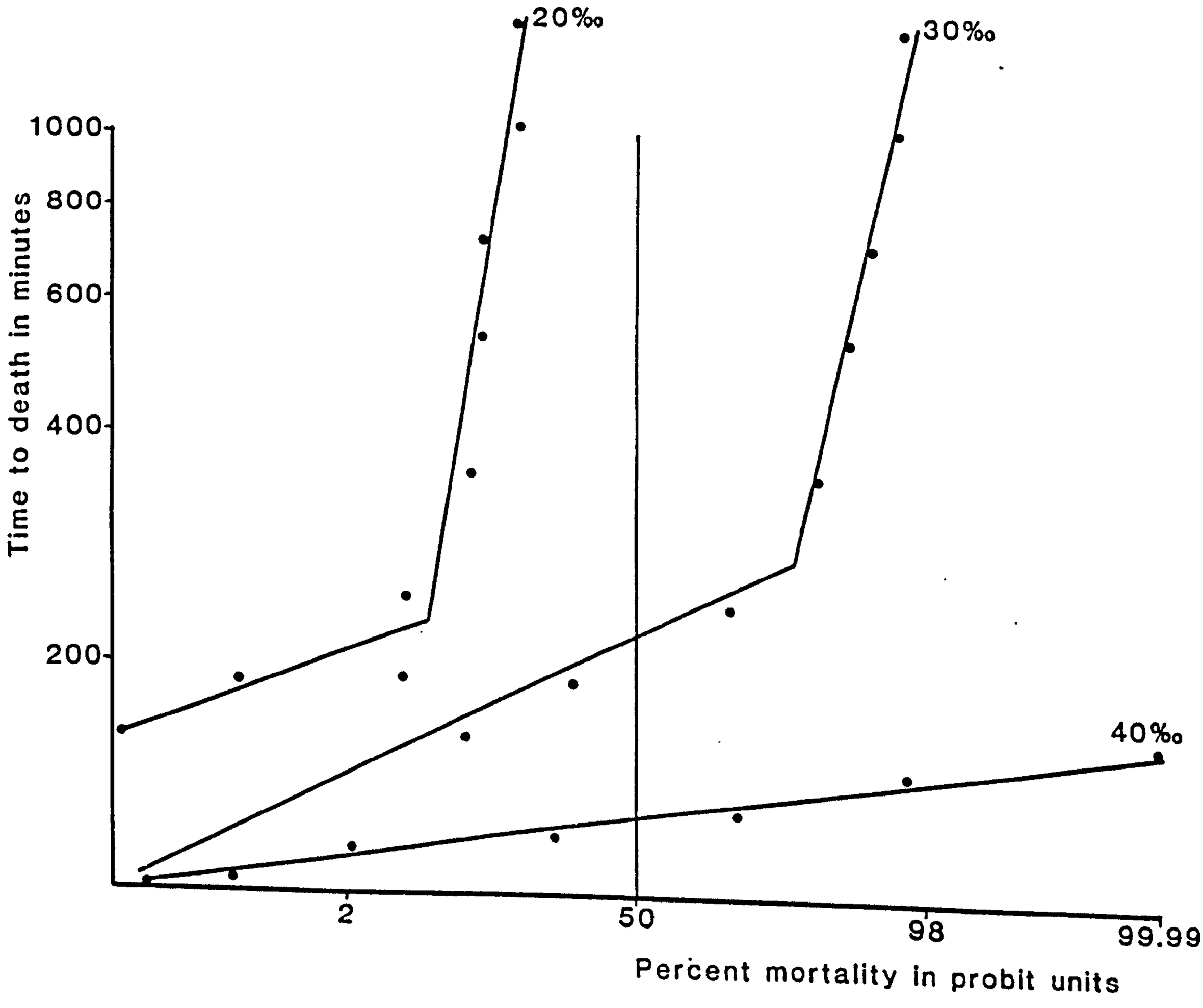
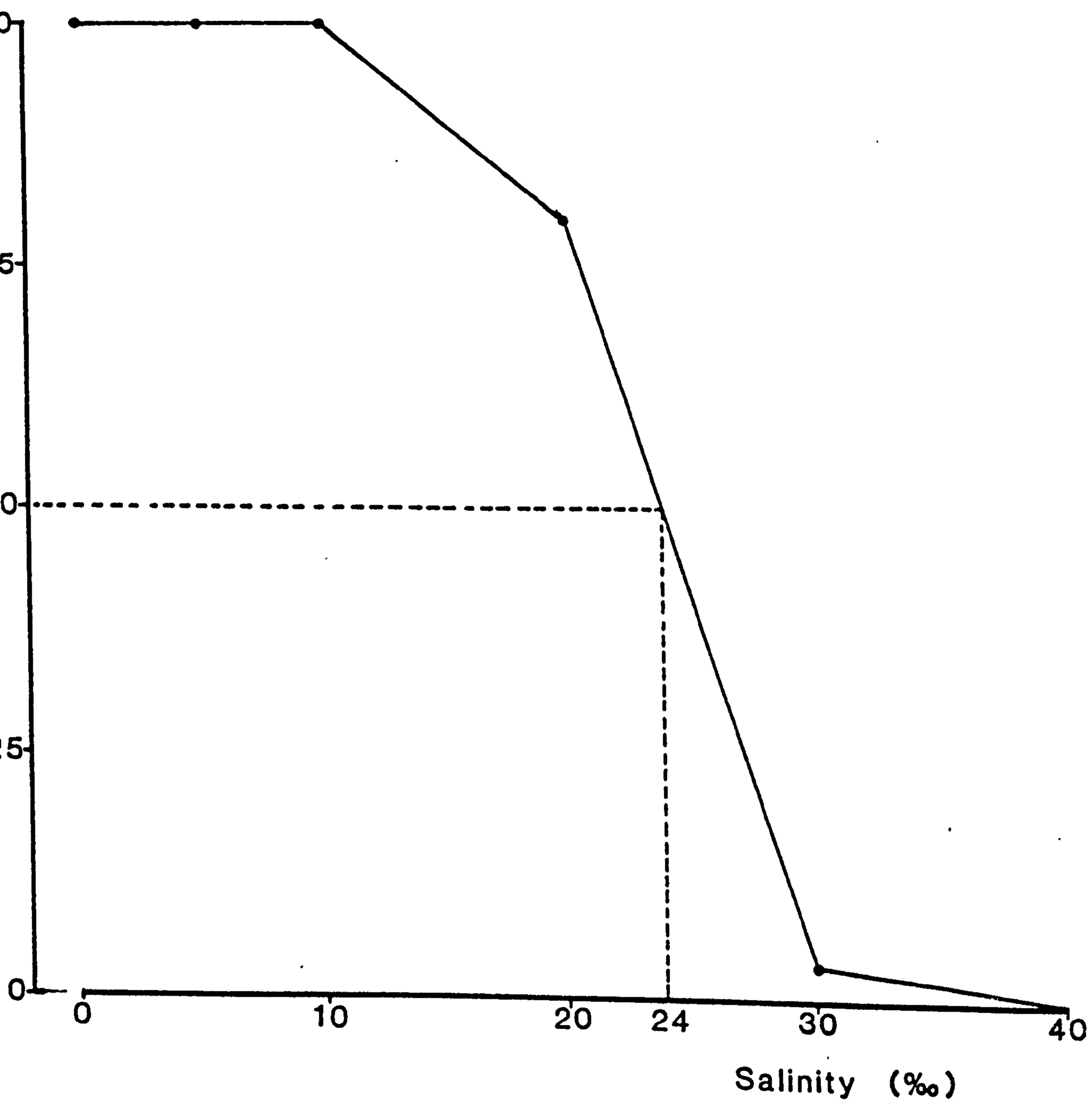

20‰
30‰
40‰
1000
800
600
400
200
Time to death in minutes
2
50
98
99.99
Percent mortality in probit units

Figure 3.9.

Survival pattern of C.urophthalmus fry reared at fresh water and transferred directly to different salinities after 144 hrs.


100
75
50
25
0
Survival (%)
0
10
20
24
30
40
Salinity (‰)

B.-Second experiment: Assessment of growth rate at different salinities.

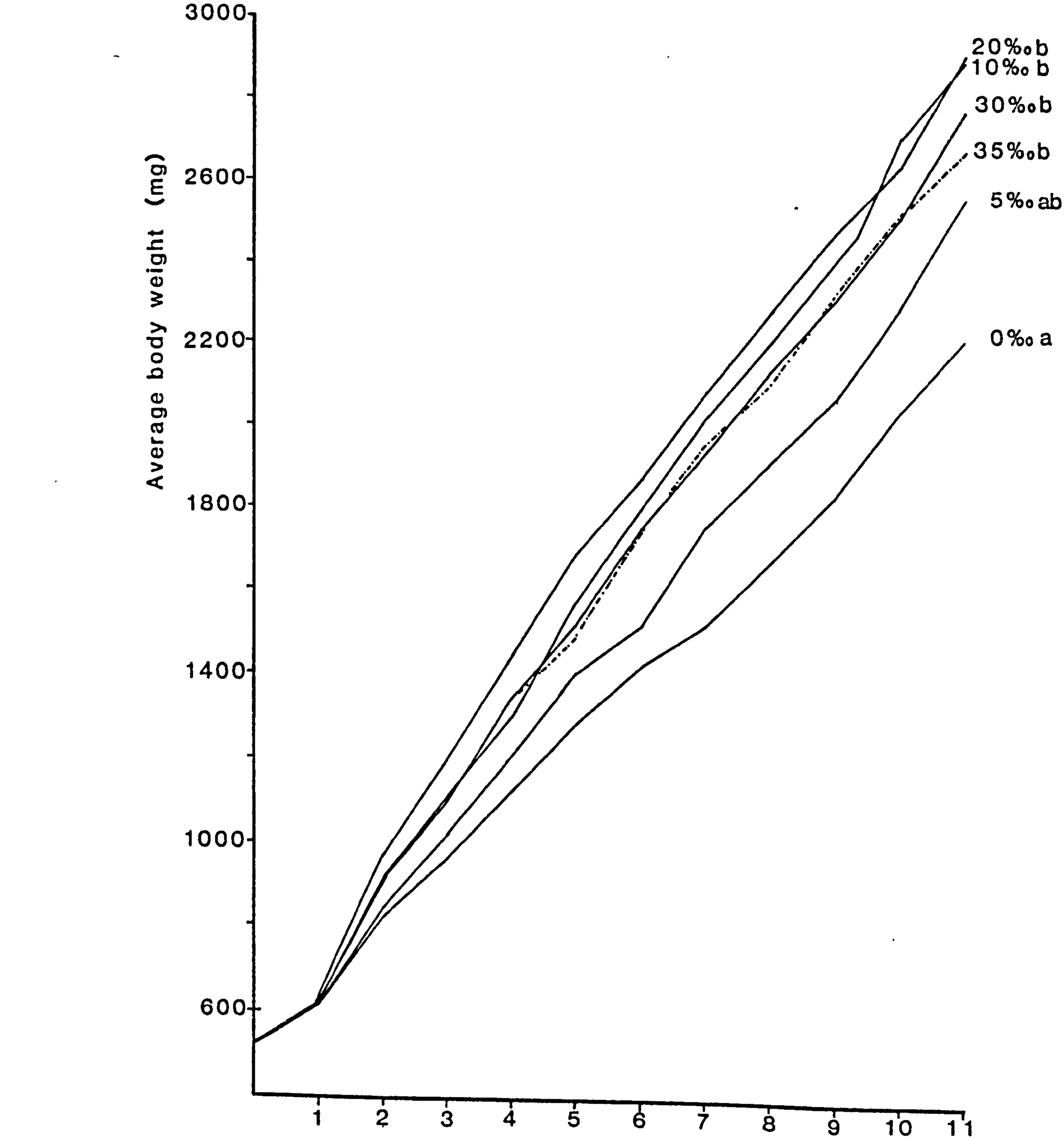
The acceptance response of the fish for the diet in the different salinities was similar with no difference being observed between treatments. The fish ate voraciously during the entire experimental period. The growth response at the different salinities is summarized in Table 3.8 and Figure 3.10. The best growth was observed with the fish at 20‰ but no significant diference ($P<0.01$) was observed between this salinity and 5, 10, 30, and 35‰. A significantly lower ($P<0.01$) final body weight was found between the fish at 0.0‰ and those at all other salinities, with the exception of 5‰. A similar response was observed in terms of weight gain (%) and specific growth rate (%/day). With respect to daily weight gain (mg/day) the highest value was recorded at 20‰ with no significant difference between 30 and 10‰. A significantly lower weight gain was attained at salinities of 5 and 35‰ but the lowest value of all was in freshwater. Comparisons on a dry weight basis may be more valid in this type of experiment because salinity can influence the weight of the fish by reducing or increasing body water content. However, it can be seen in Table 3.8 that no significant difference were found in final body water contents between treatments.

Table 3.8.
Mean growth performance, feed utilization and carcass composition of C.urophthalmus at six different salinities for twelve weeks.

	0 ‰	5‰	10 ‰	20 ‰	30‰	35‰
INITIAL BODY WT. (MG)	519.667 a	507.333 a	516.33 a	513.83 a	518.17a	531.67a
FINAL BODY WT. (MG)	2,228.333 a	2,578.50 ab	2,913.50 b	2,936.67 b	2,792.60b	2,694.77b
WEIGHT GAIN (%)	329.822 a	408.40 b	463.94 b	471.52 b	439.54b	406.75b
SPECIFIC GROWTH RATE (%/DAY)	1.880 a	2.11 b	2.25 b	2.26 b	2.18b	2.12b
FOOD INTAKE (MG/DAY)	508.093 a	539.83 ab	593.12 b	625.84 b	579.42b	584.23b
WEIGHT GAIN (MG/DAY)	155.243 a	197.33 b	214.38 cd	222.09 d	218.81d	196.12bc
FOOD CONVERSION RATIO	3.29 a	2.89 b	2.77 b	2.82 b	2.65b	2.98b

CARCASS COMPOSITION (% WET WEIGHT)

	INITIAL						
MOISTURE	82.66	74.85	74.81	73.93	74.67	73.98	73.93
CRUDE PROTEIN	12.30	15.43	15.43	15.95	15.64	16.19	16.20
LIPID	1.52	4.62	4.63	4.85	4.59	4.79	4.76
ASH	3.56	3.58	3.47	3.57	3.58	3.79	3.68


3000
2600
2200
1800
1400
1000
600
Average body weight (mg)
1
2
3
4
5
6
7
8
9
10
11
Time (weeks)
20‰b
10‰b
30‰b
35‰b
5‰ab
0‰a

The fish were fed to satiation and some of this food was wasted. This wastage is clearly reflected in the generally high food conversion ratios observed in Table 3.8. Despite feeding to satiation, there is a trend in FCR with the optimum between 10 and 30 ‰ again, the poorest FCR was in 0‰.

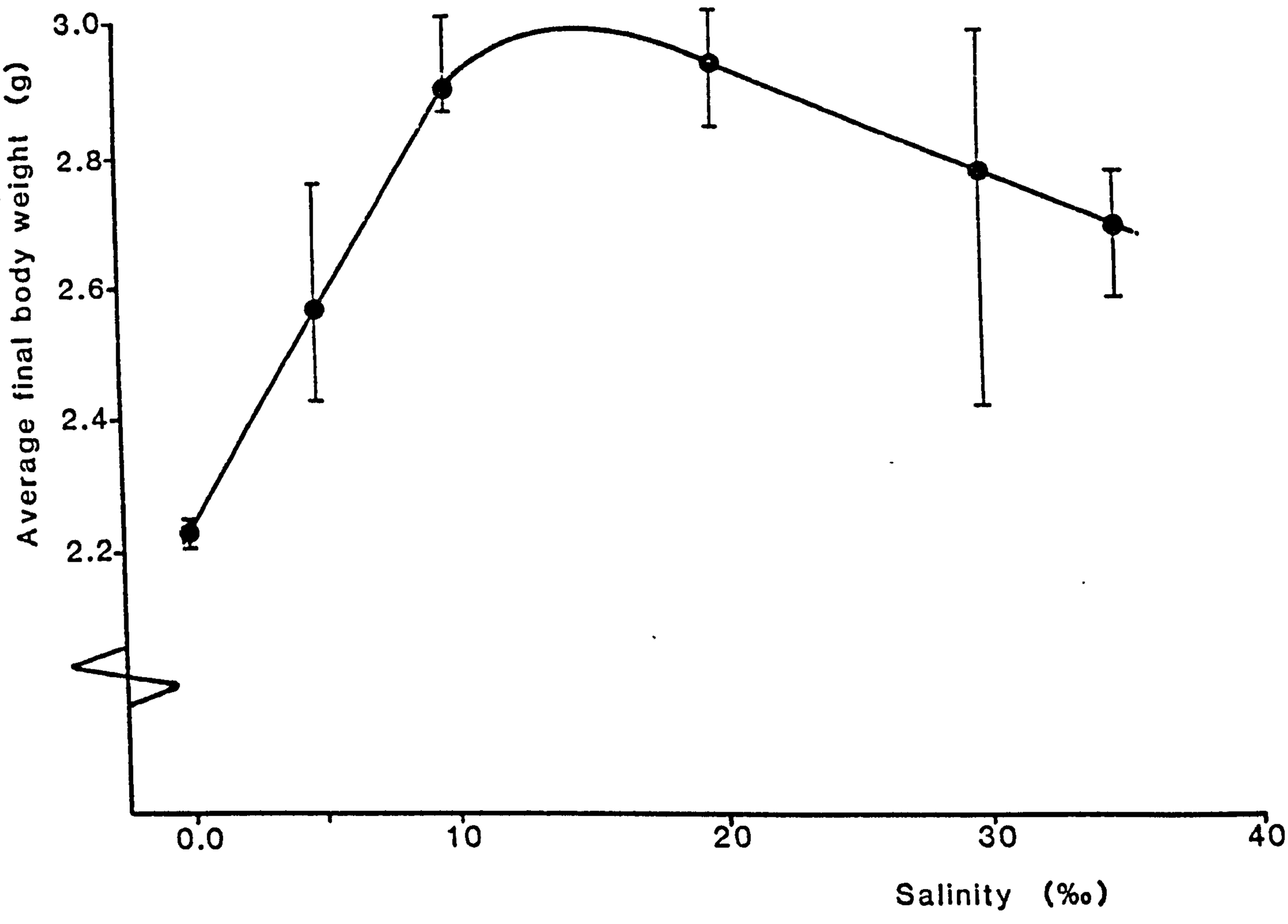
When the average final body weight was plotted against each salinity tested (Figure 3.11), it is clear that the best growth is found between 10 to 20‰ where the medium is isotonic with the fish thus reducing energy losses in osmoregulation.

The proximate analysis of the fish carcass at the start and at the end of the experiments is shown in Table 3.8. It is clear that over the experimental period protein and lipids show an increase in all treatments, with moisture level decreasing. The final carcass composition of the fish in terms of moisture, crude protein, lipids and ash content was not significantly different ($P<0.01$) between treatments.

Few mortalities were registered, and there was no relationship between mortality and salinity treatments. It is clear that the highest salinities tested (20, 30 and 35‰) did not increase the mortality of the fish, when the fish are slowly acclimated .

Figure 3.11.

Average of the final weight at different experimental salinities with their respective variation range. The curve was fitted by eye.


Average final body weight (g)
3.0
2.8
2.6
2.4
2.2
0.0
10
20
30
40
Salinity (‰)

DISCUSSION.

As the coastal lagoons and rivers from the Gulf of Mexico and the Caribbean area are the natural habitat of Cichlasoma urophthalmus (Miller, 1966; Resendez, 1981; Chavez et al., 1983 and Martinez and Ross, 1986), it may be expected that this species would be quite resistant to rapid changes in salinity. It is, neverthless very important to determine the tolerance ranges and optimum salinities of any species for aquaculture purposes. This type of euryhaline tolerance has been described in other estuarine species such as Mugil capito, which can better survive rapid changes in salinity than abrupt changes in temperature (Mires, 1970). Tolerance of abrupt changes in salinity up to 20‰ without mortality, has also been noted in a number of brackish water species, such as Poecilia reticulata, (Chervinski, 1984b), Gambusia affinis, (Chervinski, 1983), and Sarotherodon mossambicus (Balarin and Hatton, 1979). By contrast Oncorhynchus kisutch, shows high mortalities when juveniles during the early portion of the pre-smolt period are subjected to abrupt changes (Otto, 1971).

This study has shown that C.urophthalmus, can tolerate direct transfer from fresh water to 20‰ with very low mortality, and is also able to resist gradual

transference from freshwater to fully marine water (35‰), and the reverse, without mortality. No difference in survival was found between 18 and 144 hours in the range of salinities tested. The median lethal salinity (MLS) at 50% survival at 144 hours in C.urophthalmus was 24‰ and this compares favorably with the median lethal salinity of O.aureus and O.niloticus with values of 21‰ and 19.2‰, respectively, at 96 hours. This finding shows that C.urophthalmus has a slightly higher resistance to abrupt changes in salinity than the other two cichlids and this is only matched by an MLS of 25.2‰ found for O.mossambicus x O.niloticus hybrids (Watanabe et al., 1985).

Normally, both for research purposes and in culture, fish are adapted to new salinities by gradual acclimation. It is useful, however to have some Knowledge of the effect of instantaneous transfer on a given species as such as transfer may be necessary, for example in prophylactic saline treatment or in transfer of fingerling from a fresh water or brackish hatchery to more saline on-growing facilities. The ability of C.urophthalmus to survive rapid transfer to salinities up to about 15‰ without mortality and MLS of 24‰ is a reflection of the euryhaline adaptation of the species and clearly contributes to its usefulness as a cultured fish.

Cichlids, in general are euryhaline. It is known that Oreochromis niloticus can survive and grow in salinities up to 30‰ without mortality (Chervinski, 1961a,c and Beamish, 1970). On the other hand, the latter species did not show differences in growth when tested in brackish to full sea water with high survival (Alava et al., 1987), while in hybrids of O.hornorum x O.mossambicus better growth was found in high salinities (Watanabe et al., 1987). Similarly, O.mossambicus, performs well at salinites up to 33‰ (Juerss et al., 1984), has been reported to grow well even at 40‰ (Vaas and Hofstede, 1952) and can survive up to 69‰ (Potts et al., 1967). Oreochromis aureus, has been adapted to 35‰ in 14 days succesfully without mortality (Teng et al., 1980 and Finale and Brito, 1984) and has been grown in ponds at 36.47‰ to 44‰ (Chervinski, 1972). Tilapia zillii can survive salinities of 45‰ (Balarin and Haller, 1982). Hybrids of O.mossambicus x O.urolepis hornorum fry and juveniles tolerate transfer from fresh water to 19‰ without mortality or apparent stress but suffered 100% mortality at 29‰ (Perschbacher and McGeachin, 1987). This study has shown that C.urophthalmus is well adapted to brackish water achieving its best growth between 10 and 20‰, in this respect this species has very similar salinity tolerance to the other euryhaline members of the cichlid family.

From the field observaions, (Chapter 1), it is known that C.urophthalmus can spawn and that its fry can grow without problems at up to 20‰. These results are similar to observations on O.mossambicus, which is able to reproduce from freshwater to full sea water, and up to 49‰ (Popper and Lichatowich, 1975 In: Stickney, 1986). Similarly, O.niloticus is able to succesfully produce eggs at 20‰ (Al-Asgah, 1984). Oreochromis aureus is able to developing ovaries at salinities from 0 to 35‰ (Stickney, 1986).

Although C.urophthalmus can be adapted to a wide salinity range, this study has shown that the best growth was in a near isotonic medium, at about 12-15‰, although no significant difference ($P<0.01$) in growth was found from 5 to 35‰. Similar observations were reported by Canagaratnam, (1966) and Juers et al., (1984) for O.mossambicus which grew better at salinities of 10 and 30‰ than in fresh water. O.niloticus performed best at 11.6‰, its isosmotic point, where the energy for maintaining homeostasis for this particular species is minimal (Farmer and Beamish, 1969). Similarly, Job, (1969) suggested that at 12.5‰ O.mossambicus was able to osmoregulate more efficiently, again, the consequence being that energy expenditure for osmoregulation is reduced and consequently more energy is available for production (Febry and Lutz, 1987).

The efficiency of food conversion depends on many factors but the best response is probably strongly related to optimising the environment to approximate that to which the fish is accustomed. Otto, (1971) found that for Onchorhynchus kisutch growth rate, food intake and gross food conversion efficiency had the highest values at salinities of 5-10‰ throughout the pre-smolt period. For rainbow trout (Salmo gairdneri), the gross diet efficiency was not significantly affected by the environmental salinity (Zeitcun et al., 1973).

The results obtained in the present work show that final body weight, weight gain, specific growth rate, food intake and food conversion ratios were always better in saline environments (5, 10, 20, 30 and 35‰) than in fresh water, again showing that C.urophthalmus is well adapted to life in brackish water. With respect to changes in the body composition of C.urophthalmus at different salinities, all of the parameters measured show no significant differences ($P<0.01$), and the slight variations are probably atributable to normal experimental error. Similar results were found by Zeitoun et al., (1973) for Salmo gairdneri, while in O.niloticus body protein decreases and fat increases as salinity increases and ash content is lowest in fresh water (Alava et al., 1987). In the present study in spite of a full 12 weeks duration of the growth

experiment there were no observed differences in carcass composition, as found by Alava et al., (1987) in their 8 week experiment with O.niloticus.

In conclusion C.urophthalmus has the ability to reproduce and to grow in a range of salinities. It is thus a classically euryhaline species and as consequence has great potential in terms of aquaculture with the scope to be grown in freshwater, brackish and marine environments.

THE EFFECTS OF TEMPERATURE, BODY WEIGHT AND HYPOXIA ON THE OXYGEN CONSUMPTION OF *Cichlasoma urophthalmus* (Gunther).

INTRODUCTION.

Fish, like other animals, require oxygen in their tissues in order that oxidation can occur, obtaining by this means enough energy to maintain their metabolic requirements (Lagler et al., 1977; Nikolsky, 1968.). The solubility of oxygen in water is low (Spotte, 1979) and thus oxygen can be a limiting factor on the distribution of aquatic animals. This has created a selective pressure on fish to develop different strategies and structures in order to obtain sufficient quantities of this vital gas from the aquatic environment (Nikolski, 1968; Lagler et al., 1977; Randall, 1970; Smith, 1982).

Oxygen in the aquatic environment is much scarcer than in air in natural conditions, due mainly to its low solubility in water (Spotte, 1979). Other important environmental parameters affecting thus solubility are temperature and salinity, and the oxygen solubility decreases when the water temperature rises, and when salinity increases. For example, the oxygen content of sea water is normally 20% less than the oxygen content of freshwater at the same temperature (Smith, 1982).

For these reasons oxygen availability is more critical for tropical fish living in brackish water environments than for temperate species (Smith, 1982).

It is notable that salmonids have high requirements for oxygen and that they are well adapted to low temperatures, whereas cichlids are adapted to high temperatures, and have relatively low oxygen requirements and are also quite resistant to low levels of oxygen saturation (Sarig, 1971; Morgan, 1972; Kutty, 1972; Perez and Maclean, 1975; Balarin and Hatton, 1979; Ross and Ross, 1983; Stickney, 1986). Thus Oreochromis mossambicus and O.niloticus are species that can resist levels of oxygen as low as 0.1 mg/l for a period (Mayurama, 1958; Magid and Babiker, 1975; Melard and Philippart, 1980). This resistance enables the cichlids to invade, survive, reproduce and grow in shallow lakes and lagoons, where strong reductions of oxygen occur at different times of day and in various periods of the year which would be fatal for other less well adapted species (Philipart and Ruwet, 1982). Generally, the cichlids are capable of survival at low oxygen levels with generally low rates of oxygen consumption and the additional ability of obtaining oxygen from the saturated air-water interface (Stickney, 1986). Kutty, (1972) has further suggested that these species may be capable of anaerobic respiration which could sustain life for short periods of time even in total oxygen depletion.

Fish under culture conditions are usually held at high densities and may be subjected to strongly limiting

environmental conditions. Often these environmental parameters can not be closely regulated or maintained at adequate levels. Oxygen is an environmental parameter that can reduce the growth rate of fish, when available only at low levels, normally less than 3mg/l (Melard and Philippart, 1980; Ross and Ross, 1983), and it may be required in higher quantities when the organisms are subjected to normal handling conditions (Ross and Ross, 1983).

Temperature and salinity, produce similar reduction effect in the growth when these parameters are sub-optimal, but in most cases these parameters are very difficult to manipulate in economic terms. By contrast, oxygen can be increased with fewer problems and at considerably lower costs and in aquaculture conditions it is usually easy to control by means of mechanical or electrical systems (Stickney, 1986).

Thus, when a new species such as Cichlasoma urophthalmus, is being investigated for culture, knowledge of its oxygen requirements at different temperatures is vital in order to permit the adequate management of this important gas during maintainance, transportation, reproduction and commercial growth in semi-intensive and intensive culture.

The objective of the present experiments was to investigate the effects of temperature, body weight and hypoxia on resting respiratory rates of Cichlasoma urophthalmus in order to provide basic information for its aquaculture management.

MATERIALS AND METHODS.

Fish.

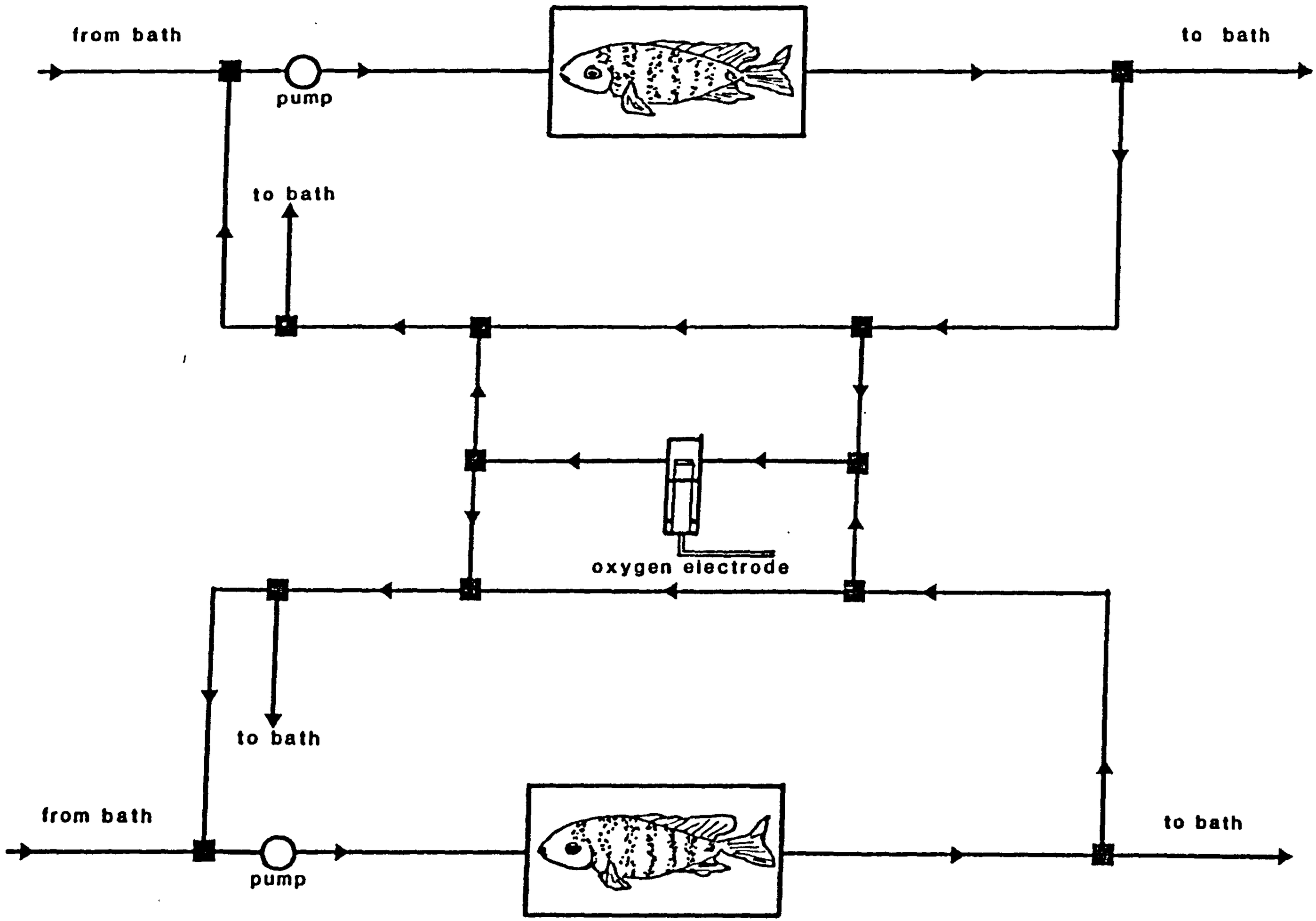
Original stocks were obtained and identified as described previously and juveniles were obtained from these original stocks for use in the present experimental work.

Respirometers.

A closed system respirometer as described by Ross and Ross, (1983) was utilized (Figure 3.12). Two different size chambers were used, one for small animals of 300 cm3 capacity and another of 1540 cm3 used for the larger fish. The total volume of the systems were 550 cm3 and 1760 cm3 respectively. Water was supplied to the chambers by water pumps through a two-way valve system. The water from water bath was strongly aerated by air stones to achieve 100% saturation. The oxygen was

Figure 3.12.

Schematic diagram of close circuit respirometer with two chambers used in the present study. Arrows show the waterflow direction and the black squares represent three-way valves.


from bath
pump
to bath
to bath
oxygen electrode
to bath
from bath
pump
to bath

measured inside this chamber, with a polarographic probe (YSI model 57) and the output of the oxygen meter was recorded on a Cole-Palmer flat-bed chart recorder.

The respirometer system was maintained in a controlled temperature room at 20°C, with a 12 hours light and 12 hours dark photoperiod. The respirometers with the experimental fish were covered by aluminium foil in order to avoid any fish disturbance while recording the data. Temperature, pH and ammonia levels were maintained closely throughout the experiments.

Effect of body weight and temperature on oxygen consumption.

Fish in the weight range of 1.1 to 195g were acclimated to the experimental temperature for three days and then transferred to the experimental chamber were they were allowed to rest for a further 12 hours. During this period the water system ran in an open circuit taking water at the required temperature from the water bath through the chamber and returning it to the bath. After the acclimation period the system was closed and the decrease in oxygen concentration was recorded over a 30 minute period. The fish were starved throughout this procedure. Every day an empty chamber was monitored to be used as a blank value, which was subtracted from the total fish oxygen consumption. From

47 to 57 fish covering a wide weight range were monitored at each experimental temperature (20, 25, 30, and 35°C) and a total of 209 fish were utilized.

Effect of low dissolved oxygen concentration.

Eighteen fish were utilized in the weight range 20.3 to 68.5g. These were allowed to deplete the oxygen in the chambers at 28°C. The oxygen concentration in the chamber was monitored continuously and the trial was terminated when the oxygen consumption had virtually ceased. Twelve further fish were allowed to fully deplete the oxygen concentration in the chambers and the duration of tolerance to virtual anoxia was estimated.

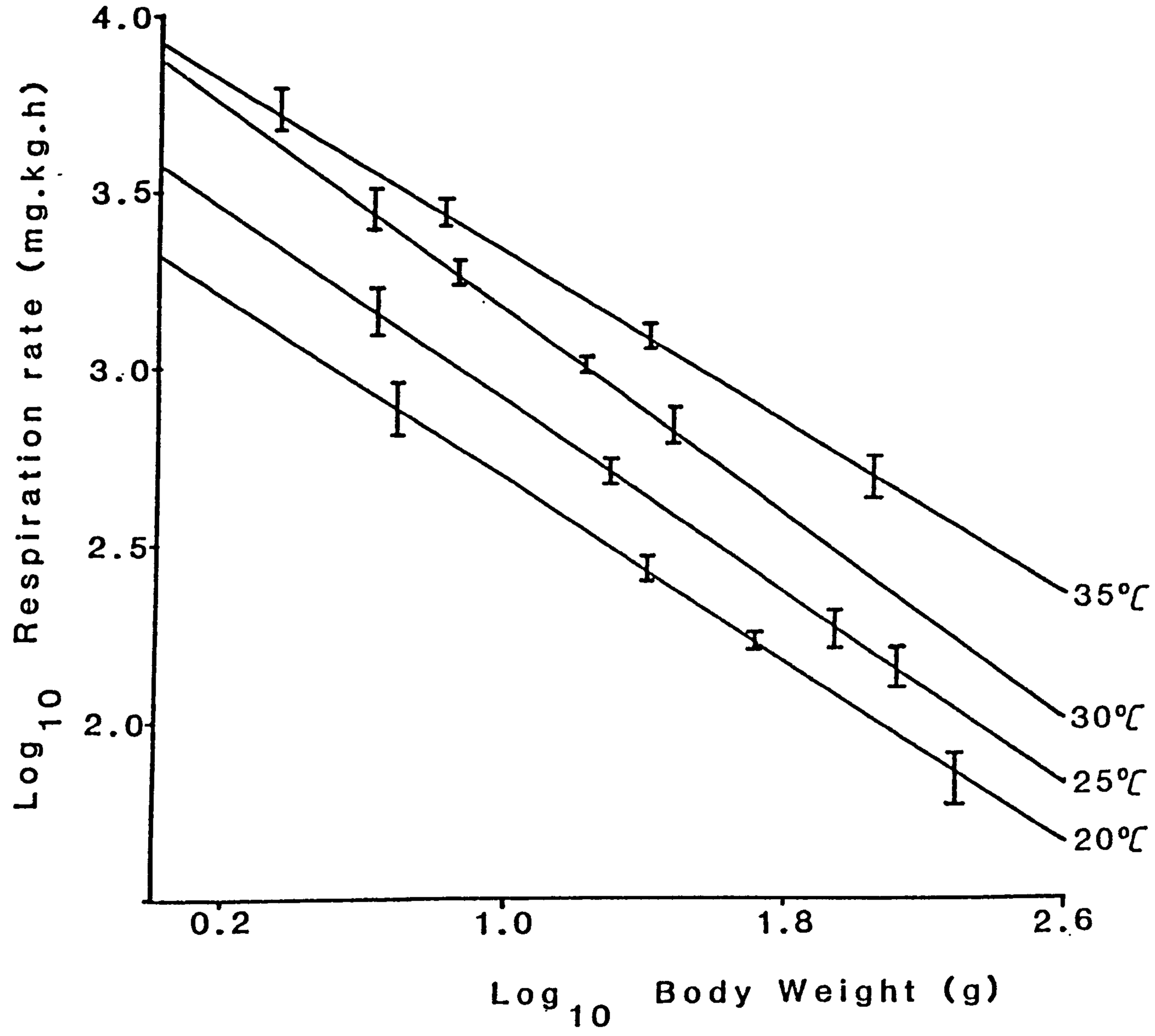
RESULTS.

Effects of body weight and temperature on oxygen consumption.

Analysis of the resting respiratory data showed that there was an inverse relationship between log10 body weight and log10 respiration rate for all temperatures tested. As may be expected the rate of oxygen consumption in C.urophthalmus increases with

Figure 3.13.

Relationship between log10 respiration rate and log10 body weight at four temperatures in C.urophthalmus. The vertical bars show 95% confidence intervals.


4.0
3.5
3.0
2.5
2.0
Log10 Respiration rate (mg.kg.h)
35℃
30℃
25℃
20℃
0.2
1.0
1.8
2.6
Log10 Body Weight (g)

increasing temperature (Figure 3.13). The regression equations for each temperature tested and its significance values in terms of r-squared, are shown in Table 3.9, where it is also clear that the Q10 is temperature dependent, increasing while temperature increases.

Effect of low dissolved oxygen levels on oxygen consumption.

Fish used in the present work varied considerably in size from 1.1 to 195.0g and in order to compare and pool the oxygen consumption in all these fish the data obtained were expressed as per cent decrease in rate against the dissolved oxygen concentration. This is shown graphically in Figure 3.14 and it is clear that the change in respiratory behaviour of this fish is the classical response of an oxygen conformer, where no attempt is made to compensate as the oxygen level falls from the critic level to zero.

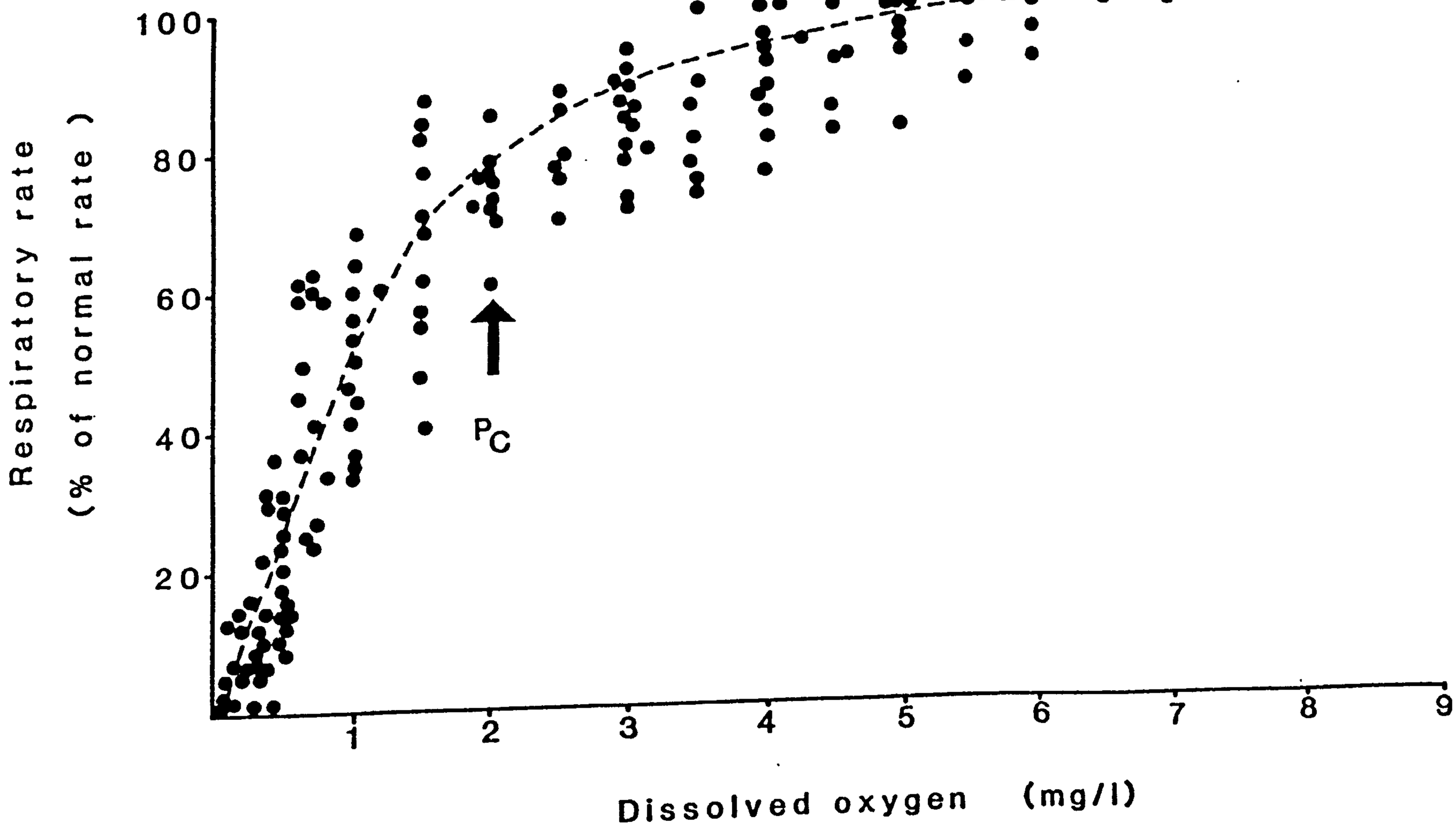
The effect of hypoxic conditions in this species is not evident until the dissolved oxygen levels reaches about 3,5 mg/l. Thus the critical oxygen concentration was estimated to be about 70mmHg at 28°C. Below this value the oxygen consumption rate decreases until at about 1.5 mg/l, a marked linear reduction with

Table 3.9.
Regression equations of respiration rate (Y) on body weight (W).

Temperature (^{o}C)	Regression equations	n	r	Significance of r	Q_{10}
20	$Y = W^{-0.648}$ ($\log_{10}Y = 3\ 33-0\ 648\ \log_{10}W$)	51	-0 889	70 001	2.02
25	$Y = W^{-0.679}$ ($\log_{10}Y = 3\ 57-0\ 679\ \log_{10}W$)	47	-0 934	70 001	2 30
30	$Y = W^{-0.72}$ ($\log_{10}Y = 3\ 87-0\ 72\ \log_{10}W$)	54	-0 883	70 001	2 65
35	$Y = W^{-0.596}$ ($\log_{10}Y = 3\ 92-0\ 596\ \log_{10}W$)	57	-0 936	70 001	

Figure 3.14.

The percentage reduction in respiratory rate of C.urophthalmus under hypoxic conditions at 28'C Pc=critical oxygen tension.


Respiratory rate
(% of normal rate)
100
80
60
40
20
P_C
1
2
3
4
5
6
7
8
9
Dissolved oxygen (mg/l)

decreasing D.O.was evident. At these low oxygen levels the fish show only small movements of the fins and a dark barred colouration due probably to the stress caused by the anoxia. During these experiments C.urophthalmus showed a great resitance to hypoxia. Some fish were maintained at levels of virtual anoxia for an extended period of time and they easily resisted 60 to 130 min. of total oxygen depletion. In these conditions the fish had an apparent respiratory rate of zero and changed the normal pale colouration tc an intense-black barred pattern. At a later the stage the fish lost equilibrium and became moribund, but only one fish was unable to fully recuperate after transfer to aerated freshwater. In general terms the fish in the present experiment were able to survive period of anoxia for up to 2 hours.

The Ammonia concentration in the respirometer did not rise above 0.312 ppm and the pH was 8.15±0.2 while the fish were tested.

DISCUSSION.

The present experiments confirm the general observations that small fish require more oxygen per unit of body weight than larger fish, as previously

shown for Carassius auratus L. (Beamish and Mookherjii, 1964), Salmo gairdneri Richardson (Muller-Feuga et al., 1978), Orechromis niloticus (L.)(Ross and Ross, 1983) and Barbus aenus Burchel (Eccles, 1985).

Increasing temperature over the range investigated, shows an increase in respiration rate, with a Q10 value of 2.49 between 20°C and 30°C. It is clear, however, that over the temperature range investigated Q10 increases from 2.02 to 2.65 (Table 3.9). This is in contrast to the work of Ott, et al., (1980), who demonstrated a decrease in Q10 between 5°C and 25°C in Cyprinus carpio. It is, interesting to note however, that between 25°C and 32°C these authors showed an increase in Q10.

When compared to other cultured species with similar temperature ranges and using similar respirometry techniques, it is clear that Cichlasoma urophthalmus has a slightly higher oxygen requirement (Table 3.10). This difference varies, depending on body weight, and this is a reflection of small differences in the slopes of the regression lines obtained by different authors. An important factor is the range of fish weights used in the experimental work, which should be as wide as possible. Clearly, trout at 25°C are at the limit of their ecological range and this probably

Table 3.10.
A comparison of respiration rates of three African cichlids and C.urophthalmus; the values are quoted for fish of 50g in mg/Kg/h at 25'C.

Species	Respiratory rate (mg/kg/h) 50-g fish	100-g fish	Author
Sarotherodon mossambicus	135	106	Caulton (1978)
Oreochromis niloticus	160	126	Ross & Ross (1983)
Tilapia rendalli	246	-	Caulton (1978)
Cichlasoma urophthalmus	261	163	This paper
Salmo gairdneri	440	380	Stevenson (1980)

accounts for the very high respiratory rate shown in Table 3.10.

The critical oxygen tension, (Pc, Fig.3.11), estimated as 70mmHg, is a little higher than the values of 60mmHg for Oreochromis niloticus (Ross and Ross, 1983) and 20mmHg for Cyprinus carpio (Ott et al., 1980). Generally, however, tolerance of acute hypoxia is good with full recovery after 2 hours in virtually anoxic conditions. The total ammonia concentrations in the respirometers rose to 0.312mg/l at pH 8.15 after about 2 hours. The unionized fraction of this is about 0.03 mg/l (Alabaster and Lloyd, 1980) and this would have little effect within the time period of these experiments, particularly as the free carbon dioxide levels will be elevated. These animals commonly occur in brackish water lagoons where oxygen concentrations can be very low and accompanied by high hydrogen sulphide and free carbon dioxide concentrations. Thus, although Cichlasoma urophthalmus is very tolerant of low dissolved oxygen concentration, it is probably inadvisable to allow dissolved oxygen to fall below about 3.5 mg/l in culture.

While resting respiratory rates provide guidelines for the minimum oxygen required by cultured animals some allowance must be made for additional routine oxygen uptake attributable to some minimum level of motor

activity and, perhaps more importantly, to dietary-induced heat increment (specific dynamic action). No data are available for heat increment in Cichlasoma but in Oreochromis spilurus increases in oxygen consumption rate of about 60% have been noted following a 2% body weight meal. This effect varies slightly in magnitude depending on protein and lipid content of the diet and the duration of the effect can be for up to 10 hours (Spencer and Ross, unpublished data). Thus although this effect should be evaluated for Cichlasoma species, some appropriate allowance can be made, based on data for tilapias, which will facilitate satisfactory system design and provision of an adequate oxygen supply. The data obtained in this study thus provide a useful basis for the calculation of carrying capacities of semi-intensive and intensive culture systems and for transportation of Cichlasoma urophthalmus over the range of temperature most commonly encountered in the field.

CHAPTER 4

NUTRITIONAL REQUIREMENTS

OF

Cichlasoma urophthalmus

GENERAL INTRODUCTION.

One of the most important targets in fish culture is to increase production per unit of culture space. As a consequence, one of the most important requirements for culture is a well balanced diet and an adequate feeding regime, avoiding undernourished or malnourished fish which will never be able to maintain their health and productivity even when an adequate environment is provided (Huet, 1986; Bardach et al., 1972; Cho et al., 1985).

Fish nutrition involves an underestanding of the chemical and physiological processes which provide the nutrients for maintenance, normal function, and proliferation of cells (Maynard and Loosli, 1969). Thus, nutrition encompasses ingestion, digestion, and absorption of nutrients and their transport within the body as well as removal of excess nutrients and metabolic wastes. In addition to these biological considerations it is important to note that the feed constituents are usually the major operating costs of rearing fish from stocking size to market size, except in specialized circumstances, (N.R.C., 1983).

Nutrition of terrestial animals has been intensively studied for many years, whereas research concerning the nutrition of fishes began relatively recently. Most of the early fish nutrition research was conducted with salmonid fishes but, more recently, attention has also turned to other important species of fish cultured in different parts of the world (N.R.C., 1983). Early fish researchers and culturists relied primarily on natural foods and fresh animal tissues to meet the nutritional requirements of the various fishes with which they worked (Davies, 1927). Because of the lack of nutritional information, the composition of the first prepared fish feeds was based largely on the proximate composition of natural foods consumed by the fish or on the nutrient requirements of other simple-stomached animals. Feeds formulated on these bases were adequate at very low fish stocking densities, however as fish stocking rates increased, the need for more precise nutritional information became apparent.

Although faced with unique problems (i.e., working in the aquatic enviroment with a poikilotherm), nutrition researchers were able to delineate the nutritional needs of various fishes by adapting research methods common in terrestrial animal research and by developing new methods. It is no exaggeration to say that the first step in the right direction for studying

fish nutrition was the establishment of an artificial diet with an appropriate vitamin mixture (McLaren et al., 1947a, 1947b; Wolf, 1951; Halver, 1957; Halver and Coates, 1957). In fact, approximately 40 chemical compounds have now been shown to be necessary for normal metabolic function in fish, and qualitatively these requirements are similar to those of land animals, (Halver, 1972; Halver, 1976; N.R.C., 1983;). Differences do exist in detailed nutrient requirements between fishes and land animals e.g., in fatty acid requirements (Henderson and Sargent, 1985; Bell et al., 1985)) and in the ability to utilize certain nutrients, such as carbohydrates, (Shimeno, 1982; Anderson et al., 1984; Kono et al., 1987). It is clear that a knowledge of the specific nutritional characteristics of a given species is important not only in ensuring survival and good growth, but also infringing on the costs and profitability of production.

Compared with terrestrial farmed animals it is notable that fish require a higher percentage of high quality protein in their diets for normal growth and health (Halver, 1976; Halver, 1972; Tacon and Cowey, 1985). Protein is the most expensive nutrient in the diet of any cultured organism and it is principally the cost of the protein that directly influences the costs of production in a fish farm (Cho et al., 1985). Thus a

knowledge of the protein requirements of the cultured fish is important in aquaculture.

The utilization of different nutrients by an organism can be measured by growth, but it is also important to know how much of a particular material is taken by the fish and incorporated through one or other metabolic pathways. This question can be answered only by a direct study of the digestibility of a particular nutrient. There have been many successful studies of digestibility of different materials in fish (Spannhof and Planticow, 1983; N.R.C., 1983; Cho et al., 1985). From these examples is known that the nutrient in some feedstuffs are well-utilized and can thus constitute a high percentage in the diet. Some other materials, however, have particularly low nutrient digestibilities and in these cases the organism is unable to digest or incorporate the material offered into its tissue.

This effect may occur for wide variety of reasons, beginning with the health of the organism , its anatomy, trophic level and obviously the availability of the nutrient. Some of these feedstuffs contain substances called antinutrients which are of different origins, chemical structures and have different mechanisms of action, and these may strongly reduce digestibility. In

yet other cases the low digestibility is related to the velocity of passage of the nutrients through the digestive tract, because if the residence time is brief, then assimilation will not take place efficiently thus reducing digestibility (Anderson et al., 1984; N.R.C., 1983). Thus, digestibility is an important element which should be incorporated in any basic study of the more usual components of the diet, with the aim of formulating reliable practical and economical diets.

The main objective of the present chapter was to evaluate the protein requirements of juvenil C.urophthalmus at two different temperatures. Experiments were also conducted, using sub-adult fish to measure digestibility of the most important materials in fish diets, protein, fat and carbohydrate.

PROTEIN REQUIREMENTS OF *Cichlasoma urophthalmus* JUVENILES AT TWO DIFFERENT TEMPERATURES

INTRODUCTION.

Proteins are extremely important in fish metabolism because they are used either to synthesise new protein for incorporation into new tissues during growth and reproduction or alternatively, they are utilized by fish directly as a source of energy. Thus if an adequate protein level or essential aminoacid conmplement is not provided in the diet, there is immediate rapid reduction or cessation of growth, because the organism withdraws protein from some tissues to maintain its most vital functions (Cowey, 1976; Halver, 1976; Davis and Stickney, 1978; N.R.C., 1983. On the other hand, recommending an appropriate protein level for a given species can be difficult particularly in view of the wide variety of possible culture practices and environmental conditions. Thus, a particular species will require different levels of proteins, depending on the fish size, environmental temperature, stocking density, daily feed allowance, amount of non protein energy in the diet and dietary protein quality (N.R.C., 1983).

Delong et al., (1958) found that the optimum protein requirements of salmon significantly increased with increasing temperature; thus at 8.4°C the fish had

a requirement for 40% of protein, while at 14.8°C the protein requirement was 55% similarly, Millikin, (1983) found that increments in temperature increased the protein requirements of Morone saxatilis, from 47% at 20.5°C to 55% protein at 24.5°C, while in Salmo gairdneri no differences were found over the temperature range of 9 to 18°C with dietary protein values of 35-45% (Slinger et al., 1977). Thus, a controversial debate about the effects of temperature on the protein requirements of fish commenced and it was suggested that the earlier basic experiments with chinnok salmon by Delong et al., (1958) lacked proper precision (Cho et al., 1985). This was partly resolved by the recent evaluation by Cho et al., (1985) who suggested that the protein requirements of fish are little influenced by the temperature, as long as the fish are maintained within their normal temperature ranges.

The protein requirements of carnivorous fish have been shown to be higher than those of omnivores or vegetarians. This is possibly due to the higher utilization of dietary carbohydrates as an energy in the latter, while carnivores use mainly fats and proteins as energy sources (Cowey, 1975; N.R.C., 1983; Cho et al., 1985; Tacon and Cowey, 1985). C.urophthalmus is a carnivorous fish with some omnivorous habits, and is quite different to the widely-cultured tilapias, which

have predominantly vegetarian to omnivorous habits (Trewavas, 1983). Thus, the protein requirements of this species are one of the major questions to resolve before being able to prepare balanced diets.

The aim of the present study was to determine the protein requirements of C.urophthalmus fry by varying the levels of dietary protein at two different temperatures, 28°C (average temperature registered in the natural habitat) and 32°C (The temperature near the optimal range observed experimentally).

MATERIALS AND METHODS

Experimental animals.

C.urophthalmus fry were obtained as described for previous experiments and separated into two batches of fry, of mean weight 0.3g for the first experiment and 0.6g for the second trial. Experimental fish were stocked at 20 fish per tank, three tanks per treatment. Prior to starting the experiments, the fry were acclimated for one week, feeding them with the basic formulation of 50% protein at a rate of 3% of body weight/day. At the start of the experiment, 150 fish were killed by an overdose of benzocaine (1:300) and stored at -15°C for subsequent carcass analysis, moisture content ans gross chemical constituents.

Experimental system.

Two independent experimental tank systems were constructed using 33 polypropylene 20l buckets and PVC plastic pipes, and these were maintained in a controlled temperature laboratory at 26+2°C, with a photoperiod of twelve hours light and twelve hours dark. The water entered each tank at a rate of one litre/minute, through a tangential pipe to give a circular flow and the exit

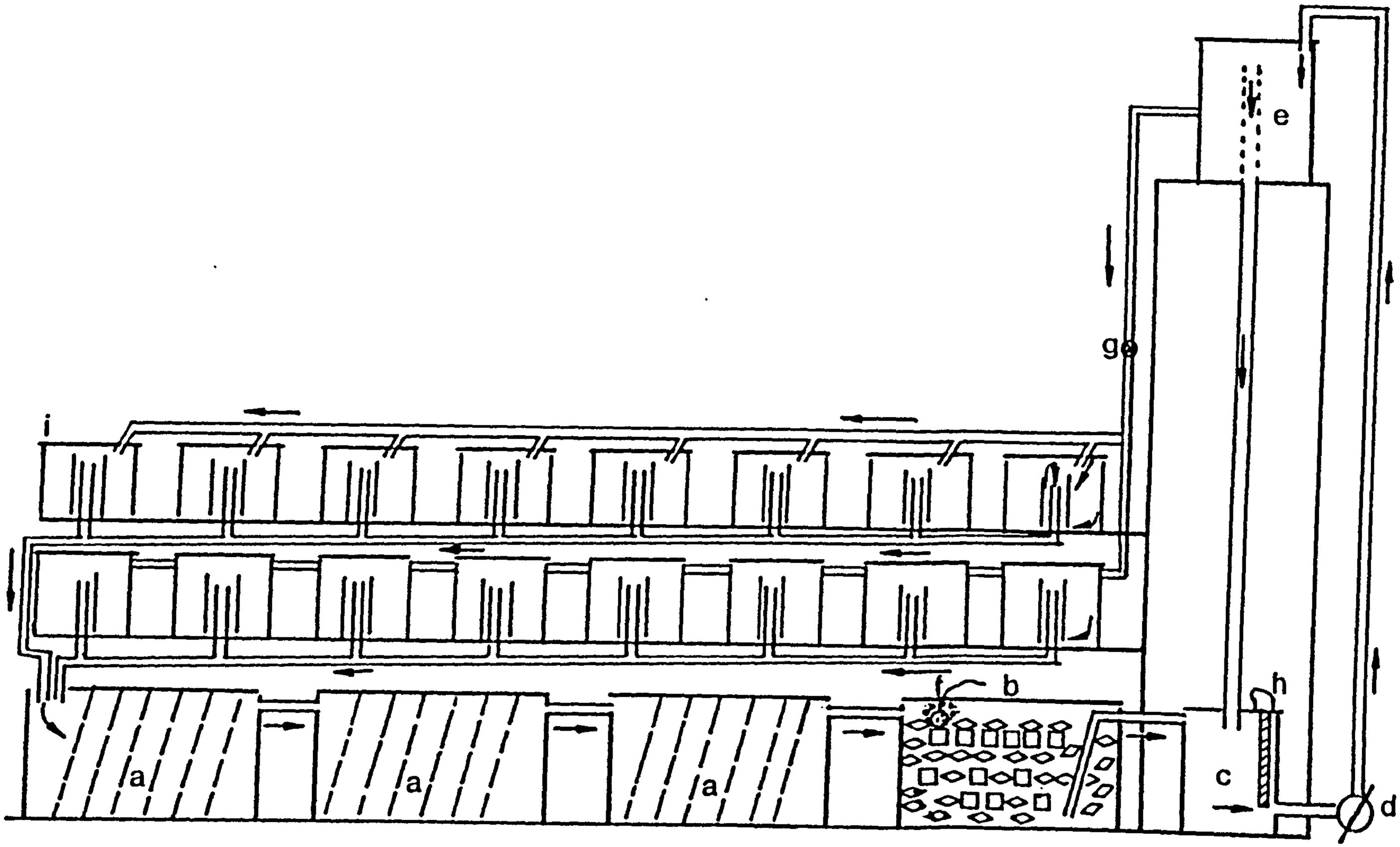
was from a central stand pipe in the bottom of the tank with a self-cleaning collar protected with a plastic net to avoid fish losses. Effluent water from each tank passed through three sedimentation tanks of 1501 capacity, each containing 12 sheets of fibre glass to aid sedimentation. Water then passed through a biological filter, which consisted of sacks of polystyrene crisps used to increase the area for bacterial action. After the filter the water was collected in a sump of 1001 from which it was pumped with a 0.5Hp centrifugal pump to the header tank (Figure 4.1.). Each system was maintained at a constant temperature of either 28° or 32°C by a 2 Kilowatt water-heater and a thermostat located in the sump tank.

Environmental parameters.

The system was filled with city water which had been allowed to settle and was oxygenated for at least two days in a concrete settling tank of 32 cubic metres capacity. The water was then aereated for a further week in the recirculation system before any use of the system. The system with its filters was then operated for at least a month with a low biomass of fish to create a biological filter prior to the start of the experiments. Replacement of the water in the experimental system to compensate for evaporation and

Figure 4.1.

Experimental recirculating system used in protein requirement experiments: a)Sedimentation tanks. b)Biological filters. c)Sump. d)Pump. e)Header tank. f)Airstone. g)Valve. h)header. i)Experimental tanks. The arrows show the direction of the water flow.


e
g
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during the weekly cleanings was made with pre-conditionated city water. Care was taken to match the temperature of the water to that required before pumping the water into the header tank.

The temperature was recorded twice a day with a mercury thermometer and with the thermistor probe of a YSI model 57 Oxygen meter. Oxygen levels were also measured frequently. The pH was measured with a Pye-Unicam pH meter model 9409. Total ammonia was measured twice during the experimental period using the method of Lind (1979). Table 4.1 show the mean values obtained for these parameters during the present experiment.

Experimental diets.

As the amino acid requirements for C. urophthalmus have still not been determined, it was neccessary to use the essential amino acid profile of C.urophthalmus fry as a reference for dietary formulation. This was based on direct correlation found between the pattern of aminoacids in the tissues as a percentage of the total essential amino acids and the dietary requirement for amino acids found, by Boorman, (1980) and described for fish by Cowey and Tacon, (1981).

Table 4.1.
Environmental parameters recorded during protein level experiments at 28'C and 32'C.

Temperature	$32^{o}C \pm 0.05$
PH	8.63 ± 0.3
Dissolved oxygen	5.8 ± 0.4 mg/l
Total ammonia (NH_3-NH_4)	0.015 ± 0.04 mg/l
Temperature	$28^{o}C \pm 0.06$
PH	9.55 ± 0.4
Dissolved oxygen	6.0 ± 0.2 mg/l
Total ammonia (NH_3-NH_4)	0.004 ± 0.001 mg/l

The amino acid profile of C.urophthalmus was obtained by killing a number of recently-hatched fry of average weight 0.133g by overdose of benzocaine. These were then dried in an oven at 105°C overnight, the fat was extracted by soxhlet with petroleum ether and the protein was then hydrolysed with 6M hydrochloric acid. Aminoacids were then analyzed in a Beckman aminoacid analyzer and tryptophan was determined in the same carcass after alkaline hydrolisis using the method of Basha and Roberts, (1977).

Table 4.2 summarises the amino acid requirements of four species as a percentage of the protein offered and as a percentage of the total diet related to the protein percentage in the diet. Table 4.2 also shows the carcass amino acid profile as a percentage of the essential amino acid for the species in the table including C.urophthalmus. This was used mainly for establishing the relationship between carcass composition and requirement. In table 4.2a the amino acid profile of Engraulis sp. meal which was used as food during the trials is shown as represents the amount of amino acid supplied in the diets at 26.8 and 40.6% protein, for reference.

The experimental diets were formulated, using brown fish meal as the only source of protein.

Table 4.2.

Percentage of amino acids in carcass of C.urophthalmus, O.mossambicus, Cyprinus carpio, Salmo gairdneri and Ictalurus punctatus compared with their known aminoacid requirements. A)Percentage of essential amino acids (EAA) as a percentage of the total essential amino acids in carcass. B)EAA in grams per 100g of dry diet. C)EAA in grams per 100g of protein. In parenthesis percentage protein in the diet.

	Orepchromis[3] mossambicus			Cichlasoma urophthalmus	Cyprinus[1,2] carpio			Salmo[1] gairdneri			Ictalurus punctatus		
	A	B	C	A	A	B	C	A	B	C	A	B	C
Arginine	13.22	1.13	2.82(40)	15.24	13.55	4.2	1.6(38.5)	12.32	1.40	3.5(40)	12.63[4]	4.3	1.03(24)[2]
Histidine	4.81	0.42	1.05(40)	5.97	4.95	2.1	0.8(38.5)	6.22	0.64	1.6(40)	5.61	1.5	0.37(24)
Isoleucine	8.81	0.80	2.01(40)	9.01	8.17	2.3	0.9(38.5)	8.35	0.96	2.4(40)	8.62	2.6	0.62(24)
Leucine	14.95	1.35	3.40(40)	17.67	14.84	3.4	1.3(38.5)	13.03	1.76	4.4(40)	19.03	3.5	0.84(24)
Lysine	16.15	1.51	3.78(40)	17.90	18.7	5.7	2.2(38.5)	18.16	2.12	5.3(40)	21.04	5.0	1.5(24)
Methionine	4.41	0.40	0.99(40)	1.87	5.59	3.1	1.2(38.5)	6.05	0.72	1.8(40)	2.81	2.3	0.56(24)
Phenylalanine	12.28	1.0	2.5 (40)	9.29	10.32	6.5	2.5(38.5)	10.65	1.24	3.1(40)	9.62	5.0	1.2 (24)
Threonine	13.35	1.17	2.93(40)	11.68	11.40	3.9	1.5(38.5)	11.48	1.36	3.4(40)	9.62	2.0	0.53(24)
Tryptophan	1.87	0.17	0.43(40)	1.17	2.15	0.8	0.3(38.5)	1.68	0.20	0.5(40)	1.60	0.5	0.12(24)
Valine	10.15	0.88	2.2 (40)	10.20	10.32	3.6	1.4(38.5)	10.68	1.24	3.1(40)	9.41	3.0	0.71(24)

1. Ogino, 1980
2. NRC, 1983
3. Jauncey et al., 1983
4. Lovell and Ammerman, 1974

Table 4.2a.
The aminoacid profile of the anchovy meal (Engraulis sp.) utilized as food ar 26.8% and 40.6% protein for comparisons.

	B	C	C
Arginine	12.43	2.61 (26.8)	4.62 (40.6)
Histidine	5.32	0.99 (26.8)	1.48 (40.6)
Isoleucine	10.23	1.90 (26.8)	3.90 (40.6)
Leucine	16.43	3.04 (26.8)	3.0 (40.6)
Lysine	16.61	3.1 (26.8)	2.61 (40.6)
Methionine	6.53	0.32 (26.8)	0.48 (40.6)
Phenylalanine	9.18	1.60 (26.8)	2.84 (40.6)
Threonine	9.08	2.01 (26.8)	4.54 (40.6)
Tryptophan	2.48	0.33 (26.8)	2.39 (40.6)
Valine	11.52	1.75 (26.8)	0.49 (40.6)

* NRC, 1983

Variations in the dietary protein level were achieved by replacement of fish meal with starch and dextrin in order to produce isocalorific diets. Proximate analysis of the anchovy meal, including determination of moisture, protein, lipid, ash and nitrogen free extractives (NFE) were performed prior to diet formulation. The composition of the experimental diet is shown in Table 4.3. for experiments at 28°C and in table 4.4 for experiments at 32°C, while Table 4.5 shows the mineral and vitamin mixtures used in both experiments. The diet components were mixed dry in a Hobart mixer for at least 30 min and later the oils were incorporated together with a small amount of distilled water to obtain a crumble mixture. The mixture was then pelleted by extrusion in a Hobart pellet mill modified for small batches (300-1000 g) and to produce pellets 2 mm thick. The pellets were then dried at 35°C in a forced air convection dryer and later broken into smaller pieces according to the size of the fish. The final diet was stored in air-tight containers in a frezeer at -15°C. Small portions (about 10g) of each diet were placed in the fridge (-4°C) and weighed daily as required. Dried samples of the prepared diets were taken for triplicate proximate analysis and values for metabolizable energy (ME) in diets were obtained by calculation, based on the assumption that proteins have an energy value of 4.5 Kcal/g (Smith, 1971), carbohydrates (as dextrin), have

Table 4.3.
Composition of the experimental diets used at 28'C and its respective proximate analysis.

INGREDIENTS (%)	DIET NUMBER								
	1	2	3	4	5	6	7	8	9
Brown fish meal	48.14	55.01	59.14	61.89	66.02	68.77	72.89	75.64	79.77
Dextrin	12.44	10.22	8.88	7.99	6.66	5.77	4.44	3.55	2.21
Corn Starch raw	24.86	20.42	17.75	14.65	13.30	11.52	8.86	7.08	4.41
Fish liver oil	3.56	3.35	3.23	4.47	3.02	2.94	2.81	2.73	2.61
Corn oil	5	5	5	5	5	5	5	5	5
Cellulose Binder	1	1	1	1	1	1	1	1	1
Vitamin Premix	3	3	3	3	3	3	3	3	3
Mineral Premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Indicator (Cr_2O_3)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
NUTRIENT CONTENT (%)									
Moisture	7.46	7.36	6.60	6.79	6.30	6.03	6.09	6.06	5.91
Crude protein (Nx6.25)	34.67	40.60	43.51	46.37	49.60	51.04	53.98	55.91	56.06
Lipid	10.36	10.00	10.98	10.91	10.56	10.66	10.41	11.69	10.79
ASH	10.79	12.04	12.57	12.57	13.67	14.28	14.79	15.07	14.91

Table 4.4.
Composition of the experimental diets used at 32'C and its respective proximate analysis,

	Diet Number										
	1	2	3	4	5	6	7	8	9	10	11
Ingredients (%)											
Brown fish meal	34.99	41.99	48.99	55.98	60.18	62.98	67.18	69.98	74.18	76.98	81.18
Dextrin	16.53	14.25	11.96	9.67	8.30	7.38	6.01	5.09	3.71	2.8	1.42
Cornstarch raw	33.1	28.51	23.92	19.35	16.59	14.76	12.01	10.18	7.43	5.59	2.85
Fish liver oil	4.38	4.25	4.13	4.0	3.93	3.88	3.80	3.75	3.68	3.63	3.55
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose binder	1	1	1	1	1	1	1	1	1	1	1
Vitamin premix	3	3	3	3	3	3	3	3	3	3	3
Mineral premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Indicator (Cr_2O_3)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
NUTRIENT CONTENT (% Wet weight)											
Moisture	6.88	6.84	6.53	6.47	6.54	6.34	6.67	6.70	6.46	6.42	6.32
Crude protein (NX6.25)	26.80	30.41	36.42	42.19	45.29	46.96	49.20	51.80	56.36	58.18	60.45
Lipid	10.22	10.84	9.96	10.18	11.40	9.62	9.64	9.86	10.18	9.78	9.44
ASH	10.06	10.27	11.10	11.22	10.78	11.46	11.82	12.04	13.12	12.23	14.05

Table 4.5.
Mineral and vitamin mixtures used in all diets.

Mineral Mixture [1] (g/kg of food)		Vitamin Mixture [1;2] (mg/kg or U.I/kg)	
$MgSO_4 \cdot 7H_2O$	10.4340	Thiamine Mononitrate (B_1)	35.0
KCl	2.0000	Riboflavin (B_2)	25.0
NaCl	2.4000	Calcium Pantothenate	90.0
$FeSO_4 \cdot 7H_2O$	1.0000	Biotin	0.6
$ZnSO_4 \cdot 7H_2O$	0.2200	Folic Acid	7.5
$CuSO_4 \cdot 5H_2O$	0.0314	Cyanocobalamin (Vit B_{12})	0.05
$MnSO_4 \cdot H_2O$	0.0769	Niacin	220.0
$COSO_4 \cdot 7H_2O$	0.0236	Pyridoxine HCl (B_6)	30.0
$CaIO_3$	0.0118	Ascorbic Acid (Vit C)[3]	1000.0
$CrCl_3 \cdot 6H_2O$	0.0051	Choline Chloride	1000.0
		Myo-Inositol	250.0
		Retinol Acetate (Vit A) UI	6500.0
		Cholecalciferol (Vit D_3) UI	1000.0
		DL-Alpha Tocopherol acetate (Vit e)	300.0
		Menadione sodium bisulfite (50% vit k_3)	12.0
		BHT	25.00
		Ethoxyquin	100.00

1. Tacon, Personal communication
2. Roche
3. Sigma

an energy value of 3.49 Kcal/g (Chiou and Ogino, 1975) and dietary lipids have an energy value of 8.51 Kcal/g (Austreng, 1978). Based on these calculations the protein to energy ratio (P:E) in mg protein/Kcal of metabolizable was estimated and is shown on a moisture-free basis in Table 4.6 for diets used at 28°C and in table 4.7 for diets used at 32°C.

Experimental protocol.

The feeding regime used throughout the trial was 6% body weight/day. The frequency of feeding was maintained at 5 times a day, 6 days a week, and twice on Sundays. Care was taken when giving the food to provide a small amount of food at a time, to be sure that the fish ate all of the diet offered. The trial were continued for 62 days at 28°C and 45 days to 32°C.

At the start of the experiment and at subsequent fortnightly intervals, fish were batch weighted to the nearest two decimal places. (0.01) on a Mettler top-pan balance (Model. PE-3600) in a dish containing preweighed water. At the end of the experiments, fish were batch weighed and dried in order to obtain total moisture and later the carcass were finely ground for subsequent chemical analysis.

Table 4.6.
Proximate analysis of the experimental diets used at 28'C on a moisture free basis with the calculated energy and protein energy ratio.

TEMPERATURE 28°C	1	2	3	4	5	6	7	8	9
Protein	37.47	43.93	46.58	49.75	52.94	54.32	57.48	59.52	59.58
Ether extract.	10.36	10.00	10.98	10.91	10.56	10.66	10.41	11.69	10.79
ASH	10.79	12.04	12.57	12.57	13.67	14.28	14.79	15.67	14.91
NFE [1]	39.51	32.06	28.13	24.25	21.67	19.04	15.16	12.55	8.54
ME (k Cal/g) [2]	3.91	3.94	4.01	4.01	4.03	4.02	4.00	4.11	3.9
P:E ratio [3]	95.83	111.24	116.16	124.06	131.36	135.12	143.7	144.82	152.77

1 NFE = Nitrogen free extractives, calculated as NFE derived from the brown fish meal + starch + dextrin.

2 ME = Metabolizable energy content.

3 P:E = Protein to energy ratio in mg protein/k cal of ME

Table 4.7.
Proximate analysis of the experimental diets used at 32'C on a moisture free basis with the calculated energy and protein energy ratio.

TEMPERATURE 32°C	1	2	3	4	5	6	7	8	9	10	11
Protein	28.88	32.64	38.97	45.11	48.46	50.14	52.72	55.52	60.26	62.17	64.53
Ether extract.	10.99	11.64	10.65	10.88	12.2	10.27	10.33	10.57	10.88	10.45	10.08
ASH	10.06	11.02	11.88	12.0	11.53	12.24	12.67	12.90	14.03	13.07	15.00
NFE[1]	51.61	45.12	38.64	32.17	28.28	25.69	21.80	19.22	15.33	12.74	8.85
ME (k cal/g)[2]	4.04	4.03	4.00	4.08	4.21	4.03	4.01	4.07	4.17	4.13	4.07
P:E ratio[3]	71.49	80.99	97.42	110.56	115.11	124.41	131.47	136.41	144.51	150.53	158.55

1 NFE = Nitrogen free extractives, calculated as NFE derived from the brown fish meal + starch + dextrin.

2 ME = Metabolizable energy content.

3 P:E = Protein to energy ratio in mg protein/kg cal of ME

Fish mortality was recorded daily as required and the weights of dead fish were discounted from the total in order to adjust the daily amount of food offered.

Chemical analysis.

Fish carcasses and all diets were analyzed for crude protein using the Micro-Kjeldhal technique with a Tecator/Kjeltec System 1003 distilling unit (AOAC, 1985). The fat content was determined by extracting dried samples for 4 hours using a soxhlet apparatus and petroleum ether (40-60) and measuring, by weight difference, the amount of ether soluble material extracted. Crude Fiber content was determined by the digestion method with diluted H2SO4 (0.22 5N) and NaOH (0.313N) (AOAC, 1985). Ash content was determined by heating a preweighed sample within a silica crucible in a muffle furnace at 450°C for 12 hours. Moisture was determined by drying a weighed sample in a drying oven at 105°C for 24 hours (AOAC, 1985).

A peroxide value test (AOAC, 1985) was conducted on the fish oil and corn oil to ensure that there were no oxidised oils in the diets. Only oils with less than 4 peroxide meq/Kg were used to prepare the diets.

Nutritional formulae.

The formulae utilized in the nutritional evaluation of the effects of protein on growth and performance of C.urophthalmus are shown in Appendix I.

Statistical Analysis.

Analysis of variance and Duncan's multiple range and F tests were employed in evaluating the significance of the experimental results (Parker, 1979) and Duncan (1955). A dose-response analysis was also used to determine the nutritional requirements of proteins (Zeitoun et al., 1976).

RESULTS

Acceptance of the experimental diets.

The acceptance response for the diets in the fish was always good. The fish ate aggressively, normally eating the food before it reached the bottom of the tank. This behaviour was maintained throughout the whole experimental period.

Growth

The growth response and fortnightly weight increase of C.urophthalmus over the experimental period at 28°C at different protein levels is shown in Figure 4.2. The best growth response in terms of final body weight was observed with the fish consuming diet 6 (51.04% protein) although no significant difference ($P<0.01$) was found between this protein level and diets 3, 4, 5, 7, 8, and 9 (43.51, 46.37, 49.60, 53.98, 55.91, and 56.06% protein respectively). No significant difference was found ($P<0.01$) in final body weight between the fish fed the lower protein diets 1 and 2 (34.67% and 40.60% protein). Growth on these diets was however, significantly lower than that on the higher protein diets, although there was some overlap with diets 3 and 4 containing 43.5%and 46.37% protein respectively (Table 4.8).

A similar response was also observed on the basis of specific growth rate and percentage weight gain, the maximum value always being found for diet 6. This again, was significantly different ($P<0.01$) from diet 1 but no differences were found among the intermediate diets (Table 4.8).

Growth responses at 32°C are summarized in Figure

Figure 4.2.

Individual growth response of *C.urophthalmus* different dietary protein levels at 28°C.

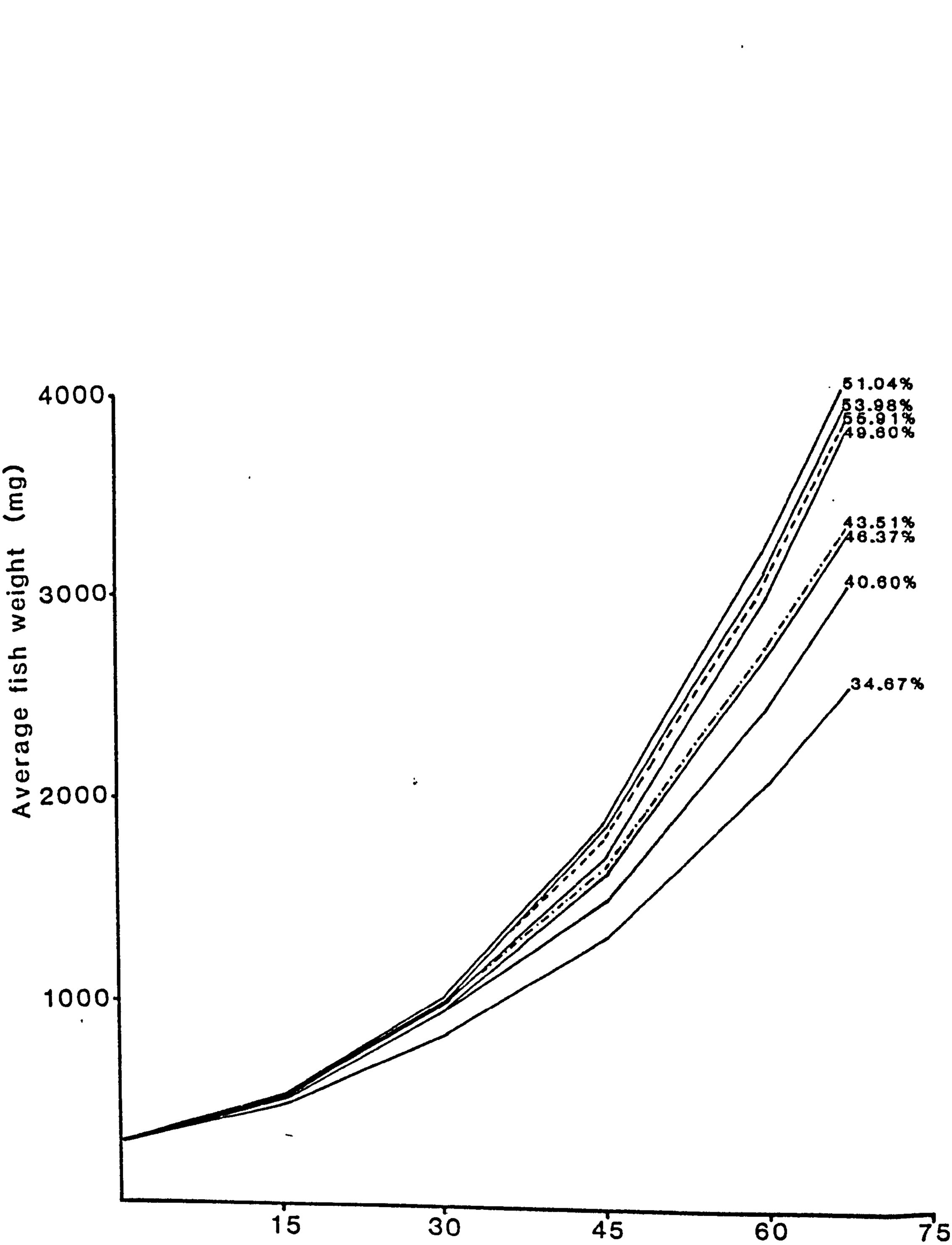

Average fish weight (mg)
4000
3000
2000
1000
15
30
45
60
75
Time (days)
51.04%
53.98%
55.91%
49.80%
43.51%
46.37%
40.60%
34.67%

Table 4.8.
Mean growth performance, feed utilization efficiency and carcass composition of C.urophthalmus fed the experimental diets at 28°C.

MEAN VALUES RESULTS	DIET NUMBER								
	1	2	3	4	5	6	7	8	9
PROTEIN IN FOOD (%)	34.67	40.50	43.51	46.37	49.60	51.04	53.99	55.91	56.06
INITIAL BODY WT. (MG)	303.333a	296.667a	310.00a	306.67a	300.00a	300.00a	306.67a	303.33a	300.00a
FINAL BODY WT. (MG)	2,561.283a	3,068.50ab	3,360.83abc	3,328.17abc	3,927.83bc	4,050.50c	3,950.67c	3,865,61bc	3,757.82bc
WEIGHT GAIN (%)	741.957a	934.14ab	984.14abc	986.18abc	1,175.95bc	1,250.17c	1,199.01bc	1,173.31bc	1,152.61bc
SPECIFIC GROWTH RATE (%/DAY)	3.429a	3.77b	3.84bc	3.84bc	4.10bc	4.20c	4.12bc	4.09bc	4.07bc
FOOD INTAKE (MG/DAY)	53.927a	60.26ab	65.70bc	63.39bc	69.50c	72.28c	71.95c	71.29c	69.53c
WEIGHT GAIN (MG/DAY)	48.793a	58.26ab	66.72abc	66.25abc	76.21bc	82.06bc	79.01c	77.90c	75.31c
FOOD CONVERSION RATIO	1.12c	1.04ab	0.99ab	0.96ab	0.91ab	0.88a	0.91b	0.93ab	0.93ab
PROTEIN EFFICIENCY RATIO	2.60d	2.33cd	2.33bcd	2.25abcd	2.21abcd	2.22abcd	2.03abc	1.94ab	1.93a
NITROGEN INTAKE (MG/DAY)	2.991a	3.91b	4.57c	4.70c	5.52d	5.90de	6.21de	6.39e	6.24e
CARCASS NITROGEN DEPOSITION (MG/DAY)	0.895a	1.27b	1.33b	1.29b	1.46b	1.64b	1.57b	1.59b	1.53b
APPARENT NITROGEN UTILIZATION (%)	29.81cd	32.52d	29.02bc	27.33abc	26.53abc	27.72abc	25.29ab	24.63ab	24.50a

CARCASS COMPOSITION (% WET WEIGHT BASIS)

	INITIAL								
MOISTURE	77.50	74.35 b	71.40a	73.17b	73.42b	74.01b	73.40b	74.13b	74.04b	74.43b
CRUDE PROTEIN	14.92	15.30a	17.50a	16.68a	16.34a	15.98a	16.76a	16.57a	16.99a	16.96a
LIPID	3.59	7.09bc	8.1c	7.21bc	6.76abc	5.79ab	6.07ab	5.66ab	5.33ab	4.05a
ASH	3.65	3.32a	3.70a	3.72a	3.54a	3.59a	3.72a	3.95a	4.03a	4.01a
MORTALITY (%)		5	1.67	0.0	0.0	5.0	0.0	3.33	1.67	3.33

4.3 and in Table 4.9. The best growth response at this temperature in terms of final body weight achieved was observed in the fish consuming diet 9 (56.36% protein) although no significant difference (P<0.01) was found between this protein level and diets 3, 4, 5, 6, 7, 8, 10, and 11 (36.42, 42.19, 45.29, 46.96, 49.20, 51.80, 58.18 and 60.45% protein respectively). No significant difference was found (P<0.01) in final body weight between the fish fed the lower protein diets 1 and 2 (26.8 and 30.41% protein), although growth on these diets was significantly different (p<0.01) from the higher protein diets, (Table 4.9). A similar response was also observed on the basis of specific growth rate and percentage weight gain (Table 4.9).

A trend of rapid linear increment in specific growth rate with dietary protein level was observed both at 28°C and 32°C (Figure 4.4). The upward linear trend was not maintained above 51.6% protein at 28°C and 56% protein at 32°C and at aproximately these points the SGR oscillates around an apparent asymptote.

Protein gain per individual fish over the trial period at both temperatures is shown in Figure 4.5. Again, there is generally a rapid increase with dietary protein level, which approaches the asymptote at 40% protein at 28°C and 50% protein at 32°C.

Figure 4.3.

Individual growth response of C.urophthalmus different dietary protein levels at 32°C.

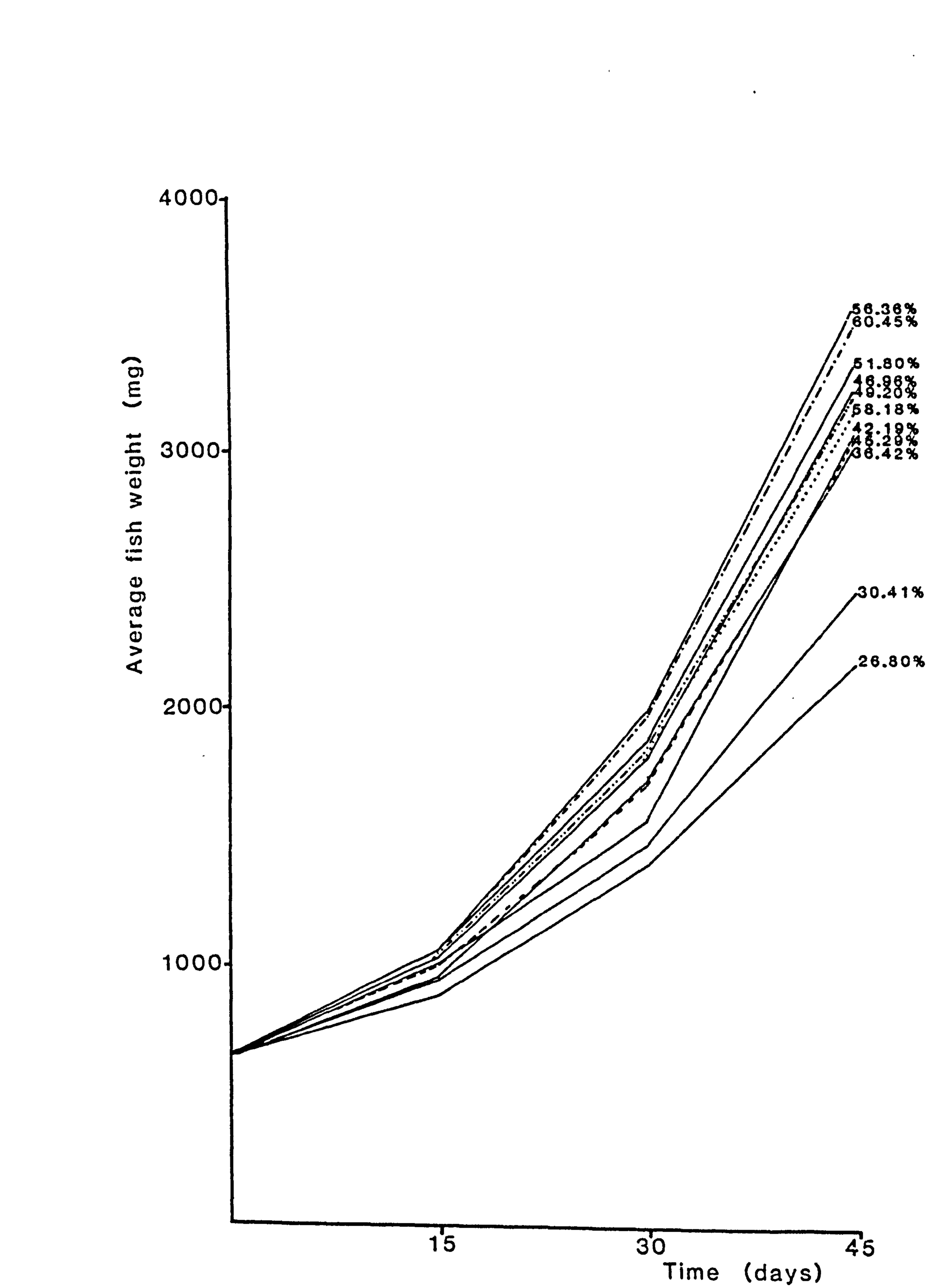
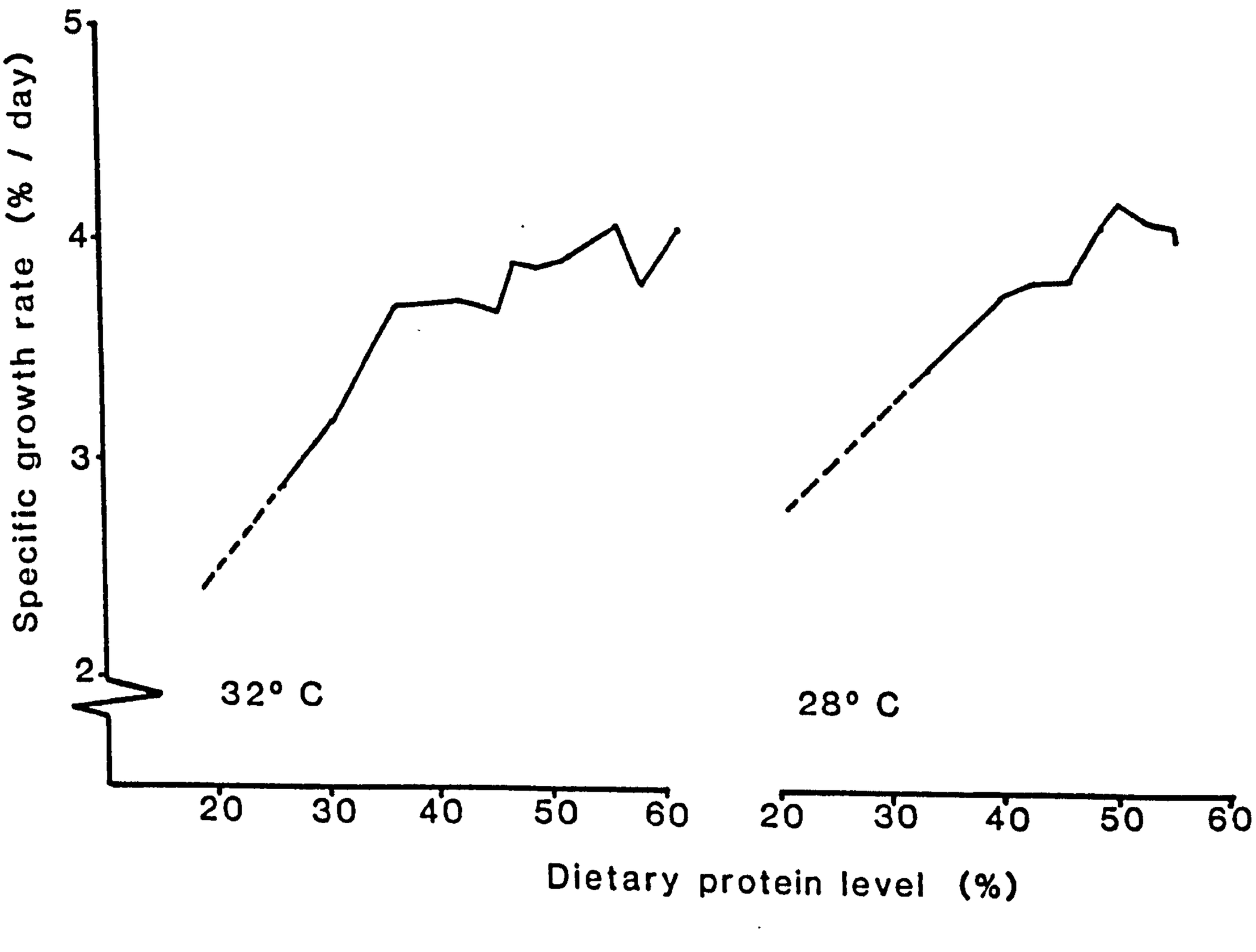

4000
3000
2000
1000
Average fish weight (mg)
56.36%
60.45%
51.80%
46.96%
48.20%
58.18%
42.19%
45.29%
38.42%
30.41%
26.80%
15
30
45
Time (days)

Table 4.9.
Mean growth performance, feed utilization efficiency and carcass composition of C.urophthalmus fed the experimental diet at 32°C.

MEAN VALUES RESULTS	DIET NUMBER										
	1	2	3	4	5	6	7	8	9	10	11
PROTEIN IN FOOD %	26.8	30.41	36.42	42.19	45.29	46.96	49.20	51.80	56.36	58.18	60.45
INITIAL BODY WT. (MG)	663.333a	666.667a	660.00a	666.67a	660.00a	660.00a	663.33a	670.00a	663.33a	663.33a	660.00a
FINAL BODY WT. (MG)	2,176.667a	2,456.67a	3,010.00b	3,060.00b	3,003.33b	3,266.67b	3,223.33b	3,330.00b	3,560.00b	3.183.33b	3.493.33b
WEIGHT GAIN (%)	228.203a	268.53a	356.06b	359.16b	355.05b	394.95b	395.89b	397.02b	436.89b	379.98b	429.29b
SPECIFIC GROWTH RATE (%/DAY)	2.992a	3.18a	3.70b	3.71b	3.69b	3.90b	3.96b	3.91b	4.09b	3.82b	4.06b
FOOD INTAKE (MG/DAY)	63.527a	68.02a	89.72a	71.82a	70.89a	77.94a	72.49a	82.15a	84.86a	72.46a	76.66a
WEIGHT GAIN (MG/DAY)	38.797a	43.79ab	59.94c	57.98bc	57.55bc	64.27c	62.51c	65.91c	70.84c	62.39c	70.24c
FOOD CONVERSION RATIO	1.64b	1.55b	1.51b	1.24a	1.24a	1.21a	1.16a	1.24a	1.20a	1.17a	1.09a
PROTEIN EFFICIENCY RATIO	2.29d	2.12cd	1.83abc	1.91bc	1.80abc	1.76ab	1.75ab	1.59ab	1.48a	1.48a	1.51a
NITROGEN INTAKE (MG/DAY)	2.724a	3.31a	5.23bc	4.95b	5.14bc	5.86 bc	5.71bc	6.81cde	7.65e	6.75cde	7.41de
CARCASS NITROGEN DEPOSITION (MG/DAY)	0.974a	1.11a	1.57b	1.52b	1.56b	1.74bc	1.69bc	1.98bc	1.95c	1.67bc	1.87bc
APPARENT NITROGEN UTILIZATION (%)	35.702a	33.53de	30.04abcde	31.30cde	30.34bcde	29.64abcde	29.43abcde	29.44abcd	25.52abc	24.68a	25.16ab
CARCASS COMPOSITION (%WET WEIGHT BASIS)											
	INITIAL										
MOISTURE	76.97	72.16a	72,88a	72.34a	73.5a	72.99a	72.82a	74.21a	73.35a	74.74b	74.79bc	74.91c
CRUDE PROTEIN	14.56	15.90a	15.51a	16.57a	15.89a	16.48a	16.57a	16.37a	17.39a	16.72a	16.45a	16.44a
LIPID	3.76	9.11d	8.87cd	7.65bcd	7.70bcd	7.41bc	6.96b	6.35ab	6.26ab	5.29a	5.04a	4.66a
ASH	3.99	2.9ab	2.82a	2.94ab	3.05abc	3.23abc	3.40abc	3.35abc	3.32abc	3.37abc	3.61bc	3.74c
MORTALITY (%)		18.33	15.0	43.33	10.0	10.0	18.34	6.67	18.33	25	3.33	8.33

Figure 4.4.

Specific growth rate of C.urophthalmus against the protein level at 32°C and 28°C.


Specific growth rate (% / day)
5
4
3
2
32° C
28° C
20
30
40
50
60
20
30
40
50
60
Dietary protein level (%)

The data on % weight gain and absolute gain in protein by the fish over the experimental period were replotted for 28°C in Figure 4.6 and 32°C in Figure 4.7. This data was analysed using the "broken-line" technique of Zeitoun et al., (1973) in which the significantly different values at the lower protein levels are expressed by a linear regression and a second, horizontal, line is derived from the mean values at the higher protein levels which are not significantly different from each other. The intersection of these lines is taken as an indication of the dietary protein requirement of the fish. It can be seen that at 28°C this suggests a protein requirement of 45.33% and 42.66%, based on % weight gain and absolute protein gain respectively (Figure 4.6) while at 32°C the protein requirements are 39.3% and 39% (Figure 4.7).

Food Conversion Efficiency

At 28°C diets 2 to 9 show significantly better food conversion ratios and there were no significant difference between them ($P<0.01$). These were however, significantly different ($P<0.01$) from diet 1 which had the highest FCR of 1.12. At 32°C better conversion ratios were obtained in the higher protein diets 4, 5, 6, 7, 8, 9, 10 and 11 and these were significantly different from the lower proteins diets 1, 2, and 3,

Figure 4.5.

Protein gained in mg individual *C.urophthalmus* at different dietary protein levels at 28°C and 32°C.

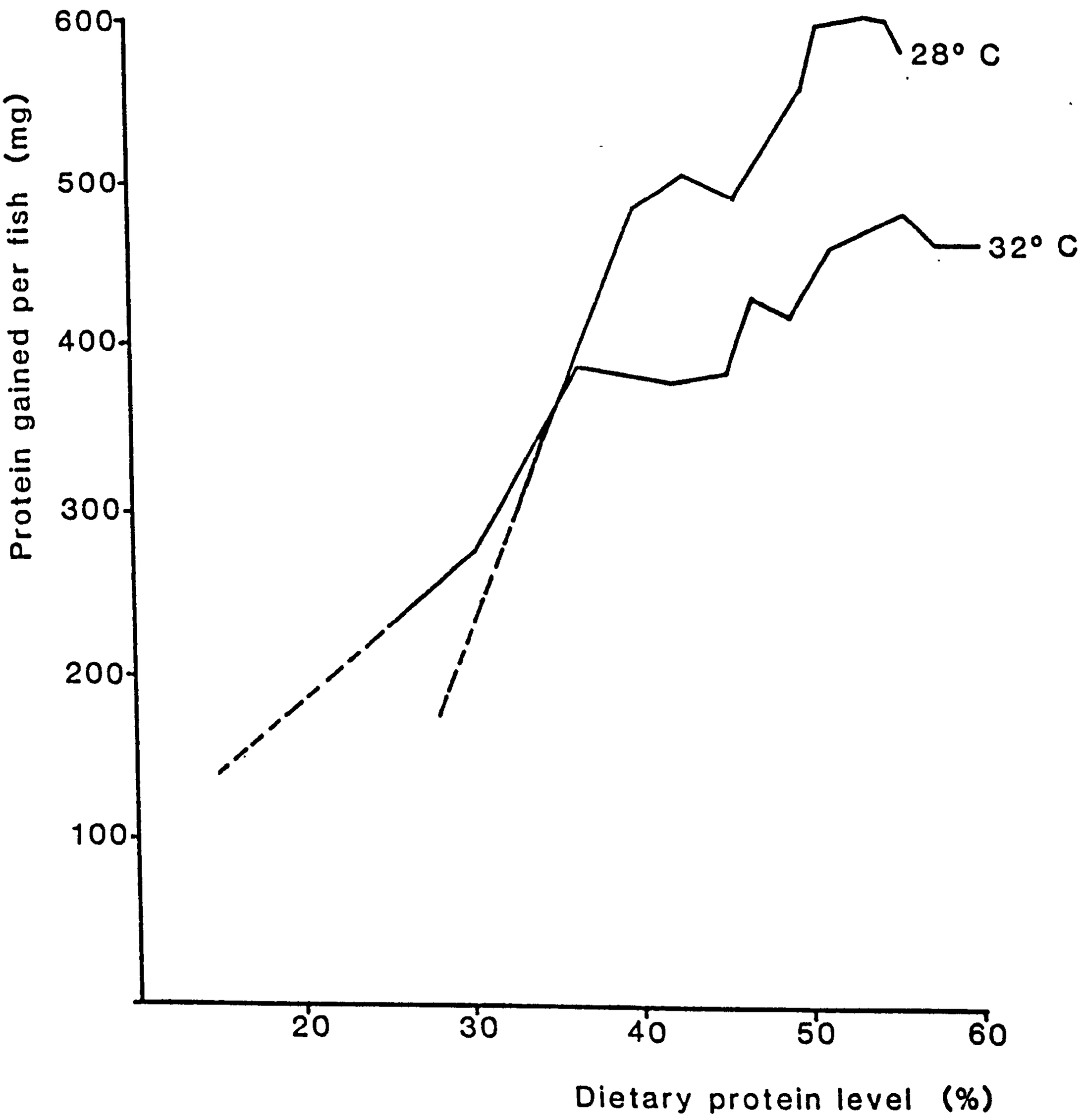

600
500
400
300
200
100
Protein gained per fish (mg)
28° C
32° C
20
30
40
50
60
Dietary protein level (%)

Figure 4.6.

Dose response analysis for 28°C experiment. a)Weight gain dietary protein. b)Protein gain (mg) against dietary protein.

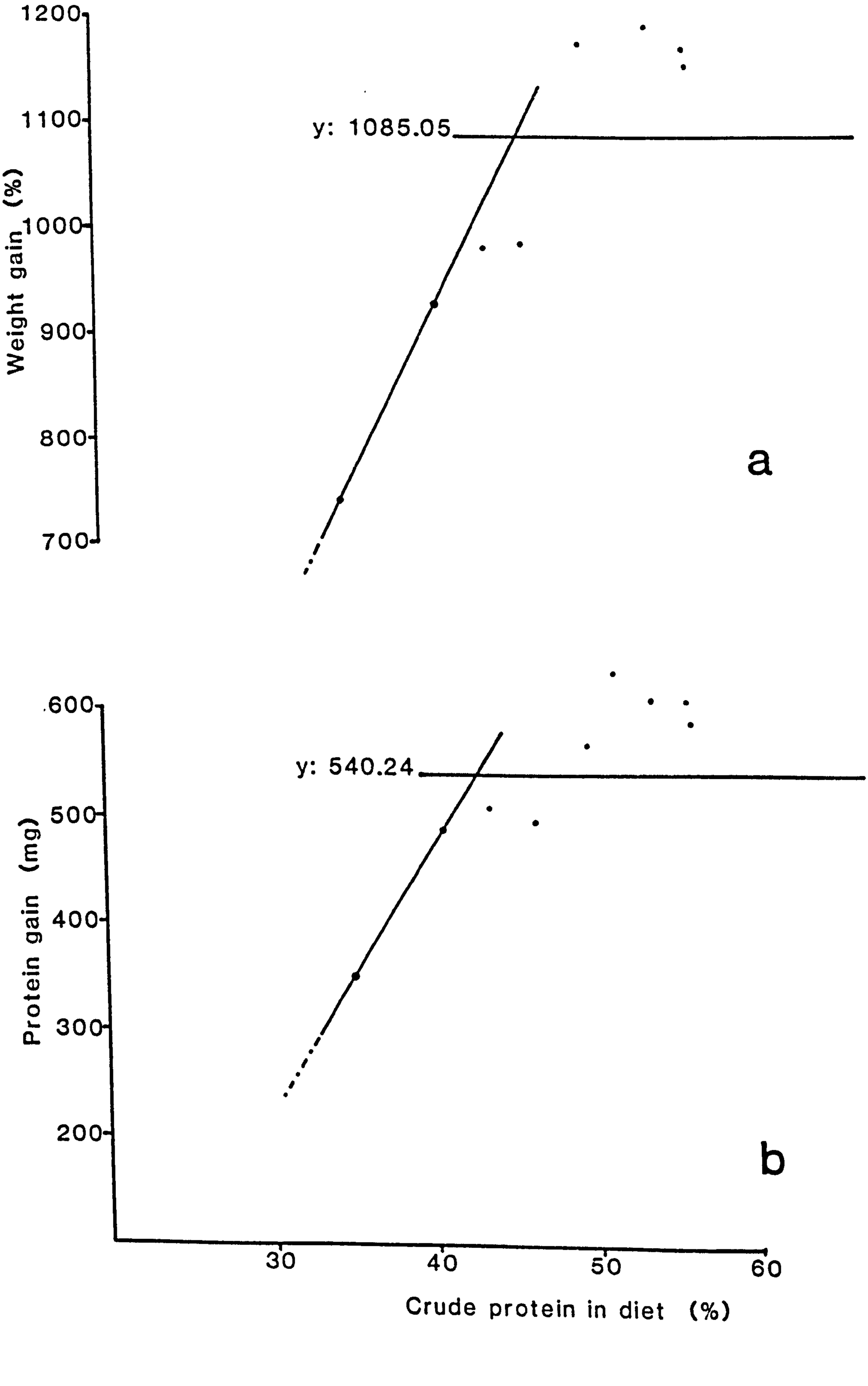
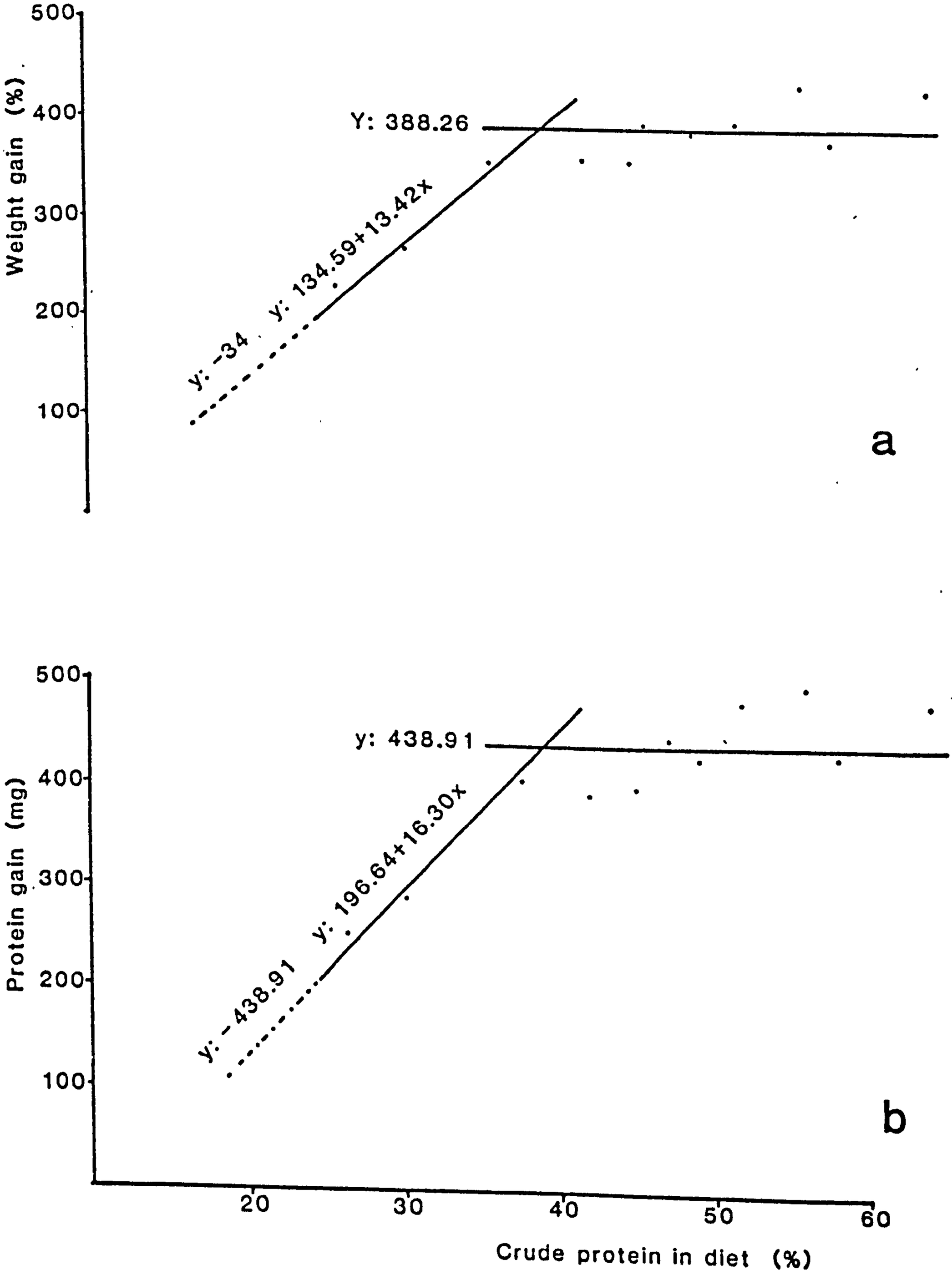

1200
1100
1000
900
800
700
Weight gain (%)
y: 1085.05
a
600
500
400
300
200
Protein gain (mg)
y: 540.24
b
30
40
50
60
Crude protein in diet (%)

Figure 4.7.

Dose response analysis for 32°C.

a)Weight gain against dietary protein.

b)Protein gain (mg) against dietary protein.


Weight gain (%)
500
400
300
200
100
Y: 388.26
y: 134.59+13.42x
y: -34
a
Protein gain (mg)
500
400
300
200
100
y: 438.91
y: 196.64+16.30x
y: -438.91
20
30
40
50
60
Crude protein in diet (%)
b

whose values did not show a significant difference between them ($P<0.01$).

Protein efficiency ratio

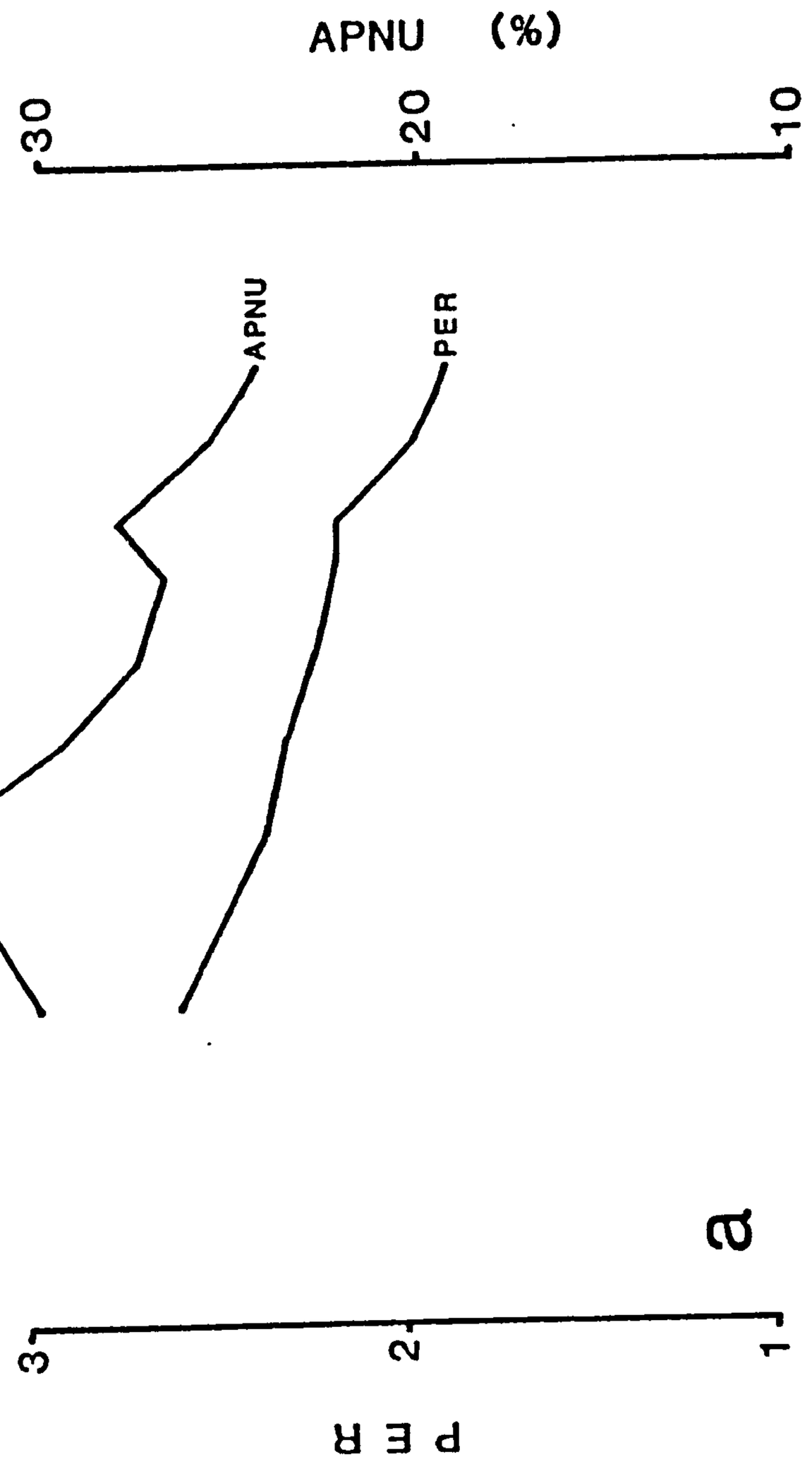
Protein Efficiency ratio (PER) is reduced with increasing dietary protein. At 28°C the lowest value was obtained for diet 9 (59.58% protein) although this was not significantly different from the values for diets 4 to 8. The highest PER was found for diet 1 (34.67% protein) although, again this was not significantly different from the values for diets 2 to 6 (Figure 4.8; Table 4.8). At 32°C, the highest values were for diets 1 and 2, and the lowest group was in diets 5 to 11, with diets 3 and 4 being intermediate (Table 4.9).

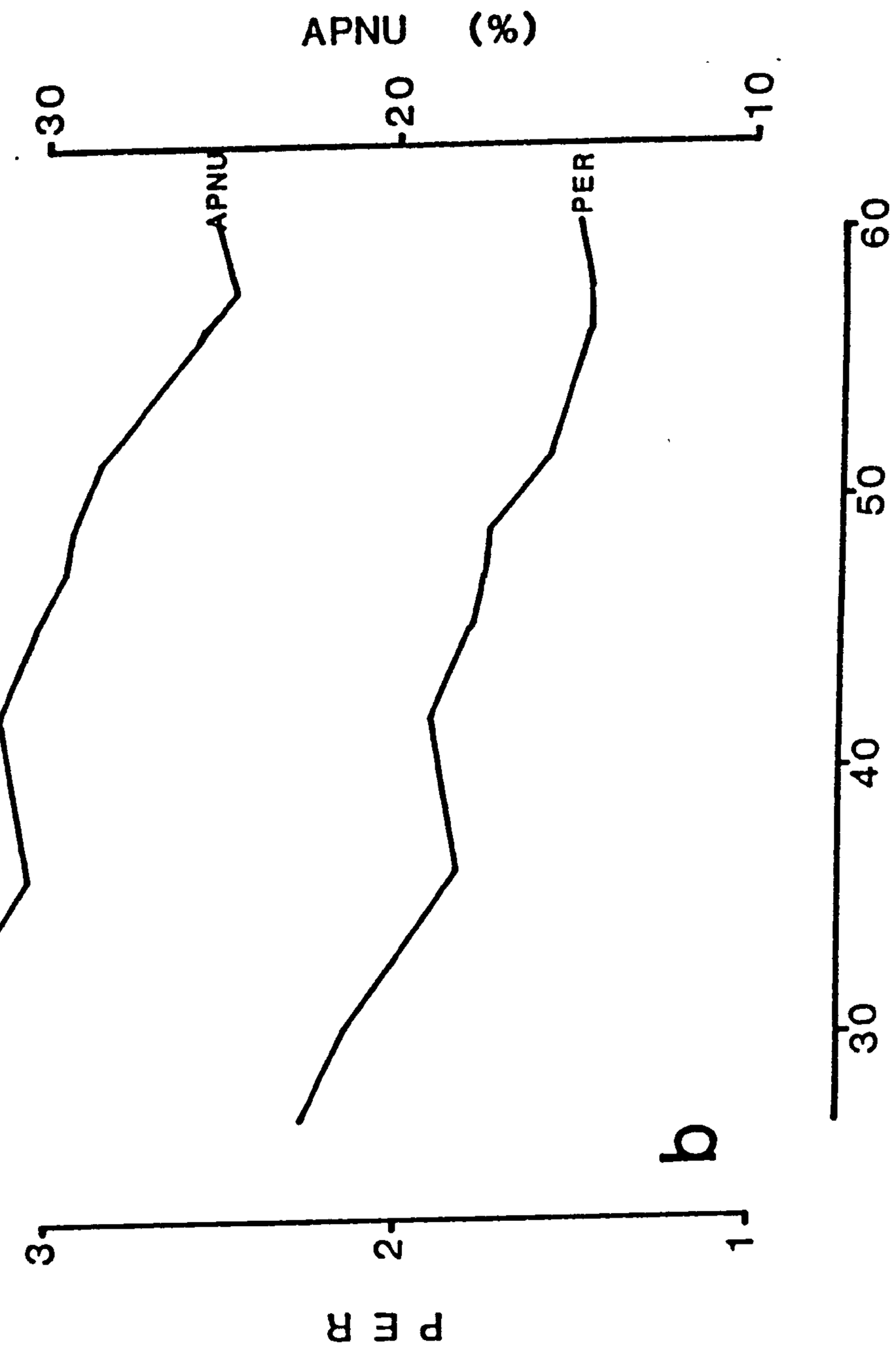
Apparent net nitrogen utilization

The values of Apparent net nitrogen utilization (APNU) obtained show that, as well as PER, there is a strong reduction in APNU with the increment of protein in the diets. Thus, at 28°C the lcwest values of APNU were found in diet 9 with a high value of protein in the diet (56.06%) while the highest was in diet 2 with a dietary protein level of 40.60% both being significantly different ($P<0.01$). The remainder of the diets were in

Figure 4.8.

Protein efficiency ratio (PER) and apparent net nitrogen utilization (APNU) of C.urophthalmus at different dietary protein levels. a)28°C water temperature b)32°C water temperature.


APNU (%)
30
20
10
APNU
PER
3
2
1
PER
a


APNU (%)
30
20
10
APNU
PER
60
50
40
30
Protein level (%)
3
2
1
PER
b

between these values generally decreasing as the dietary protein increased (Figure 4.8; Table 4.8). A similar trend in APNU was found at 32°C, the lowest value being in diet 10 and the highest in diet 1, these being significantly different ($P<0.01$) (Figure 4.8a; Table 4.9).

Carcass composition.

The proximate composition of the whole fish carcass at the start and end of the experiment is shown in Tables 4.8 and 4.9. In general, the body composition at both temperatures was not greatly affected by the administration of different levels of protein in the diets. The fish fed the lowest protein levels tend to have significantly lower moisture contents, higher lipid and lower ash than the highest protein inclusion. With respect to carcass protein, higher levels of protein inclusion in the diets appear to result in higher values of carcass protein but they were not significantly different from the lower dietary inclusion levels ($P<0.01$).

Mortality

At 28°C mortality varied from 0 to 5% (Table 4.8) and survival was thus excellent. At 32°C mortalities

varied from 25% (diet 9) to 3.33% (diet 10) with the notable exception of 43.3% mortality in diet 3 (Table 4.9). Although much higher than at 28°C, these mortalities can be considered as normal at this temperature (Chapter 3).

DISCUSSION

In both experiments at 28°C and 32°C the protein supplied by the Engraulis meal adequately provides the aminoacid requirements of C.urophthalmus, based on a reasonable growth performance, food conversion ratio and the lack of deficiency signs observed during the experiments. It is, however, pertinent to point out that the low value for methionine in the aminogram of C.urophthalmus (Table 4.2) was probably due to the denaturation of this aminoacid during the evaluation method.

In comparing Figures 4.2 and 4.3 the optimum growth of the fish in the present work seems to be influenced directly by the environmental temperature, thus at 32°C the best growth was obtained with 56% dietary protein, while at 28°C the best growth was obtained with 51% dietary protein. These two apparent

maxima were not however significantly different from 36.42% at 32°C and 43.51% at 28°C respectively (Tables 4.8, 4.9). The dose-response analysis of the data showed that the requirement at 32°C is about 39.3% protein when the weight gain is used and 39% protein when the protein gain is plotted. The same analysis applied to the data for 28°C gives values at 45.33% and 42.66% respectively. This slightly higher protein requirement found at the lower temperature using the broken-line dose-response analysis suggests that temperature does have a slight influence on the protein requirement, within the temperature range used in the present experiment. While these differences may be due to the normal range of experimental variation or to the genetic variation of the fish, the findings are in agreeement with the observations of Cho et al., (1985) who suggested that temperature has a very small influence on the protein requirements of the fish, as fish appear to be well adapted to dealing with temperature changes within their thermal tolerance limits. Tacon and Cowey, (1985) stated that the effect of water temperature does not lead to an increased protein requirement. It is clear that increments in temperature accelerate many physiological functions, but the efficiency with which protein and energy are digested are probably not substantially altered within the normal range of temperatures. Possibly the ranges used by DeLong et al., (1958) for

Oncorhynchus tshawytscha were wide enough to produce the strong differences found. However Cho et al., (1985) by analysis of dose-response curves derived from the DeLong experiments, suggested that the experimental design was inadequate, with no plateauing of the response curve being evident at high protein intake in some of the experiments, and that no conclusive results could be taken from such experimental work. On the other hand, in Morone saxatilis, the requirement of protein was increased from 47 to 55% when the temperature was increased from 20.5 to 24.5°C (Millikin, 1982), while in Salmo gairdneri there was no difference in growth at temperatures of 9, 12, 15 and 18°C at protein levels of 35, 40 and 45°C (Slinger et al., 1977). These observations suggest that wide differences in protein requirement can be found if a wider temperature range is used (in the limits of the normal ranges of the species). In terms of aquaculture, however, the investigation of such wide limits has little relevance as the fish will normaly be maintained near the optimum ranges of environmental parameters, in order to obtain the best growth possible in the minimum time, and the extreme temperatures that normally occur in ponds, or other aquacultural systems are occasional.

Both specific growth rate and food conversion efficiency increased linearly at both temperatures up to

the apparent optimum protein requirement level and this was followed by a clear decrease when the protein was increased above that point. A similar response has been observed for S.mossambicus (Jauncey, 1982), Cyprinus carpio (Ogino and Saito, 1970), Pleuronectes platessa (Cowey et al., 1972), Chrysophrys aurata (Sabaut and Luquet, 1973) Channa micropeltes (Wee and Tacon, 1982); and O.niloticus (Wang et al., 1985) and this effect is widely interpreted as being due to a lack of dietary energy required for growth because of the extra energy needed to deaminate an excess of aminoacids in the diet (Jauncey, 1982; Tacon and Cowey, 1985; Cho et al., 1985).

The protein efficiency ratio (PER) and apparent net nitrogen utilization (APNU) showed a clear reduction at both temperatures after they reached 40% protein. Similar observations were described in Cyprinus carpio (Ogino and Saito,1970), Tilapia zillii (Mazid et al, 1979), Oreochromis mossambicus (Jauncey, 1982) and Ctenopharyngodon idella (Dabrowski, 1977).

The carcass composition at both temperatures responded similarly, where the moisture and ash were inversely related to the carcass fat while there was no notable trend in protein content. Similar results have been found for other species, such as Pleuronectes

platessa (Cowey et al., 1974); Salmo gairdneri (Dabrowska and Wojno, 1977); Ictalurus punctatus (Murray et al., 1977); O.aureus (Davis and Stickney, 1978; Winfree and Stickney, 1981); Sarotherodon mossambicus (Jauncey, 1982); O.niloticus (Wang et al., 1985). C.urophthalmus is thus no different from other species in this respect.

The Protein/Energy (P:E) ratio calculated for 28°C at the levels of protein of 42.6 and 45.3 were 111.24 and 116.16 mg protein/Kcal respectively and for the experiment at 32°C at 39% protein level 97.42 mg protein/Kcal was calculated. These values are in the range obtained for the optimum growth of O.mossambicus with 40% protein and a P:E of 116.6 (Jauncey, 1982); O.aureus with 56% protein and P:E of 123 mg Protein/Kcal (Winfree and Stickney, 1981), and T.zilli where 35% dietary protein with a P:E of 103 mg Protein/Kcal was found to give the best growth (Mazid et al., 1979). By contrast, DeSilva and Perera, (1985) working on O.niloticus found that a higher amount of energy in the diet produced a sparing effect on the protein. They found optimum growth for the latter species at 28-30% dietary protein but they did not mention either the P:E utilized or the analysis of fat in the final carcass. Thus, the increase of the weight at high dietary protein inclusion level could be due to excces of fat deposition and not necessarily true proportional tissue growth

(Reinitz et al., 1978; Watanabe, 1977; Watanabe et al., 1979). The use of extra energy in diets should be based on the real requirements of the species, by actually determining the best protein energy ratio (P:E) which will minimize the oxidation of protein as an energy source (Cowey and Sargent, 1979). On the other hand, it must be borne in mind that in terms of practical aquaculture an excess of dietary lipid could result in excessive deposition of lipid within the fish and this may be quite unacceptable to both the consumer and the producer (Cowey and Sargent, 1979), furthermore the possibility of diseases due the fatty fish are not excluded.

From the present experiment it is also possible to observe, from the nature of the diets offered, that the species apparently has the ability to tolerate higher levels of dietary carbohydrates than some of the other members of the cichlid family. However, a specific investigation of this subject would be required to be conclusive.

The mortality of the fish during the trials at both temperatures (28°C and 32°C) was not affected by protein level. The slightly different mortality found in some of the trials at 32°C was due mainly to the effect of the high temperature on growth, and the probable

production of "jumpers" which attack the smaller fish (see Chapter 3).

Comparisons between the protein requirements of different fish are sometimes impossible due to the great variation of technique and approach utilized by different authors. Of prime importance in assessing these differences is the fact that the species and its feeding habits will significantly affect the observed requirements. The source of protein is a further very important variable in this respect. Many authors utilize such different materials as casein, gelatin, white fish meal, brown fish meal and other protein sources that some times are enriched with free aminoacids. It has been demonstrated for some fish species that free aminoacids are not adequately utilized because of their different residence time compared with aminoacids bonded into polypeptide chains, (Cowey and Sargent, 1979). Other sources of variation are different dietary energy levels, different environmental conditions, different feed ratios and one of the most important in terms of the protein requirements is the size of the fish as it is well known that bigger fish tend to require less dietary protein than their fry (Halver, 1976).

Table 4.10 shows the protein requirements for some cichlids by different authors to date. It can been seen that old-world cichlid fry of up to 3g have protein requirements that vary from 35% to 56% with the exception of the work of De Silva and Perera, (1985) who found a 28-30% protein requirement for O.niloticus. Fish from 3g to 30g generally have a reduced requirement of 30 to 35% dietary protein and, as shown by Leong and Tuan (1987), O.niloticus grows adequately when fed between 27.5 to 35% dietary protein at pre-spawning size. All these results are for omnivores or planktivores which have fine pharyngeal teeth to triturate plant material and long intestines with low stomach pH to break down the plant cell walls for later digestion (Trewavas, 1983; Payne, 1986; Weatherley and Gill, 1987). It is interesting to compare this result with the requirements obtained in the present work for C.urophthalmus because the latter species is clearly a carnivore with some occasional omnivorous habits (Chapter 1). As shown in the present work the protein requirements of C.urophthalmus fry are in the same range as these herbivorous cichlids although generally higher than many of the results shown in Table 4.10. It is difficult, however, to make a conclusive comparison due to the different fish sizes used by the different authors. The protein requirements of larger C.urophthalmus are expected to be reduced as has been

4.10.
Protein requirements to date of different species of old world cichlids compared with the protein requirements of Cichlasoma urophthalmus.

PROTEIN REQUIREMENT (%)	INITIAL WEIGHT (g)	EXPERIMENTAL TEMPERATURE (°C)	PROTEIN SOURCE	FEO RATIO	SPECIES	REFERENCE
35 - 40	1.35	25	casein	—	T. zillii	Teshima et al., 1978
35	1.2	25 ± 1	casein	10	T. zillii	Mazid et al., 1979
56	fry to 2.5	31 ± 5	casein	10 - 20	O. aureus	Winfree and Stickney, 1981
34	7.5	31 ± 5	casein	10 - 20	O. mossambicus	Winfree and Stickney, 1981
40	1.8	27 ± 1	white fish meal	6	O. mossambicus	Jauncey, 1982
30 - 35	6 - 30	29 ±	fish meal	3	O. mossambicus	Jauncey and Ross, 1982
29 - 38	3 - 4 cm	—	mixed proteins	3	O. mossambicus	Cruz & Laudencia, 1977
30	3.4	24 - 25	casein	10	O. niloticus	Wang et al., 1985
28 - 30	0.024	24 - 30	fish meal dextrin	6	O. niloticus	De Silva & Perera, 1985
35	0.02	24 - 30	mixed proteins	10 - 15	O. niloticus	Santiago et al., 1982
27.5 - 35.0	Pre-spawing	—	—	—	O. niloticus	Leong and Tuan, 1987
40 %	juvenile	—	—	—	S. galilaeus	Bob-Manuel, 1987
39	0.6	32	brown fish meal	6	C.urophthalmus	This study
42.6 - 45.3	0.3	28	brown fish meal	6	C.urophthalmus	This study

observed in general for most fish (Halver, 1976; N.R.C., 1983).

Overall, these findings show that the dietary protein required by C.urophthalmus is only slighthly greater than the rest of the members of the cichlid family already investigated. Hence, the cost of production of diets for intensive culture of the species will be substantially similar to tilapia diets already in use around the world. This lack of apparent strong difference between the protein requirements of the tilapia species with vegetarian habits and the carnivorous C.urophthalmus further underlines the need for more research on the protein requirements of carnivorous species, as suggested by Cowey, (1975) and Cho et al., (1985), to establish whether there is a clear difference in the dietary protein requirements of vegetarian, carnivorous and ichthiophagous fish.

DIGESTIBILITY STUDIES IN JUVENILES

OF

Cichlasoma urophthalmus

INTRODUCTION.

In the last hundred years nutritionist have shown that many nutrients are essential for the compilation of a complete diet for culture organism, and many feedstuffs have been evaluated as nutrient sources. These materials have been studied not only in terms of nutritional value and chemical composition but also based on the ability of the organisms to digest them. Digestion involves a series of processes in the alimentary tract by which the feeds are broken down in particle size and finally rendered soluble so that absortion is possible (Maynard and Loosli, 1969).

It has been shown that considerable variation exists in the nutrient digestibility of different feedstuffs between fish, and depending on different environmental conditions (Shrable et al., 1969; Lovell, 1977; Stickney, 1979). It is clear that fish are well able to digest fats and proteins (Takeuchi, 1979; Law et al., 1983) but are poorly adapted to digest carbohydrates (Cowey and Sargent, 1979; Furuichi and Yone, 1980; Shimeno, 1982; Spannhof and Platinkow, 1983; Anderson et al., 1984; Kono et al., 1987). Dietary carbohydrates are known to be utilized by various fish, but only limited information is available on their digestibility and metabolism (Neuhaus and Halver, 1969;

Nose, 1971; N.R.C., 1983). A factor which has a major effect on carbohydrate digestibility is the degree of polymerization, thus monosacharides are well absorbed by fish, while dextrin is only moderately digestible and crude starches have comparatively low digestibilities (Singh and Nose, 1967; Neuhaus and Halver, 1969; N.R.C., 1983). Other carbohydrates such as fibres, hemicellulose, lignin and pentosans generally form undigestible fractions in the feed. The growth of some fish species tends to be depressed by the presence of about 8% of dietary fibre and is highly depressed when the fibre content reaches 20%, probably due to the dilution of digestible nutrients through increased bulk or by obstruction of enzyme action (Hilton et al., 1983; Bromley and Adkins, 1984; N.R.C., 1983.

In addition to these problems in utilizing the carbohydrate fraction of a diet, there may also be anti-nutrients found in natural feedstuffs, which can produce problems in the absortion of nutrients through the inhibition of some of the chemical mechanisms utilized during digestion. These factors may inhibit the action of some hydrolases, as for example, the trypsin inhibitors present in many feedstuffs of plant origin,such as raw beans (N.R.C., 1983) or the amylase and protease inhibitors found in raw wheat (Tacon, 1985). In addition one of the most characteristic

chemical stores of minerals found naturally in plants is phytic acid which binds minerals in the form of a complex protein-phytate molecule. Minerals such as Zn, Mn, Cu, Mo, Ca, Mg, Fe, may be bound, effectively lowering the dietary availability of these essential elements because the animals lack phytase (N.R.C., 1983).

Plant materials, such as soybean meal, are cheaper sources of protein that have been utilized traditionally in fish diets. Some plant materials must be restricted in their use as they contain these factors which affect digestibility, either as fibre in the cellulose walls of the plant cells or by the occurence of different antinutrients (NRC, 1983; Tacon, 1985). These factors affect different fish species in different ways (Tacon, 1985), for example, Salmonids are extremely sensitive to the trypsin inhibitors in soybean (Sandholm et al., 1976) and cyprinids are also affected in a similar manner (Dabrowski and Kozak, 1979). By contrast, Reinitz et al., (1978) and Tacon et al., (1983) have suggested that in Salmo gairdneri soybean can adequately replace up to 75% of the dietary fish meal with no deleterious effects.

Thus, in the investigation of a new species for culture where the protein requirements are already

known, such as C.urophthalmus, the question immediately arises of balancing a commercial diet in economical terms based not only on the use of expensive fishmeal but also on cheaper bio-available protein which meets the species requirements. Thus, in a search for appropiate substitutes, a feedstuff may appear from its chemical composition to be an excellent source of nutrient, but will be of little actual value unless it can be digested and absorbed by the fish (NRC, 1983).

The aim of the present study was to investigate, by a simple digestibility assay, the ability of C.urophthalmus to digest different materials which by their origin could present different causes of indigestibility. The materials investigated were soybean meal, containing a trypsin inhibitor; wheat, containing an amylase and protease inhibitors; and the different carbohydrates pure raw starch, dextrin and polypropylene.

MATERIALS AND METHODS.

Experimental animals.

C.urophthalmus juveniles ranging between 30 to 50g mean weight were reared in the aquaculture laboratory at CINVESTAV from wild brood stock as previously described (see Chapter 1).

Experimental system.

An independent experimental recirculated water system was built consisting of six 30l glass aquaria with an inclined glass bottom. The water entered each tank through a pipe with 2mm orifices, situated near the surface and orientated along the width of the aquarium. The water flow was directed along the inclined bottom of the tank, and with this disposition faeces were always washed along the bottom of the aquarium towards the deeper end. In the deeper zone was an cutlet pipe which was as long as the width of the aquarium , so that all the faeces which were carried by the water flow to this end of the tank were collected. The outflow upwelled through a settling column on the right side of the aquarium and faeces settled to the base of these columns

because of its relative density. The faeces could then be collected at the bottom of the column in 150ml conical collection flasks. This method of faecal settling columns was selected as there is a close agreement between the values obtained by the latter method and intestinal dissection or anal suction (Talbot, 1985). The overall system is shown in Plate 4.1.

The faeces-free effluent water from each tank was drained from the top of the sedimentation columns and passed through 3 sedimentation tanks of 150 l. capacity, each containing 6 sheets of fibreglass to aid sedimentation. The water then passed through a biological filter, which consisted of sacks of polystyrene crisps used to increase the surface area for bacterial action. After the filter the water was collected in a sump of 100l from which it was pumped by a centrifugal pump of 0.5 HP to the header tank.

The overall system was maintained in a controlled temperature laboratory at 25± 2°C, with a photoperiod of 12 hours light 12 hours dark. The water temperature in the system was maintained at 27°C± 1 through the use of a 2 kilowatt water-heater in the sump tank.

Plate 4.1.

Two aspects of the experimental recirculated water system a used during the digestibility trials.A) aquaria with inclined bottom. B) Settling columns. C) Valves where the faeces were collected. D) Settling tanks of the recirculated system. E) Biological filter. The arrows show the flow direction of the water.

B

A

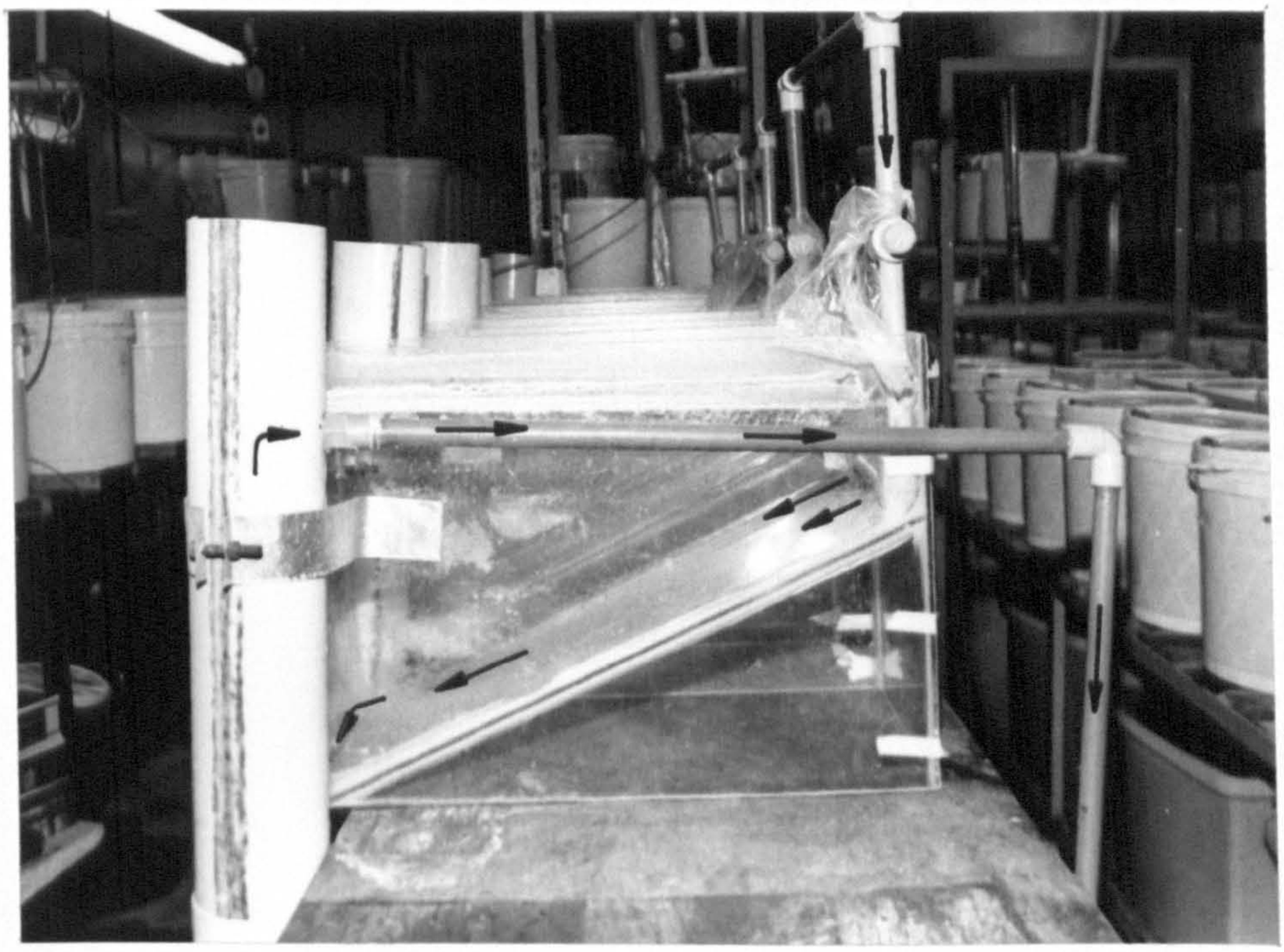
A

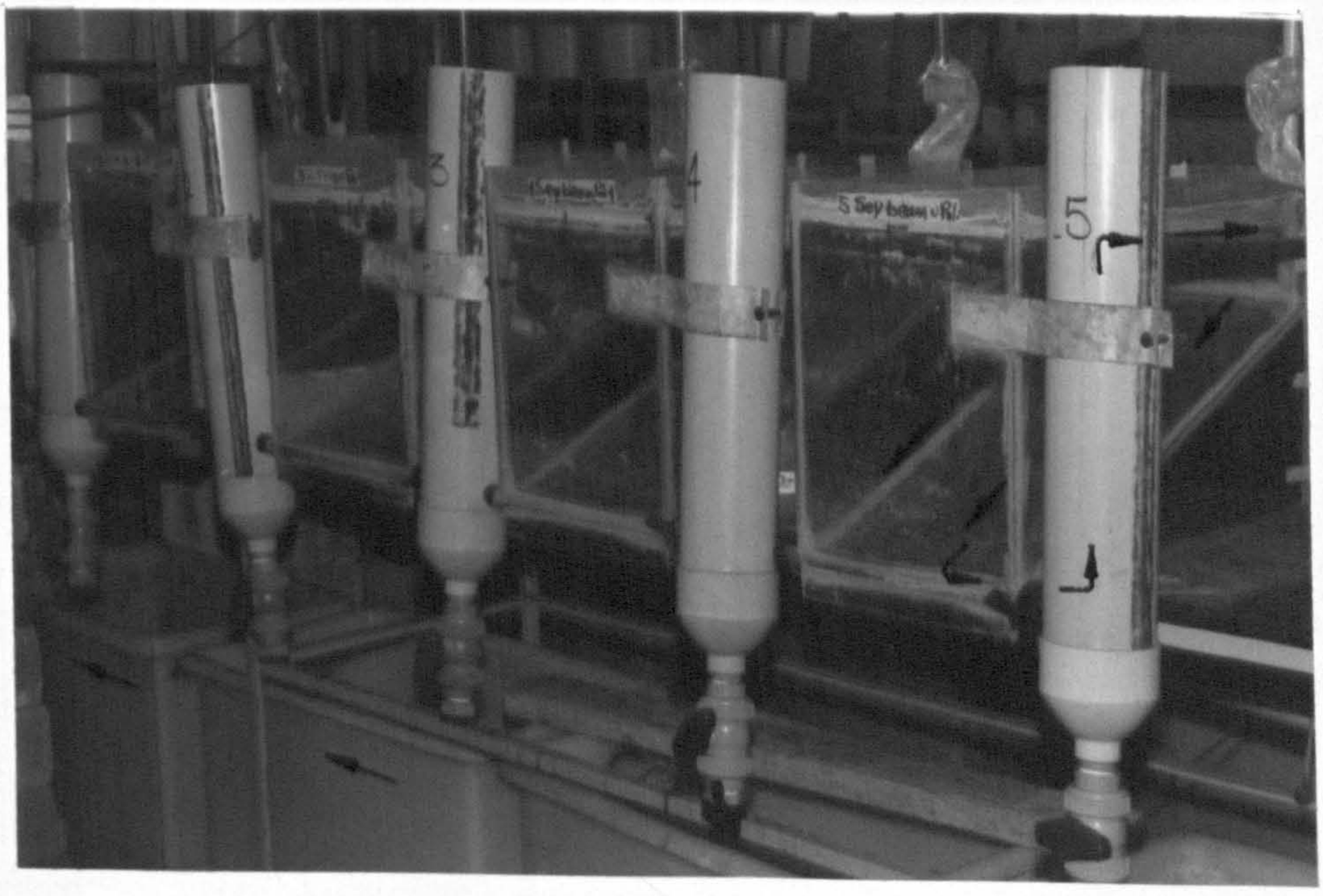
B

E

D

C

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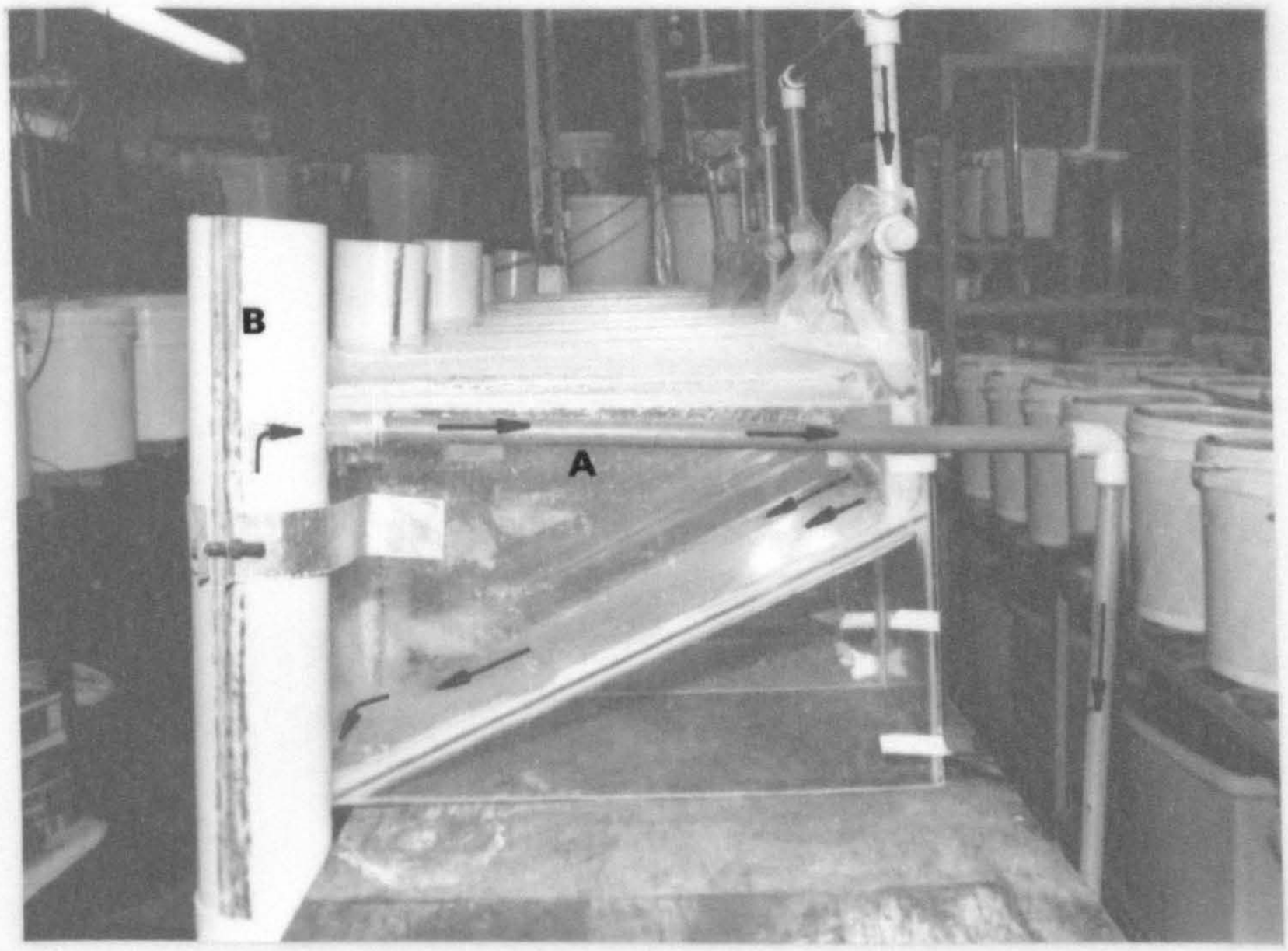
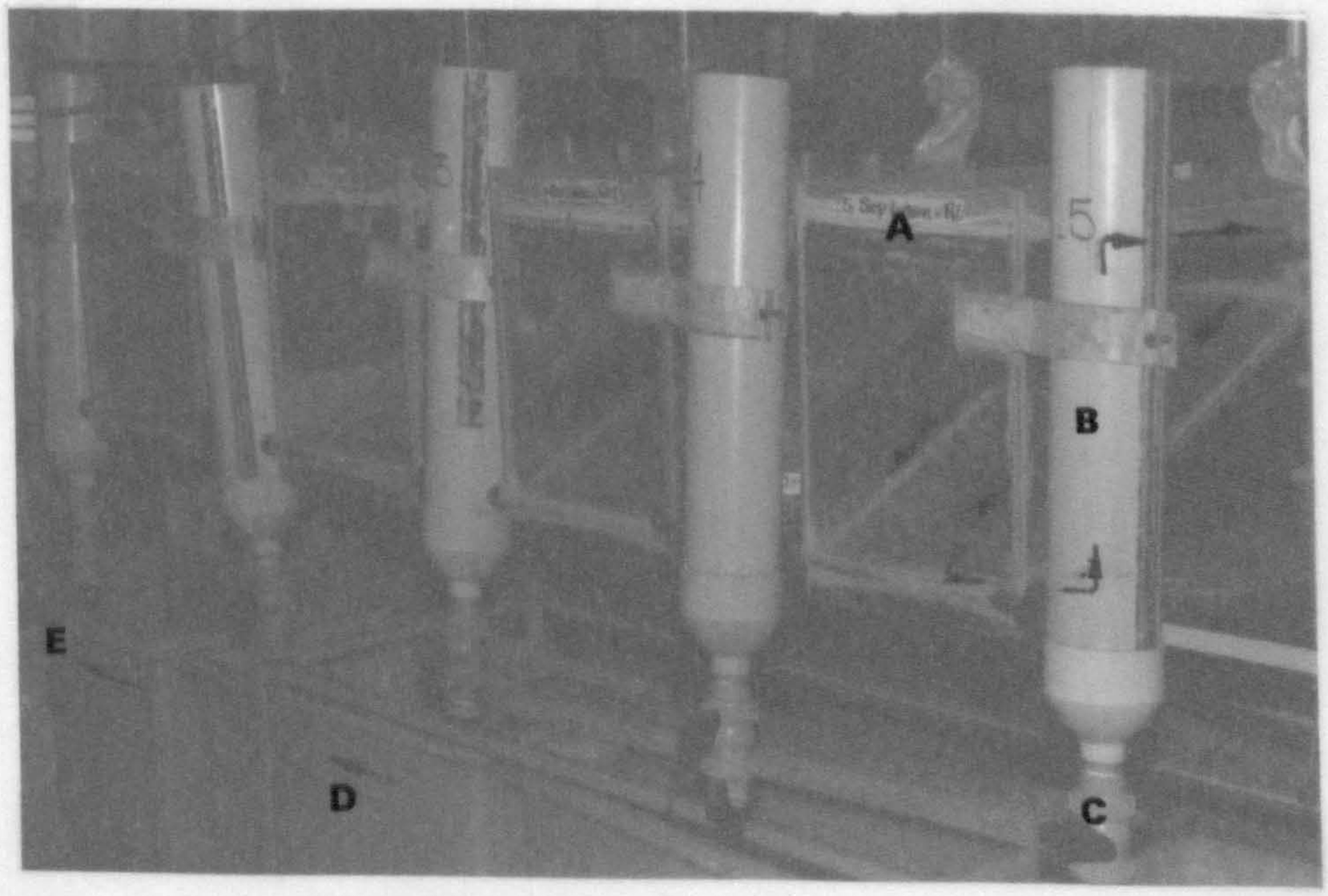

B
A


A
B
E
D
C

Environmental parameters.

The system was filled with city water which had been allowed and oxygenated as in the previous experiments. Replacement of the water in the experimental systems to complete for evaporation and during the weekly cleanings was made with previously aged city water.

Diet preparation.

The experimental diets were formulated, containing brown fish meal as the main source of protein. Variations in the diets were achieved by replacement of fish meal by starch and dextrin, solvent extracted soybean meal, finely ground raw wheat or polypropylene dust. 0.5% chromic oxide was used as a marker in all diets to evaluate the digestibility coefficients.

Proximate analysis was performed on all materials utilized prior to diet formulation and moisture, protein, lipid, ash and nitrogen free extractives (NFE) were determined. The composition of the experimental diets is shown in Table 4.11. Based on the results from Chapter 3 and from the protein level experiments, six diets were prepared and balanced with a protein level of

42%. The inclusion level of mineral and vitamin mixtures was the same as that used in the protein experiment.

Diet 1 was designed to show the effects of wheat inclusion in the diets, but with 3.99% of added dextrin. Diet 2 was identical to diet 1 but in this case the wheat was the only source of carbohydrate with 3.99% polypropylene instead of dextrin. Diet 3 was used as a control for diets 1 and 2 and as a comparative control for diet 4 where the carbohydrate was supplied by raw starch. Diet 5 was designed to show the role of soybean as a source of either carbohydrate or protein, where the carbohydrates were subtituted as in diet 2 by polypropylene. Finally diet 6 was designed as a comparative control for diet 5 using soybean, but the source of artificial fibre (polypropylene) was substituted by dextrin (Table 4.11). All diets were maintained in air tight containers in a freezer at -15°C. Small portions (about 10g) of each diet were placed in the fridge (-4°C) for daily feeding.

Experimental protocol.

The experimental fish were stocked at 15 fish per tank, three tanks per treatment. Prior to starting the experiments, fish in tanks were acclimated for one week,

Table 4.11.
Composition of the experimental diets utilized for the digestibility studies and their respective proximate analysis.

INGREDIENTS %	DIET NUMBER 1	2	3	4	5	6
Brown fish meal	57.56	57.56	63.96	63.96	44.77	44.77
Wheat meal	26.7	26.7	-	-	-	-
Dextrin	3.99	-	22.22	-	-	14.0
Raw starch	-	-	-	22.22	-	-
Polypropylene [1]	-	3.99	-	-	14.0	-
Defatted soybean meal	-	-	-	-	25.81	25.81
Cod liver oil	.44	.44	-	-	1.42	1.42
Corn oil	3.31	3.31	5.82	5.82	6.0	6.0
Cellulose binder	3	3	3	3	3	3
Vitamin premix	3	3	3	3	3	3
Mineral premix	1.5	1.5	1.5	1.5	1.5	1.5
Indicator (Cr_2O_3)	0.5	0.5	0.5	0.5	0.5	0.5
NUTRIENT CONTENT	(% WET WEIGHT)					
Moisture	7.08	5.26	7.06	4.77	4.57	5.56
Crude protein (NX6.25)	42.47	43.31	42.54	41.64	42.29	41.02
Lipid	8.55	12.90	12.32	14.14	8.35	10.46
Ash	11.18	11.62	11.31	11.80	11.47	10.49
Fibre	1.24	6.00	1.046	0.55	17,27	1.81
Carbohydrates	28.95	20.48	25.25	26.26	15.48	30.24
Cr_2O_3	0.53	0.43	0.47	0.44	0.57	0.42

1. Aldrich Chemical

feeding them with the experimental diets to satiation. Faeces were not collected until the third day of feeding to be sure that the faeces truly corresponded to the diets offered; in other words it is important to feed the fish with the diets containing the indicator for a period longer than the gut evacuation time (Talbot, 1985).

The feeding regime used during all trials was to satiation. The frequency of feeding was maintained at 2 times a day, seven days a week. Care was taken when giving the food, to clean the whole system, starting from the bottom of the aquaria to the settling cones to avoid any mixing of faeces and unconsumed food.

Faeces were collected every morning and afternoon before the meals by closing the valves and removing the 150ml collecting flasks. The fish were then fed and the systems were washed out as described above. After collection the faeces were placed in glass petri dishes and dried overnight at 105°C. The dry faeces were placed in a vacuum drier until cool and later the faecal material was carefully removed from the petri dishes, powdered in a mortar and pooled with the previous collections corresponding to each diet. This material was then stored in air-tight containers in a freezer until the end of the experiment for chemical analysis.

The fish were maintained under the experimental conditions for 60 days on each diet to ensure that enough faecal material was available for all analyses.

Chemical analysis

Proximate analysis of fish carcasses and diets were conducted as previously described. Fibre content was determined after digestion with diluted H2SO4(0.225N) and NaOH (0.313N) (AOAC, 1984). Digestibility was determined using the wet acid method of Furukawa and Tsukahara, (1966). Each diet and their respective faeces was tested on three occasions and all analyses were performed in triplicate. The calculation of digestibility was made using the digestibility evaluation proposed by Maynard and Loosli, (1969), the relevant formulae are shown in Appendix 1.

Statistical Analysis.

Analysis of variance and Duncan's multiple range and F tests were employed in evaluating the experimental results (Parker, 1980; Duncan,1955).

RESULTS.

Diet acceptance.

As in previous experiments the experimental diets were taken without difficulty. The fish ate aggressively and this behaviour was maintained throughout the whole experimental period.

Environmental parameters.

Average water quality for the experimental period were: Temperature 27.5±1.2°C; pH 8.67±0.2; Oxygen 6.2±0.5 mg/l and ammonia 0.004±0.002 mg/l.

Digestibilities.

Table 4.12 shows the results of proximate analysis of the faeces for each diet, together with the results of dry matter digestibility, and the digestibilities of protein, fat and carbohydrate.

The highest value for dry matter digestibility was found in diet 3 (82.59%) where the only source of carbohydrate was dextrin. However no significant

Table 4.12.
Proximate analysis of fish faeces when fed on different diets.

NUTRIENT CONTENT IN FEACES (% Dry matter)

	DIET NUMBER					
	1	2	3	4	5	6
Crude protein	26.20	21.76	28.51	23.76	12.78	19.99
Lipid	.59	1.70	0.73	1.89	1.93	2.05
ASH	36.73	28.65	38.41	44.87	17.81	34.29
Fibre	7.73	26.00	4.59	4.64	52.74	10.31
Cr_2O_3	2.81	2.26	2.70	2.23	1.35	1.84
Carbohydrata	25.94	19.63	25.06	22.61	13.39	31.52
Dry matter Digestibility	81.14bc	80.69bc	82.59c	80.14bc	57.86a	77.18b
Protein digestibility	88.37a	90.22a	88.33a	88.67a	87.24a	88.88a
Fat digestibility	98.70c	97.40c	98.97c	97.34c	90.25a	95.52b
Carbohydrate digestibility	83.10b	81.54b	82.71b	83.15b	63.51a	76.21b

difference ($P<0.01$) was found between diets 1, 2 and 4, where the main sources of carbohydrates were wheat (diets 1 and 2) and raw starch (diet 4). Dry matter digestibility in diets 1,2 and 4 was not significantly different ($P<0.01$) from diet 6, where the main source of carbohydrate was supplied by soybean meal and 14% of dextrin. The lowest value of dry matter digestibility was found in diet 5 and thus was significantly different from the other diets ($P<0.01$). In this diet the source of carbohydrates was soybean meal and 14% polypropylene used as a filler to test the capacity of the soybean as a source of digestible carbohydrate.

A high protein digestibility (more than 87.24%) was found in the six diets tested and no significant difference ($P<0.01$) was observed between the protein digestibilities, irrespective of the protein origin (wheat, soybean and fish meal). No deleterious effects in the protein digestibility due to the presence of anti-nutrient factors could be detected in any diet.

High values for fat digestibility were recorded, all diets being in excess of 90%. However, diet 6, where soybean was used (25.81% substitution) showed a significant difference ($P<0.01$) from diets 1, 2, 3 and 4 and the lowest value (90.25%) was obtained in diet 5 in which 25.81% of soybean meal was substituted and 14%

polypropylene was used as a filler. This was significantly different (P 0.01) from the rest of the diets.

The values obtained for carbohydrates, digestibility were high in diets 1, 2, 4 and 6 with no significative difference between them (P<0.01). However, diet 5, again, had a signifficantly lower (P<0.01)carbohydrate digestibility.

DISCUSSION.

In terms of protein digestibility, it is clear from the high values observed that no appreciable effects of antinutrients were shown in any diet. Diets 1 and 2, containing 26.7% of crude wheat meal and diets 5 and 6 containing 25.81% of solvent extracted soybeanmeal all performed well. This result opens the possibilities of utilizing wheat as a main source of carbohydrate and soybean meal as a partial fish meal protein substitute. Experimental work must, however, be done to determine the maximum inclusion level of soybean meal in practical diets, to reduce costs.

The high digestibilities observed for the fish

and corn oil demonstrate that this species has the ability to utilize these dietary fat sources efficiently. These results compare favourable with similar data obtained for carp, where 90% digestibility was measured using similar oil sources (Takeuchi, 1979). The relatively low value for diet 5 (90.25%) was possibly due to the high amount of fibre in this diet, increasing the velocity of the chyme in the intestinal tract, thus reducing residence time and consequently the digestibility (Buhler and Halver, 1961; Smith, 1971; Learly and Lovell, 1975; NRC, 1983). The low fat digestibility of diet 6 (95.52%) in comparison with diets 1, 2, 3 and 4 was possibly again due to the high carbohydrate level, and this may have acted similarly to the fibres, increasing the chyme velocity and reducing the digestibility (Spannhof and Plantikow, 1983).

As with protein digestibility, values for carbohydrates digestibility were generally high. The low digestibility found in diet 5 where the only source of carbohydrates was soybean was probably due to the low digestibility of soybean carbohydrates (54%) as was observed previously by Cho and Slinger, (1979) and secondly due to the reduced residence time of the chyme,as pointed out above, caused by the polypropylene in this diet which acts as a fibre (Anderson et al, 1984). It is interesting to observe that there were no

deletereous effects in diets 1 and 2 (26.7% wheat meal) and diets 5 and 6 (25.81% soybean meal) due to anti-nutrients when compared with diets 3 and 4 which contained antinutrients-free sources of carbohydrates.

The dry matter digestibility is the combination of the partial digestibilities of all the materials in the diets. In the present experiment the origin of the carbohydrates affected the digestibility of the dry matter directly. Thus, diet 3 containing a high percentage of dextrin had the highest digestibility values to dry matter. This was followed by diet 4 in which the principal carbohydrate source was raw starch, diets 1 and 2 in which wheat meal was added and diet 6 in which soybean meal and 14% dextrin were utilized. The lowest value for dry matter digestibility was obtained in diet 5, again, due to the low digestibility of the soybean carbohydrate and the inclusion level of fibre.

It is evident, on the basis of these experiments, that no effects of anti-nutrients were shown on the digestibility of protein and carbohydrates with the materials used and at the inclusion levels in the present experiment. It was also demonstrated that dextrin had a higher digestibility compared with raw starch, and similar results have been observed by other authors (Spannhof and Pantikow, 1983; Singh and Nose,

1967; Smith, 1971; Rychly and Spannhof, 1979; Bergot and Breque, 1983; Anderson et al., 1984).

Clearly, the digestibility of diets is reduced in C.urophthalmus when high percentages of fibre are used in the formulations. Similar results were observed in other fish such as Ictalurus Punctatus (Learly and Lovell, 1975), Salmo gairdneri, (Bergot, 1981; Hilton et al., 1983), Chrysophrys major and Seriola quinqueradiata (Shimeno, 1982; Kono et al., 1987) where more that 10% of dietary fibre considerably reduced growth.

This study has provided guidelines for future work in which it will be neccessary to test different regionally-available protein sources in order to reduce food costs for culture of C.urophthalmus.

GENERAL DISCUSSION

The most general problems associated with introduction of an exotic species to a region for aquaculture purposes have been outlined throughout in this thesis and it is time to say that all of these difficulties have been encountered at various times in different parts of the world. In addition to the serious biological impacts of introductions there can also be substantial problem of creation of a new market, based upon the acceptability and assured availability of a new product. For these reasons it is important that the developing world aquaculture industry considers the suitability of existing unexploited species within a region and much research remains to be done to break away from what could easily become a sterotyped industry based on a limited number of species. With these premises in mind this project has attempted to evaluate some of the important features of Cichlasoma urophthalmus for aquaculture in Central and South America.

Among the most important and desirable features of a species with potential for aquaculture, is the range of tolerances to environmental factors, which will simplify problems of husbandry and may permit wider utilization of such species in different geographical areas. These conditions include variations in oxygen tension, temperature, salinity, sun light intensity, pH,

residual metabolites such as ammonia or nitrites and pollution wastes that can affect water quality (Webber and Riordan, 1976). It is very well known that species with wide tolerance limits for more than one factor such as euryhalines and eurytherms have been succesfully cultured at different scales in many parts of the world. Examples of these are the eels, some trouts, carps and tilapias. Other species with very narrow ranges of tolerances tend to be restricted to specific geographical areas.

In the present study Cichlasoma urophthalmus, has been shown to survive and grow well in the temperature range of 20 to 36°C. It's optimum growth was at 33°C and its maximum production (Growth and survival) was at 28°C. This characteristic is similar to many of the African cichlids actually cultured, such as O.aureus, O.mossambicus, T.rendalli and O.niloticus (Avault and Shell, 1966; Stickney, 1986). The species is thus well suited to culture in tropical and subtropical regions, but is probably not sufficiently adaptable to low temperatures to warrant consideration in areas where overwintering may be required.

In terms of salinity tolerances, C.urophthalmus showed a substantial resistance to abrupt changes from fresh water to different brackish water

concentrations and displayed a good adaptability and growth at different salinities, up to, and including, full strength sea water. These results suggest that this species can be cultured from freshwater to a wide range of salinities, as is the case in many African cichlids (O.aureus, O.mossambicus, O.niloticus, and T.zillii; Smith, 1986), with the clear option of using this native species in lagoon systems along most of the Atlantic and Pacific coasts of Southern Mexico and Central America.

The oxygen requirements of C.urophthalmus are not substantially different from the requirements of other Cichlids and they can probably be grown succesfully in D.O.'s down to 3.5 mg/l. They have also demonstrated a high resistance to anoxic environments, resisting total oxygen depletion for almost two hours. This is an important characteristic, of relevance in culture offering a buffering capacity while the normal conditions of culture are restored.

The tolerances and optima derived from field and laboratory studies of the basic parameters of temperature and oxygen in this work, demonstrated that Cichlasoma urophthalmus is a highly versatile species for the tropics and this characteristic is shared with the other members of the family studied and cultured, such as O.mossambicus, O.niloticus, O.macrochir,

O.aureus and Tilapia rendalli (Caulton, 1982; Chervinski, 1982; Balarin and Haller, 1982). With this basis it is meaningful and possible to continue with a variety of further studies of its potential in semi-intensive, and intensive aquaculture in different environments.

One of the most relevant characteristics of the old-world cichlids is their reproductive precocity and consequent stunting, which causes a declination in the progress of its culture. Recently, many new techniques have had to be developed in order to combat this problem such as hybridization, the utilization of different strains and the use of hormones for monosex cultivation (Guerrero, 1982; Huet, 1986). In this respect C.urophthalmus has the advantage that the minimum size of maturity appears to be about 50g (102mm SL). The species thus invests energy in growth rather than in reproduction, at least up to this size. Another advantage in the type of reproduction of this species is the strong parental protective behaviour towards the fry and also the space required for the brood to reproduce, because in crowded culture conditions reproduction is drastically inhibited. On the other hand, in cage culture the geotactism of the fry will avoid any chance that the recently hatched fry will remain near the parents since they will cross the net when swimming to the bottom.

Most of the more important tilapias in aquaculture are mouth brooders (O.mossambicus, O.niloticus, O.aureus) and this characteristic has some disadvantages over the substrate spawners. Generally, the former do not stop reproducing even in intensive systems in which the bottom spawners cannot reproduce (Balarin and Haller, 1982; Rana, 1986). C.urophthalmus is a bottom spawner and is strictly dependant upon access to the substrate for reproduction, as this species has sticky eggs. Furthermore their recently hatched fry have a strong geotactic response and they maintain this substrate dependance for several days. For these two reasons C.urophthalmus is probably unable to reproduce in cages and in intensive systems where the spawning and essential mating behaviour is virtually destroyed by the lack of an adequate surface for incubation and fry development.

A further characteristic of the mouth brooders, compared with substrate spawners, is their reduced fertility in terms of egg production per spawn for females of equivalent weight (Table D.1). The high fecundity of C.urophthalmus (4 to 6 times, that of mouth brooders) gives this species the advantages of reduced management and reduced number of brood stock to produce the same number of eggs and fry compared with the mouth brooders in hatchery production (Table D-1).

Table D.1.
Predicted fecundities of three sizes of O.niloticus, O.mossambicus and C urophthalmus females in relation to number of spawnings required for target production of 100,000 egg/month (=1.2 millions/year).

Female Weight (g)	Total fecundity (no.eggs/spawning)			Number of spawnings required/month		
	O.niloticus[2]	O.mossambicus[2]	C. urophthalmus[1]	O. niloticus[2]	O.mossambicus[2]	C.urophthalmus[1]
100	529	621	3025	189	161	33
200	782	818	4550	127	122	21
300	983	960	6075	101	104	16

1 Fecundities determined from regression equations (given in chapter 1)

2 Data of tilapias taken from Rana (1986)

The reduced number of degree days to free swimming of the bottom-spawners (144-168) compared with that of the mouth brooders (216-264), means that effort, costs, time and management in the hatcheries will be less for the same production of eggs and fry of this type of cichlid. This is clearly a further advantage of C.urophthalmus over the introduced mouth brooders currently cultured in Mexico.

The behaviour of C.urophthalmus during care of the fry, make this species an ideal fish for production of fry with the minimum facilities and high viability, it only being necessary to provide the brood stock with adequate space, free of predators. This approach could be used to produce enough fry for low-technology systems or even for intensive systems. The mouth brooders in intensive systems require special spawning techniques and incubation systems to keep eggs and fry out of the mouth of the parents to increase viability. This management directly affects the incubation period (Rana, 1986). The minimal territorial requirements of C.urophthalmus in intensive conditions and its ability to spawn throughout the year with some temperature control reduces the costs of specially designed equipment, cost of maintenance of brood stock and fry handling. At the simplest level, only simple multipurpose tanks of almost any material available are

required for maintainance of brood stock, reproduction, incubation and ongrowing of fry for supply to the ongrowing systems.

The behaviour of cleaning, digging and color pattern of mature fish and their special barred colouration during coupling may be of practical importance in hatchery management as the putative couple can be easily recognized. Similarly, in systems of lower intensity the ability to recognise those animals which are ready to spawn means that the formation of the couple can be followed and the fry can be collected easily when required after hatching, or even later, and thus an efficient extensive fry production system can be easily envisaged.

The investigations of the dentition, gut structure and the food habits of C.urophthalmus in the natural environment revealed it to be a carnivorous fish eating mainly invertebrates, and with some sporadic omnivorous habits. In this respect this species is somewhat different to the cultured tilapias which are generally vegetarians feeding on plankton and bottom algae with some opportunistic carnivorous habits (Jalabert and Zoar, 1982; Trewavas, 1983). This habit often confers to the latter a typical muddy off-flavor especially in extensive culture and for this reason the

acceptability of C.urophthalmus in local markets is higher when offered together with the tilapias. These factors must be taken into account carefully when planning developments in aquaculture because acceptability and market price could be the ultimate deciding factors in assessing the potential of a species to be cultured (Chaston, 1987).

The dietary protein requirements found for C.urophthalmus were 39-45% crude protein and these values are similar to those found for the other commercially cultured cichlids at the same stage. The growth rate of the species is also similar to other fast growing cichlids cultured commercially. Thus, the Central American cichlids can be considered as alternatives for culture using fully balanced diets in intensive systems. It is clear that the species adapts well to artificial diets and that the entire life cycle of the species could be grown in this way with excellent food conversion ratios. The feasibility of formulation of diets where fish meal must be reduced to make the diets commercially viable is clearly possible, and is enhanced by the good digestibility of the major low-cost components used normally in fish diets by juveniles of C.urophthalmus.

Based on the findings in this thesis, a series of formulations for the culture of fry, juveniles and adults of C.urophthalmus are proposed in Appendix 2, based on the understanding that the proposed diets are just the first step towards a fully balanced diet for this species and that much more work must be done to produce better, reliable diets with lower costs. Fish meal and soybean meal have become widely used materials for fish foods, but the great demand for these materials has increased the cost of the food. Thus it is preferable to utilize alternative sources of protein. Unfortunately the cost of the diet proposed will be relatively high because no sources of high quality substitute materials such as meat and bone meal, blood meal, feather meal, or other non-conventional high protein sources are produced in Mexico with enough quality and quantity to be included in fish diets. This is a major pitfall in the Mexican intensive fish culture industry. These tentative formulations immediately underline the necessity for a series of experiments on dietary protein substitution to reduce costs of production of diets and to utilize sources of protein currently available in the region.

Table D-2 is an attempt to summarize the important features of C.urophthalmus and to allow comparisons of these with other cultured cichlids.

Table D.2.

Summary of the main characteristics, environmental requirements and nutritional requirements of two species of tilapia and C.urophthalmus.

SCIENTIFIC NAME	Cichlasoma urophthalmus	Oreochromis niloticus	Tilapia zillii
COMMON NAME	Mojarra casterica	Nile tilapia	Tilapia
GEOGRAPHICAL NATIVE DISTRIBUTION	Souther Mexico until Nicaregua (1)	Central and North Africa (2)	Central West and North Africa (2)
NATURAL HABITAT	Fresh water rivers and brackish water lagoons of Gulf of Mexico and Caribean	Fresh water lakes,lagoons and rivers (3)	Rivers,lakes and lagoons fresh and brackish water (3)
TEMPERATURE RANGE	22-40°C	11-36°C (2)	11-36°C (2)
TEMPERATURE OPTIMA	32-33°C	31-36°C (Preffered temp.) (2)	20-31 (preffered temp.) (2)
CRITICAL OXYGEN TENSION	70mm Hg (5)	60 mm Hg (4)	——
MAXIMUM TIME OXYGEN DEPLETION	2 hrs.	1.5-2 hrs (4)	——
SALINITY RANGE RESISTANCE	0-40 ‰	up to 35 ‰ (3)	0-45 ‰ (3)
SALINITY OPTIMA	15 ‰	11.6 ‰ (6)	11-29 ‰ (7)
SALINITY TOLERANCE RAPID CHANGE 50%	24 ‰	18.9 ‰ (8)	24-27 ‰ (7)
NATURAL DIET	Carnivorous with some vegetal habits eating preominantly invertebrates	Herbivorous with oportunistic carnivorous habits feeding on invertebrates (9)	Herbivorous with oportunistic carnivorous habits feeding on invertebrates (7)
MATURATION AGE	3-4 months	2 months (11)	2 months (11)
MATURATION WEIGHT	50 g	10 g (11)	10-20g (10)
FECUNDITY RANGE	2000-7000	500-1000 (11)	up to 1000 (12)
GROWTH	300-450 g/year	300-450 g/year	300-450 g/year
INCUBATION SYSTEM	Bottom spawner	Mouth brooder (7)	Bottom spawner (7)
DEGREE DAYS TO HATCH	60.6 - 61.3	112 (11)	56 - 60.76 (10)
DEGREE DAYS TO FREE SWIM	195 - 196	252 - 308 (11)	182 - 187 (10)
SEED SUPPLY	Artificially reared and natural collections	Artificially reared (7)	Artificially reared (7)
HANDLING RESISTANCE	High	High	High
ACCEPTABILITY TO BALANCED DIETS	High acceptability of pelleted food.	Readlily take pelleted food (3)	Readily take pelleted food (3)
PROTEIN REQUIREMENT OPTIMA AT 28°C	42%	35% (13)	35-40 (14,15)

1. Miller, 1976
2. Philippert and Ruwet, 1982
3. Balarin and Hatton, 1979
4. Ross and Ross, 1983
5. Martinez and Ross, 1986
6. Farmer and Beamish, 1969
7. Balarin and Haller, 1982
8. Wang et al., 1985
9. Treweavas, 1983
10. Rana Personal communication
11. Rana, 1986
12. Noakes and Balon, 1982
13. Santiago et al., 1982
14. Teshima et al., 1978
15. Mazid, et al., 1979

Clearly, the species is similar in many respects to the cultured old world cichlids, but it has advantages in many cases, making it a strong candidate for regional aquaculture development.

Based on the preliminary findings of this thesis a basic scheme for implementation of culture of C.urophthalmus may be summarized as follow:

1. Trapping of wild genetically-defined, brood stock.

2. Maintenance in a general purpose tank to define mating couples.

3. Transference of bonded couples to isolated 1m square tanks for breeding.

4. Feed brood stock with high level protein in diets (40-50%) in order to have high quality spawns with high survival. The frequency of feeding must be maintained at at least 2 times a day, ad libitum or 2 to 3% body weight daily, divided in to three meals.

5. Search for spawning in the tanks. Care must be taken to maintain an adequate range of temperature (26-32°C) and sudden changes must be avoided during this critical period. Oxygen must be maintained above 4.5 mg/l.

6. The tank out flow must be gentle and covered with a screen to avoid fry looses.

7. Once the fry start to swim they must be offered finely ground first fry food (48-50% crude protein) ad libitum or at 20-30% body weight avoiding either food dust, or large crumbs that produce gill diseases and starvation effect. The food must be offered as frequently as possible during the first month (5-6 times a day or by continuous automatic feeder). The tanks for the recently hatched fry must no be deeper than 40cm. to avoid long swimming distances for the fry.

8. After reaching 200 to 400mg body weight the fry must be removed from the parental tank and pooled with other fry of the same size, avoiding big differences in size to minimize canibalistic behaviour.

9. The adults without the fry must be maintained in constant observation, because there is a high risk that instead proceeding to spawn, aggressive behaviour will develop. A safe method is to return the couple to a general tank with more space to avoid fighting and from this a new mature and bonded couple can be identified. Food with high quality protein must be offered to maximize egg production.

10. Fry of 1-3 grams body weight must be fed with 35% crude protein in the diet until they reach 8-15g, it should then be possible to reduce the protein level to 30% for bigger fish. The feeding frequency must be maintained at least 3 meals/day to commercial size.

11. Reproduction, fry and juveniles growth can be achieved in either fresh or brackish water (5-20‰) and eventually the growth to commercial size could be realized in marine waters.

12. Juveniles of 10-15g body weight could be used to stock cages or ponds for intensive culture, with high survival.

Because of its easy handling, as in other members of the cichlid family, C.urophthalmus could be cultured using basic and easy techniques and highly trained personnel would not be required to start a modest scale hatchery for this species.

In conclusion, it can be seen that this native Mexican species has many characteristics which suggest that culture of the organism should be considered further, at least to pilot scale, in a range of appropriate environments and at different levels of intensity. A sound knowledge of the biology and culture

attributes of a native species such as C.urophthalmus could provide a base for future studies on other important native cichlids in many regions of Tropical America, as in the Cichlasoma genus alone there are more than 100 species. It is hoped that this thesis may provide guidelines for, and a stimulus for, future research aimed at regional aquaculture development without the need for exotic species introductions.

APENDIX I

Table A.1.

Nutritional formulae.

The following formulae were utilized in the nutritional evaluation of the protein effects in growth and performance of C.urophthalmus.

For growth:

INDIVIDUAL WEIGHT GAIN (IWG mg/day)

$$IWG = \frac{\text{total weight gain each 15 days}}{\text{n at the end of the 15 days}} \times 1000$$

Total time (days)

where n=number of fishes

WEIGHT GAIN (%)

$$\text{Weight gain (\%)} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight.}} \times 100$$

SPECIFIC GROWTH RATE (SGR %/day).

SGR.Measure the change of weight fish in percent per day (Brown, 1957)

$$SGR = \frac{\text{Log e Final weight} - \text{Log e Initial weight}}{\text{Time (days)}} \times 100$$

FEED EFFICIENCY.

INDIVIDUAL FOOD INTAKE (IFI mg/day)

$$IFI = \frac{\text{Total food intake during each 15 days}}{\text{n of fish at the end of each 15 days}} \times \frac{1000}{\text{time (days)}}$$

FOOD CONVERSION RATIO (FRC)

$$FRC = \frac{\text{Individual food intake mg}}{\text{Individual weight gain mg}}$$

PROTEIN EFFICIENCY

PROTEIN EFFICIENCY RATIO (PER)

PER.Measure the ability of fish to utilize dietary protein (Osborne et al.1919)

$$PER = \frac{\text{Individual weight gain mg}}{\text{Individual protein fed in mg}}$$

NITROGEN UTILIZATION

App.N.U. Measure the capacity of the fish utilize nitrogen in the diet (Nose, 1962).

APPARENT NET NITROGEN UTILIZATION (%)

$$App.N.U.\% = \frac{\text{Nitrogen deposition (mg)}}{\text{Nitrogen intake (mg)}} \times 100$$

DIGESTIBILITY

Measures the amount of nutrient absorved across the gut wall of the fish by diference of the amount of nutrient ingested and amount of nutrient egested, using in the food a inert marker as cromoic oxide (Cr203).

NUTRIENT DIGESTIBILITY (%)

$$D(\%) = 100 - \left(100 \times \frac{\%\ \text{indicator in food}}{\%\ \text{indicator in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in food}}\right)$$

TOTAL DIGESTIBILITY(%)

$$\text{Total digestibility} = 100 - \left(100 \times \frac{\%\ \text{indicator in food}}{\%\ \text{indicator in faeces}}\right)$$

Table A.2.
Proposed C.urophthalmus feed formulae based on feed stuffs commercially available in Mexico.

INGREDIENTS (% of diet)	DIET 1 First feeding to 1g	DIET 2 1g-35g	DIET 3 35g and over
Brown fish meal	52	20	16
Soy bean meal	20	36	29
Surgum meal	—	1	9
Son flower	—	8	12
Rice polishing	—	9	9
Corn gluten	5.0	5	5
Vegetal oil	5.66	4.31	4.32
Fish oil	3.67	4.12	4.27
Mineral mix	3.0	3	3
Vitamin mix	2.0	2	2
Binder	2.0	2	2
COMPOSITION (% of diet)			
Protein	48.00	35.34	30.21
Lipid	12.00	10.17	10.17

APPENDIX II.

Table A.2.(Follow).
The vitamin mixture formulation proposed is adapted from the studies on vitamin requirements for C.urophthalmus by Chavez, 1987.

Vitamin Mixture (mg/kg or U.I/kg)	
Thiamine Mononitrate (B_1)	35.0
Riboflavin (B_2)	25.0
Calcium Pantothenate	160.0
Biotin	0.6
Folic Acid	7.5
Cyanocobalamin (Vit B_{12})	0.05
Niacin	220.0
Pyridoxine HCl (B_6)	10.0
Ascorbic Acid (Vit C)[3]	2000.0
Choline Chloride	1000.0
Myo-Inositol	250.0
Retinol Acetate (Vit A)	6500.0
Cholecalciferol (Vit D_3)	1000.0
DL-Alpha Tocopherol acetate (Vit E)	300.0
Menadione sodium bisulfate (50% Vit k_3)	12.0
BHT	25.00
Ethoxiquin	100.00

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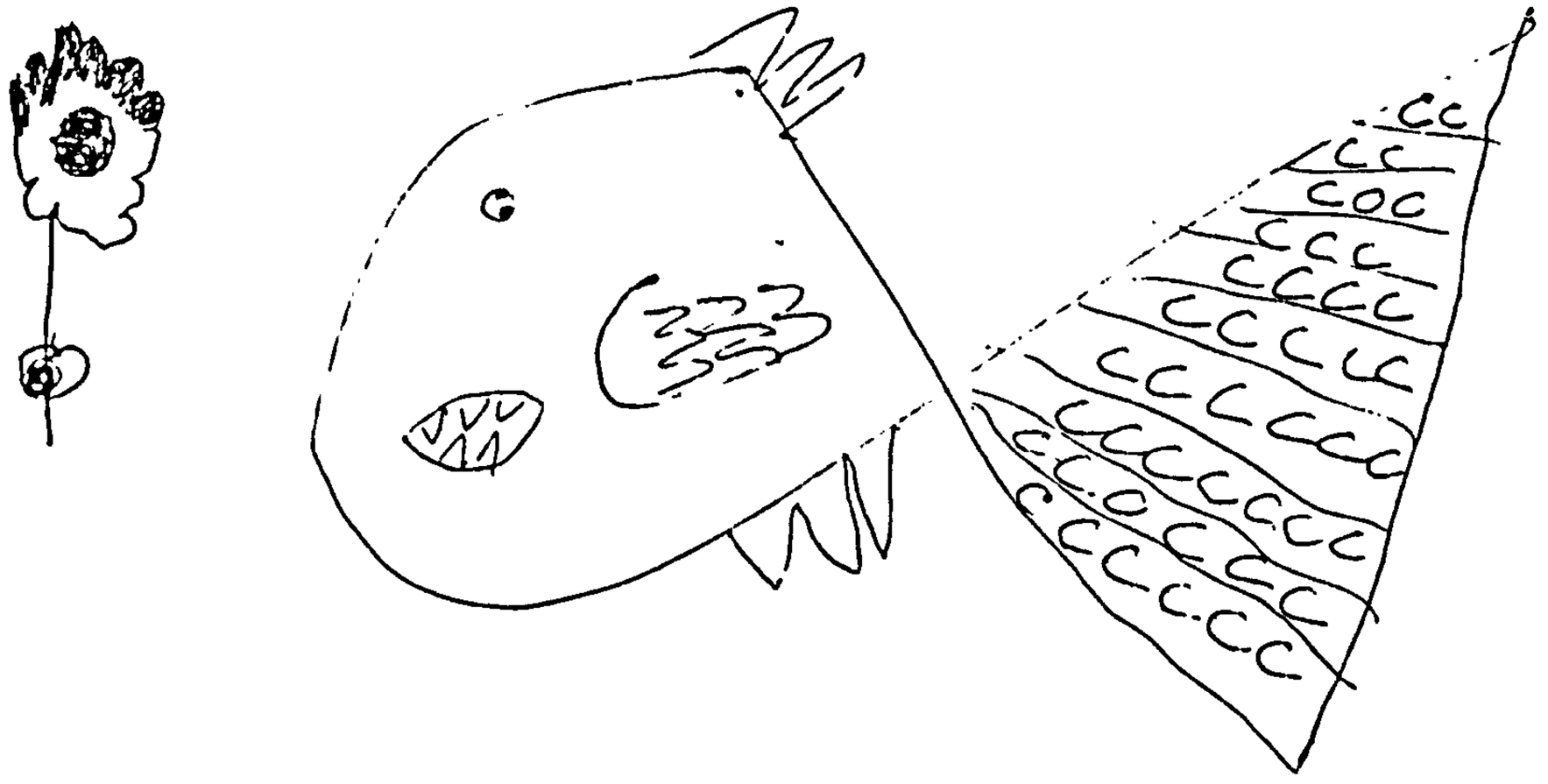

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