BIOENERGETICS AND GROWTH OF WHITE STEENBRAS, *LITHOGNATHUS LITHOGNATHUS*, UNDER CULTURE CONDITIONS.

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University of Cape Town

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BY

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Submitted as a requirement for the fulfilment of the degree of Master of Science.

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ABSTRACT

The bioenergetics and growth of white steenbras, *Lithognathus lithognathus*, under culture conditions were determined to assess its suitability for mariculture. Fish were captured by seine netting, from nearby estuaries and transported to the laboratory where they were successfully kept for the duration of the experimental period. The fish were initially fed on white mussels and then weaned onto a diet of commercial trout pellets.

The bioenergetics of *L. lithognathus* was examined using a large, simple closed system respirometer in which nitrogen excretion and oxygen consumption were measured simultaneously. This system permitted the use of groups of fish for experiment which simulated aquaculture conditions. The effects of fish size, ration size and temperature on faecal and nonfaecal excretion, and on oxygen consumption of *L. lithognathus* were examined.

Faecal production rates were variable with peaks, and subsequent return to prefeeding levels, varying with fish size and ration size. Faecal production was shown to increase linearly with food consumption. Faecal losses, as a percentage of the ingested nitrogen, amounted to 3.59%. Gross, carbon and nitrogen absorption efficiencies ranged from 86-98% and were not significantly affected by fish size, ration size or temperature. Net protein utilization (NPU) and biological value (BV) ranged from 78-95% and were not significantly affected by fish size, ration size or temperature.

Ammonia and urea constituted 77.93% and 10.07%, respectivley, of the total exogenous nitrogen excretion, the balance being made up of other dissolved organic nitrogen (DON). Endogenous (nonfeeding) ammonia excretion rates were significantly greater in small fish compared to large fish. Total nonfaecal losses as a percentage of the ingested ration amounted to 21.86%. Fish size and ration size increased nitrogen excretion, but temperature had no affect. Maintenance rations, calculated from the relationship between total daily nitrogen excretion (E_N) and total nitrogen in the ingested ration (I_N) and absorbed ration (pI_N), were 0.76% dry body wt. and 0.60% dry body wt. for small (57 ± 14g) and large fish (282 ± 48g), respectively.

The oxygen consumption rates were not significantly affected by the ration and temperature ranges used in the experimental trials. The mean routine (prefeeding) metabolic rate was $0.061mgO_2.g^{-1}$ dry $wt.h^{-1}$ for small fish and $0.035mgO_2.g^{-1}$ dry $wt.h^{-1}$ for large fish. Respiration, in excess of routine, due to feeding, in terms of carbon and energy, contributed approximately 2-6% and 3-14% for small and large fish respectively. Total carbon losses, as a percentage of the ingested ration, were 10.77% and 18.75% for small and large fish, respectively. Using groups of fish, in a large respirometer, produced lower estimates of oxygen consumption rates compared to other studies.

Long-term growth and feeding experiments indicated that *L.lithognathus* has the capacity to ingest food far in excess of its energy requirements. Ration size ranged from 4.50-11.52% dry body wt. for small fish (28 ± 2g), and 1.67-4.00% dry body wt. for large fish (250 ± 23g) depending on feeding level. Ration size for a 74g fish ranged from 1.80-4.26% dry body wt., depending on feeding level and temperature. Condition factors ranged from 1.19-2.19 for all the experiments. Specific growth rates at 16°C were $0.60\%.day^{-1}$ (28g fish), $0.29\%.day^{-1}$ (74g fish) and $0.19\%.day^{-1}$ (250g fish), and at 20°C $0.45\%.day^{-1}$ (74g fish. Food conversion (GCE) was inefficient at the high feeding levels (21.3%) compared to low feeding levels (32.6%). Proximate body composition in terms of moisture, protein, ash and energy were not significantly affected by feeding regime. Lipid levels increased significantly at higher feeding levels. These results suggested that feeding regimes should be maintained at relatively low levels to avoid overfeeding and food wastage.

The relative proportions of the energetic equation ($C = F + U + R + G$) for *L.lithognathus* were calculated to be $100 = 9 + 2 + 12 + 77$. The carbon and nitrogen budgets and growth model overestimated growth at high rations but estimations at low rations can be made.

It was concluded that *L.lithognathus* would be suitable for mariculture provided growth rates could be improved and the feeding regime could be strictly controlled.

CHAPTER 1

INTRODUCTION

Mariculture in South Africa has been slow to develop for a number of reasons. Up until the mid 1960's fish farming was not thought to be viable due to South Africa's rich harvest from its natural marine fisheries (Hecht, 1988). The lack of suitable sites, legalities involved in the use of the coastal zone and inadequate information on future demands for mariculture products (Walmsley & Bruton, 1987) were considered to be stumbling blocks.

At present, most of the fish species in commercial production are freshwater species and include catfish (*Clarias gariepinus*), tilapia (*Oreochromis mossambicus*), grey mullet (*Mugil cephalis*), carp (*Cyprinus carpio*), and grass carp (*Ctenopharyngodon idella*), with rainbow trout (*Oncorhynchus mykiss*) being farmed in freshwater and seawater (Hecht & Britz, 1990). Marine fish presently being considered as potential aquaculture species include galjoen (*Coracinus capensis*) and leervis (*Lichia amia*) and are at the experimental stage (Hecht & Britz, 1990).

The present decline in marine catches in South Africa will help promote the development of mariculture (Heydorn, 1973; Bross, 1981; Safriel & Bruton, 1984; Van Niekerk, 1988) with certain marine fish becoming high priority species. There is an immediate need to identify mariculture fish species in order to conduct the necessary basic research required to evaluate their feasability. The aim of the present study is to evaluate the biological suitability of a local marine fish, white steenbras *Lithognathus lithognathus*, for mariculture in South Africa.

White steenbras was selected on economic and biological criteria: *L. lithognathus* is an important commercial and line fish species with much demand from restuarants. At present it is in scarce supply, and is available only seasonally. It commands a retail price of about R11.00 per kg.

Large numbers of juvenile white steenbras are found in local estuaries (Mehl, 1973; Marais & Baird, 1979; Winter, 1979; Day *et al.*, 1981; Wallace *et al*, 1984) thus availability of fry, tolerance to suboptimal conditions and adaptability to mass rearing conditions are characteristics which make *L.lithognathus* a suitable candidate. *L.lithognathus* has previously been maintained in captivity without showing signs of stress (Mehl, 1973).

The bioenergetic approach and the use of predictive energetic models are of great benefit in aquaculture (Knights, 1985) and involve measuring the energy cost of growth of fish in captivity (Brett & Groves, 1979; Diana, 1983; Cho *et al.*, 1982). A major benefit of energy budgets is that they can be carried out over a short period of time and therefore rapid predictions of growth can be made (Brafield, 1985; Knights, 1985).

Few studies have measured the energetics of fish under culture conditions where groups of fish are kept in large tanks and fed a formulated diet. Most bioenergetic experiments have involved measuring only some parts of the energy budget, whilst others have calculated some of the components by difference (Braaten, 1979). The few studies that have simultaneously measured rates of food intake, growth, metabolism and excretion have used small continuous flow respirometers (Brett *et al.*, 1971; Solomon & Brafield, 1972; Elliott, 1975b,c, 1976b,c; Brett & Zala, 1975; Wiggs *et al.*, 1989). Such experimental systems do not simulate conditions on fish farms. Knights (1985) commented that laboratory-scale systems could be used to study the energetics of farmed fish provided the system could adequately mimic conditions on a farm. In other studies large open rectangular tanks have been used as respirometers and in such studies oxygen consumption was measured by the drop in dissolved oxygen between the inlet and outlet (Staples & Nomura, 1976; Knights, 1985). A serious problem in this type of system is the oxygen absorption from the atmosphere. Knights (1985) commented that more studies are needed, under conditions comparable to those on fish farms

A large, closed system respirometer which simultaneously measures oxygen consumption and excretion was used by Durbin & Durbin (1981a,b), James & Probyn (1989) and James *et al.* (1989a) to study the energetics of planktivorous fish. As this system is large and can accommodate groups of fish, the present study adopted this approach to examine the bioenergetics of *L.lithognathus*. Du Preez *et al.* (1986b, 1990) and Cockroft & Du Preez (1989) examined the bioenergetics of *L.lithognathus* but they used individual fish on a natural diet of white mussel, and did not measure nitrogen excretion and oxygen consumption simultaneously.

To evaluate the biological suitability of *L.lithognathus* for mariculture the bioenergetics approach was used, in addition to a long-term feeding and growth study. Fish size, ration size and temperature are the three most important variables affecting growth (Braaten, 1979; Brett, 1979; Elliott, 1982; Crowder & Magnunson, 1983; Shepherd & Bromage, 1988).

Chapter 2 examines effect of fish size, ration size and temperature on the nitrogen excretion and absorption efficiencies of *L.lithognathus* under culture conditions. The various components of the nitrogen budget were determined. The total amount of food consumed, faecal and nonfaecal nitrogen excretion and absorption efficiencies were investigated.

Chapter 3 examines the effects fish size, ration size and temperature on the metabolism of *L.lithognathus* under culture conditions. Measurements of routine and feeding metabolic rates were undertaken, and the amount of carbon and energy lost in respiration, as a percentage of the ingested ration, were determined.

Chapter 4 involved measuring the long-term growth of *L.lithognathus* under different experimental conditions of fish size, feeding level and temperature. Weight gain, food conversion, condition factor and proximate body composition were used as indicators of adequacy of diet and general "well-being" of the fish.

Chapter 5 constructs carbon and nitrogen budgets using the data obtained in Chapter 2 and 3. Growth models in terms of carbon and nitrogen are derived which predict growth of *L.lithognathus* for a range of ration sizes. The predicted values are then compared to the observed measurements made in Chapter 4.

CHAPTER 2

NITROGEN EXCRETION AND ABSORPTION EFFICIENCIES

INTRODUCTION

The estimation of the nitrogen budget of a fish is calculated by subtracting the proportion of the ingested ration which is lost via faecal and nonfaecal (urinary) products, from the total consumed nitrogen, the remainder representing nitrogen retained for growth (Braaten, 1979). The nitrogen budget is best described by the equation:

N consumed - faecal N = N absorbed = N retained + N excreted (Birkett, 1969).

In a nitrogen budget equation faeces represents the nonabsorbed fraction of the food (Braaten, 1979; Brett & Groves, 1979; Pandian, 1987) and thus faecal production rates are related to absorption efficiency. A proportion of the nitrogen absorbed is excreted via two fractions, endogenous and exogenous (Brett & Groves, 1979). The endogenous fraction is the lowest level (maintenance) of nitrogen excretion (Savitz, 1969) and arises from the catabolism of body proteins (Pandian, 1987). Exogenous nitrogen excretion represents the energy loss resulting from feeding (Brett & Groves, 1979; Pandian, 1987).

Few studies have measured the nitrogen budget of fish under standard culture conditions in which groups of fish are kept together in large tanks and fed a formulated, pelleted diet. Nitrogen and energy losses via faecal and nonfaecal excretion in white steenbras, *Lithognathus lithognathus*, have been previously investigated by Cockroft & Du Preez (1989). However, they studied individual fish on a natural diet of white mussel. This chapter describes the various components of the nitrogen budget of *L.lithognathus* under culture conditions fed on a formulated diet. Total amount of food consumed, faecal and nonfaecal nitrogen excretion and absorption efficiencies were investigated.

MATERIALS AND METHODS

Collection and maintenance of the experimental fish is described in Chapter 4.

Standard procedure

Fish (31-380g) were grouped into small and large size classes. The fish were kept in experimental tanks for a 6-8 week acclimatization period prior to starting experimentation. They were fed once a day on commercial trout pellets. The large fish were kept in a 2m diameter, 1.5m deep fibreglass tank and the small fish in a 1m diameter, 50cm deep fiberglass tank. Filtered seawater (5μm) was supplied on a continuous basis at a temperature of either 16 ± 1°C or 20 ± 1°C.

The fish were starved for 3-4 days (until no more faeces were produced) before each experiment to ensure that the last meal had no effect upon metabolism during the experiment. During this time the sides and bottom of the tank were thoroughly scrubbed and the water siphoned to remove any attached growth. This procedure was repeated 2-3h before the start of the experiment at 09h00, the tank was then flushed with filtered seawater and the water flow turned off. The water level was then adjusted to the required depth. A 3mm thick transparent Lexan lid, which fitted snugly to the sides of the tank, was lowered onto the surface of the water, forming a simple closed system respirometer (James & Probyn, 1989). The tank was flushed briefly after 24h to reduce excessive ammonia accumulation.

Experiments were conducted to investigate faecal production, nitrogen excretion rates and absorption efficiencies at 16°C and 20°C under the following feeding regimes (FR):

FR 1 - fed to satiation over a 30min period

FR 2 - fed to satiation over a 1h45min period

Any food remaining at the end of each feeding period was removed and reweighed to calculate the ingested ration. The biochemical composition of the food is summarised in Table 4.1 (Chapter 4).

Respiration rate were determined during the same experimental runs and this is described in Chapter 3. Where possible experiments were replicated.

Nitrogen excretion

Each experimental run began with a measurement of prefeeding ammonium and urea excretion rates over 2.5h. The fish were then fed and water samples for the determination of ammonia and urea were collected every 30min before and every 30min for 2h after feeding, then every 1-2h thereafter over a period of 20-36h. No measurements were taken during the night. Water samples were collected in 100ml syringes and filtered through a prerinsed glass fibre filter (45μm) into a clean beaker. Triplicate 5ml subsamples were subsequently stored in clean, disposable test tubes (Kimbel) at -20°C until processed. Ammonia and urea concentrations were determined in triplicate 5ml samples, according to the methods described by Koroleff (1983).

Nonfaecal loss was divided into endogenous and exogenous excretion. Endogenous excretion represents the lowest level of nitrogen excreted which approximates to maintenance levels (Brett & Groves, 1979). The increase in nitrogen excretion above endogenous levels during feeding is known as exogenous excretion (Jobling, 1981).

Faeces collection

The fish produced cohesive faecal strings in a mucous sheath which were easily collected at regular intervals by siphoning into a clean 20l bucket. The water removed from the tank with the faeces was returned by back filtration. The faeces were rinsed well in distilled water and filtered onto preweighed, oven dried (24h at 60°C), GF/F Whatman filters. Faeces were then dried for 24h at 60°C and reweighed. A subsample of each batch was used for the determination of carbon, nitrogen and ash content. Total carbon and nitrogen was measured using a Heraues Rapid CHN analyser and ash content was determined by combustion (550°C overnight). Energy values were determined by bomb calorimetry (Model CP500) after combining subsamples of each batch to obtain one value for each experiment. Energy values were expressed as $kJ.g^{-1}$ dry wt of sample. The biochemical composition of the fish and food was determined by the same methods as the faeces.

All nitrogen data were expressed as $\mu gN.g^{-1}$ dry wt. of fish.

Absorption efficiency

The terms "apparent digestibility", "assimilation efficiency" and "absorption efficiency" have been used by previous workers (Cho & Slinger, 1979; Fange & Grove, 1979) to represent that part of the consumed energy that is not rejected as faeces (Beamish *et al.*, 1975; Jobling, 1983). Pandian (1987) suggested that the term "absorption efficiency" was the most appropriate one and this term is therefore used in this study.

Dry weight, carbon and nitrogen absorption efficiencies were calculated from the total amounts of each constituent in the faeces compared to that available in the food, using the following expression:

$$\underset{(p)}{\text{absorption efficiency}} = \frac{\text{component}_{\text{food}} - \text{component}_{\text{faeces}}}{\text{component}_{\text{food}}}$$

The energy efficiency was calculated using the following equation:

$$\%\text{ E efficiency} = \frac{\text{energy intake (kJ)} - \text{energy faeces (kJ)}}{\text{energy intake (kJ)}}$$

(Pandian & Marian, 1985).

Biological value (BV) and net protein utilization (NPU) (Shepherd & Bromage, 1988) were calculated as an indication of nitrogen retention:

$$\text{BV} = \frac{\text{nitrogen retained}}{\text{nitrogen absorbed}} \qquad \text{NPU} = \frac{\text{nitrogen retained}}{\text{nitrogen ingested}}$$

Nitrogen retained is that part of the ingested ration remaining for growth.

The Mann-Whitney non-parametric test was used to test significant differences between two groups of values at the 0.05 significance level (Zar, 1984)

RESULTS

Table 2.1 summarises the experimental details of the fish, ration size and temperature of the trials conducted on small (56 ± 14g, 153 ± 13mm) and large (282 ± 48g, 259 ± 8mm) steenbras. The mean dry weight of the fish as a percentage of wet weight was 29.77 ± 1.61% (wet:dry weight regression for $n = 35$, $Y = 0.301X - 0.282$, $r^2 = 0.97$). The ration consumed increased with fish size and temperature and ranged from 2.46-11.87% dry body wt. (Table 2.1).

In the experiment it was assumed that each of the fish in the group consumed an equal portion of the available ration and contributed equally to nitrogen excretion and faecal production during the experiments. Although no conclusive proof was obtained that this was so, it seems a reasonable assumption since the fish were graded into groups of similar sizes prior to the experiments.

TABLE 2.1: Summary of experimental trials conducted (15 fish per experiment)

Expt.	Mean fish length (mm)	Total fish weight		Ration size (% dry body wt.)	Temp. (°C)
		Dry wt. (g)	Wet wt. (g)		
small					
4	143.90	209.89	698.25	4.33	16
6	143.90	209.89	698.25	9.53	16
8	162.79	301.23	1001.70	8.70	20
10	162.79	301.23	1001.70	8.37	20
12	162.79	301.23	1001.70	12.67	20
14	162.79	301.23	1001.70	11.87	20
large					
2	254.21	1119.57	3720.45	2.59	16
3	254.21	1119.57	3920.45	2.46	16
5	254.21	1119.57	3720.45	5.00	16
7	264.94	1427.90	4744.80	4.12	20
9	264.94	1427.90	4744.80	4.38	20
11	264.94	1427.90	4744.80	6.58	20
13	264.94	1427.90	4744.80	6.41	20

Biochemical composition

The biochemical composition of the fish, food and faeces is summarised in Table 2.2. The composition of the fish in terms of C, N and ash was very similar for all the experiments and also similar for small and large fish. The C:N ratio in the faeces was significantly higher than the C:N ratio in the food ($p < 0.05$). The energy content of the faeces in all the experiments ranged from 13-18kJ.g^{-1} whilst the ash content ranged from 14-26% dry wt.

Faecal elimination

Faecal elimination rates for small and large fish varied considerably over the experimental period and between replicate experiments (Figs. 2.1 and 2.2, respectively). Faeces began to appear 1-3h after feeding in small fish (Fig. 2.1) and 2-4h after feeding in large fish (Fig. 2.2). Peaks of faecal production typically occurred 4-8h after feeding then again after 20-24h (Figs. 2.1 and 2.2). The time taken for total faecal elimination varied according to fish size and ration size, but not temperature. In small fish faecal production ceased after 46h on a low ration (FR1) and after 52h on a high ration (FR2), and in large fish this ranged from 48h on a low ration to 70h on a high ration (Figs. 2.1 and 2.2). The biochemical composition of the faeces changed over time with the C:N ratio increasing over time in most of the experiments (Figs. 2.1 and 2.2).

Faecal production when expressed in energy terms (kJ.day^{-1}) increased linearly with increased food consumption (Fig. 2.3), the regression being $Y = 0.106X - 0.074$ ($r^2 = 0.97$, $n = 13$).

The slope indicates that 10.6% of energy consumed was eliminated in the faeces, thus 89.4% was absorbed.

TABLE 2.2: Chemical composition of the fish, food and faeces (± SD)

	Chemical composition (% dry wt.)			C:N	Energy ($kJ.g^{-1}$)
	C	N	Ash		
L. lithognathus					
small	49.98 (0.39)	13.55 (0.03)	14.17 (1.14)	3.70 (0.35):1	22.92 (0.82)
large	48.94 (1.79)	14.30 (0.21)	15.09 (1.63)	3.49 (0.33):1	22.56 (0.61)
Food	44.53 (1.24)	7.58 (0.80)	10.03 (1.75)	6.00 (0.61):1	20.05 (0.17)
Faeces					
small					
4	38.63	2.64	13.97	14.63:1	15.42
6	33.83	1.72	16.29	19.67:1	15.43
8	44.03	4.08	20.00	10.79:1	18.24
10	36.64	3.01	14.47	12.17:1	16.41
12	42.79	3.32	14.08	12.89:1	17.39
14	38.58	3.61	14.31	10.69:1	18.05
large					
2	33.90	2.06	20.61	16.46:1	14.62
3	30.77	2.43	25.81	12.66:1	14.17
5	33.07	2.16	21.92	15.31:1	13.13
7	39.87	2.32	14.83	17.19:1	16.51
9	40.29	1.95	16.00	20.66:1	15.81
11	41.44	2.78	15.94	14.91:1	16.33
13	39.96	2.93	17.39	13.64:1	16.32
mean	37.99 (4.05)	2.59 (0.69)	17.36 (3.66)	14.74 (3.11)	15.99 (1.47)

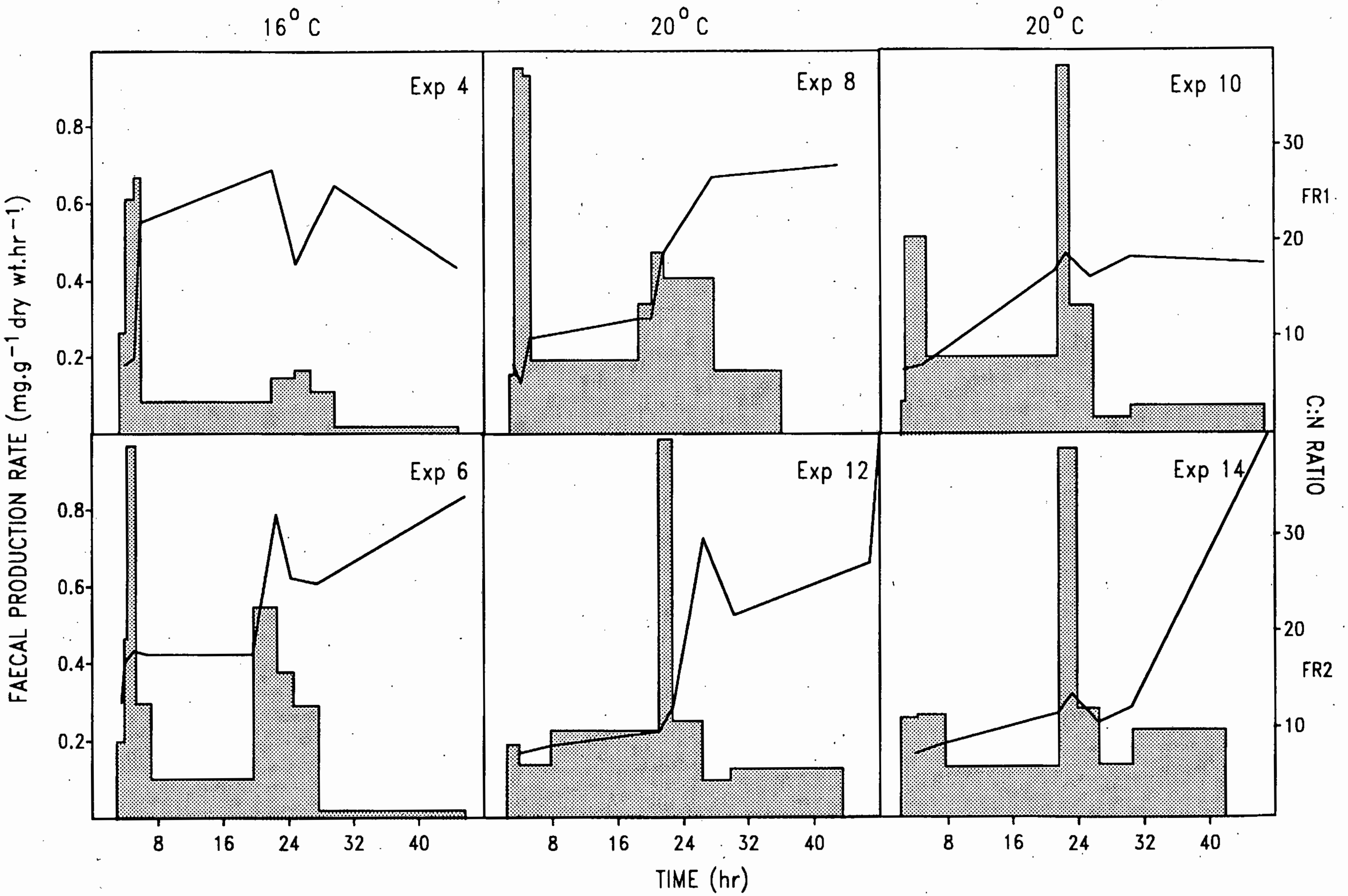

Fig. 2.1: Faecal production rates (bars) and C:N ratios (solid lines) of faeces for small steenbras during the experimental time course

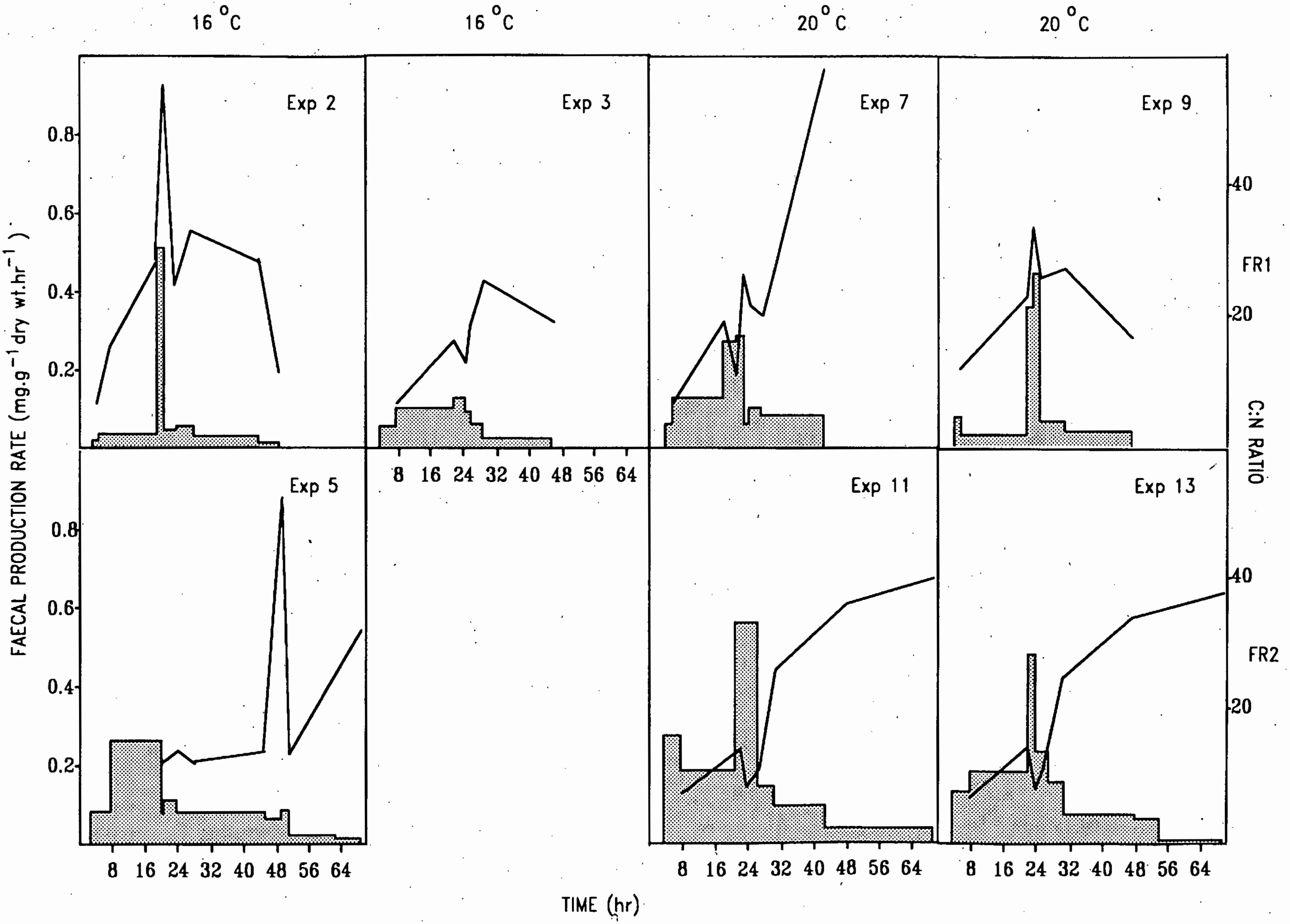

Fig. 2.2: Faecal production rates (bars) and C:N ratios (solid lines) of faeces for large steenbras during the experimental time course.

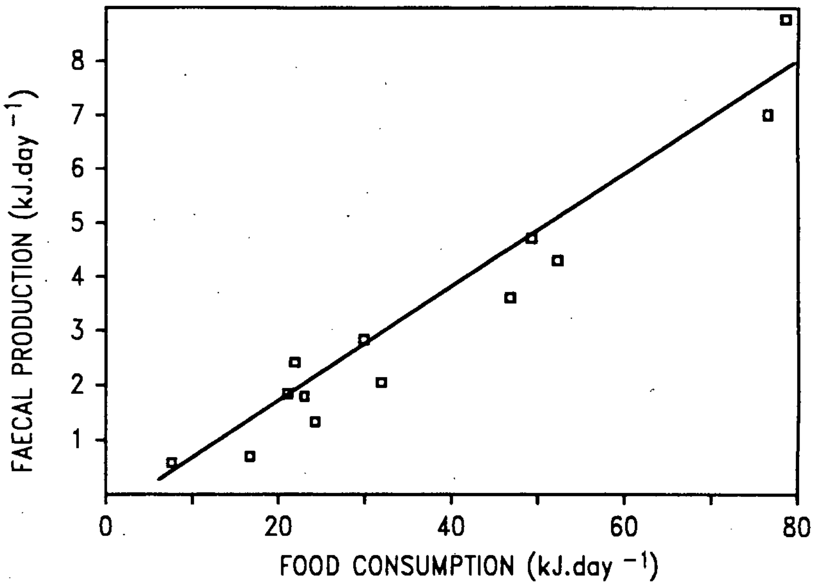

Fig. 2.3: Relationship between faecal production and food consumption.

Absorption efficiency

The gross, carbon and nitrogen absorption efficiencies ranged from 86-98%, with nitrogen absorption efficiencies being the greatest in all experiments (Table 2.3).

Although it appears that absorption efficiencies were higher at 16°C and at FR2 for small fish and at 16°C and FR1 for large fish (Fig. 2.4), the effect of these variables on the absorption efficiencies was not significant ($p > 0.05$).

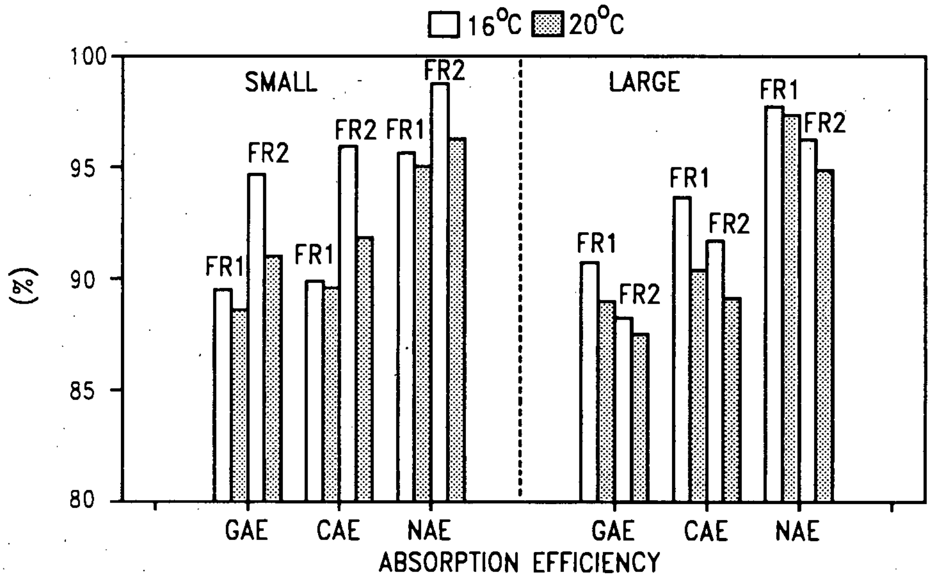

Fig. 2.4: Gross (GAE), carbon (CAE) and nitrogen (NAE) absorption efficiencies for small and large *L.lithognathus*

TABLE 2.3: Overall absorption efficiencies for the formulated diet by *L.lithognathus*

(E = energy; BV = biological value; NPU = net protein utilization)

Expt.	Ration size	Absorption efficiencies (%)			Efficiency E	BV	NPU
	(mg.g^{-1}dry wt.)	Gross	Carbon	Nitrogen	(%)	(%)	
small							
4	43.26	89.49	89.87	95.67	92.33	83.20	79.60
6	95.29	94.68	95.95	98.77	95.91	84.04	83.00
8	86.98	87.82	88.09	93.92	88.93	91.68	86.11
10	83.66	89.33	91.05	96.15	91.26	81.63	78.50
12	126.65	92.58	93.03	96.65	93.57	87.49	84.56
14	118.68	89.43	90.67	95.94	90.49	84.66	81.22
large							
2	25.90	92.48	94.44	98.81	94.52	79.32	78.40
3	24.56	88.92	92.80	96.65	92.17	79.00	76.37
5	50.02	88.22	91.70	96.26	92.29	86.86	83.61
7	41.24	88.37	89.87	97.06	90.42	79.89	77.54
9	43.79	89.56	90.88	97.59	91.77	95.13	80.03
11	65.80	86.27	87.66	94.21	88.82	84.80	79.88
13	64.14	88.73	90.62	95.56	90.83	88.20	85.02
mean		89.69 (2.27)	91.28 (2.34)	96.40 (2.04)	91.79 (2.04)	85.07 (4.81)	81.07 (3.11)

It is evident from Fig. 2.3 that absorption efficiencies in terms of energy were high. This is also shown by the range of 89-96% for energy absorption efficiencies (Table 2.3).

The biological value (BV) and net protein utilisation (NPU) were high ranging from 78-95% with the biological value being greater in all instances (Table 2.3).

Nitrogen excretion

Excretion of organic compounds other than ammonium and urea were calculated from the nitrogen excretion study by Cockroft & DuPreez (1989) for *L.lithognathus*. They found ammonium and urea accounted for 88% of the total dissolved nitrogen (TDN) excreted and other dissolved organic nitrogen (DON) was 12%. In the present study, those experiments where urea could not be measured, a value of 7% of TDN was used.

Ammonia excretion rates peaked 4-6h after feeding and were back to pre-feeding levels within 36h for all the experiments (Figs. 2.5 and 2.6). Urea excretion rates followed a similar pattern to ammonia, although at much lower levels (Figs. 2.5 and 2.6).

Nitrogen excretion (endogenous and exogenous) rates varied with fish size, excretion rates for large steenbras (Fig. 2.6) being approximately half that of the small steenbras (Fig. 2.5). Ration size increased excretion rates marginally, but temperature did not have any apparent effect (Figs. 2.5 and 2.6).

The mean endogenous (nonfeeding) excretion rates for ammonia and urea are summarised in Table 2.4. Endogenous excretion rates for small fish were significantly greater than for large fish ($p < 0.05$).

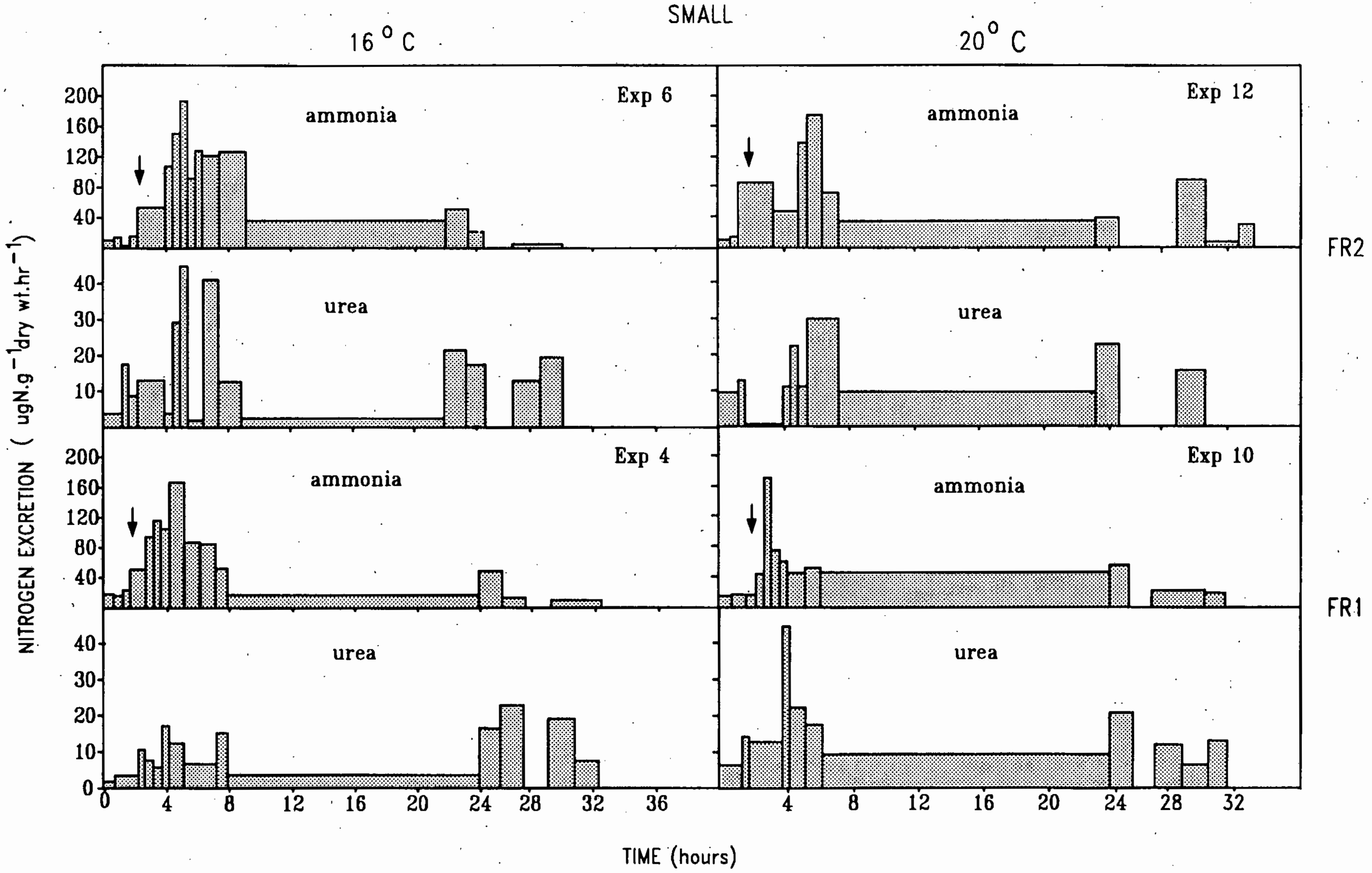

Fig. 2.5: Rates of ammonia and urea excretion by small *L.lithognathus* before and after feeding (arrows indicate when the fish were fed)

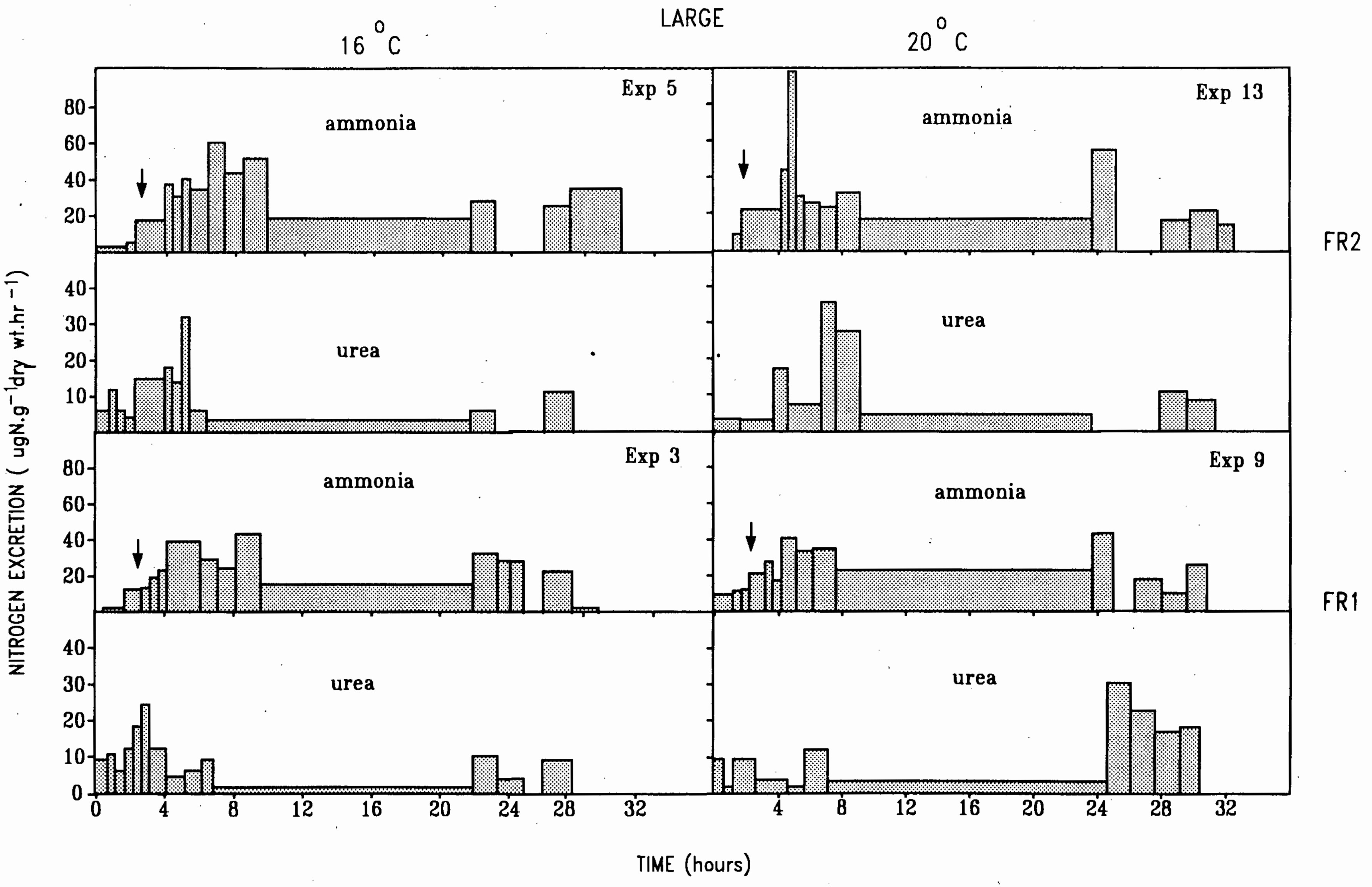

Fig. 2.6: Rates of ammonia and urea excretion by large *L.lithognathus* before and after feeding (arrows indicate when the fish were fed)

TABLE 2.4: Mean endogenous excretion rates ± 1 SD

	Ammonia	Urea	Total
	(μg N.g^{-1} dry wt.h^{-1})		
small	11.06 ± 2.79	3.08 ± 1.28	14.14 ± 4.07
large	5.43 ± 1.75	2.96 ± 1.14	8.39 ± 2.89
			11.27 ± 4.07

Total exogenous nitrogen excretion (e_N) was calculated by subtracting the endogenous rates (Table 2.4) from the total nitrogen excreted during the period of elevated excretion (the period during which excretion exceeded the upper 1 SD of the mean endogenous rate). A period of 36h was used as the minimum time for excretion rate to return to basal level.

When expressed as a percentage of ingested nitrogen (I_N) the various forms of nitrogen losses (excretion and defaecation) were similar for both small and large steenbras (Table 2.5). Ammonia and urea constituted 77.93% and 10.07% respectively of the total dissolved nitrogen (exogenous), the balance being made up of other DON.

TABLE 2.5: Faecal and nonfaecal losses as a percentage of the ingested nitrogen (± 1SD).

	small	large	mean
ammonia	13.18 ± 2.26	15.19 ± 3.39	14.26 ± 2.99
urea	2.71 ± 1.36	1.02 ± 0.59	1.80 ± 1.31
other DON	2.44 ± 0.38	2.42 ± 0.46	2.37 ± 0.41
total nonfaecal	17.84 ± 2.93	18.63 ± 3.50	18.26 ± 3.14
faecal	3.82 ± 1.57	3.40 ± 1.48	3.59 ± 1.47
Total	21.65 ± 3.08	22.03 ± 2.56	21.86 ± 2.69

Significant relationships ($p < 0.05$) exist between the total exogenous nitrogen excretion (e_N) over the duration of the experiment and both the total nitrogen in the ingested ration (I_N) and the absorbed rations (pI_N, p = absorption efficiency) for small and large fish (Fig. 2.7). The least squares regressions are:

for I_N

Small $e_N = 0.132 + 0.147\ I_N \text{mgN.g}^{-1}$ dry wt.day^{-1} ($r^2=0.88$, n=6)

Large $e_N = 0.189 + 0.093\ I_N \text{mgN.g}^{-1}$ dry wt.day^{-1} ($r^2=0.95$, n=7)

and for pI_N

Small $e_N = 0.146 + 0.152\ pI_N \text{mgN.g}^{-1}$ dry wt.day^{-1} ($r^2=0.84$, n=6)

Large $e_N = 0.180 + 0.101\ pI_N \text{mgN.g}^{-1}$ dry wt.day^{-1} ($r^2=0.95$, n=7)

Thus, small and large fish respectively, retained 85.3% and 90.7% of the ingested ration and 84.8% and 89.9% of the absorbed nitrogen. These values are within the range of BV and NPU values (Table 2.3). The rest (14.7% I_N and 15.2% I_N for small fish, 9.3% pI_N and 10.1% pI_N for large fish) being in excess of immediate requirements, was excreted.

These relationships may be expressed in terms of daily nitrogen excretion (E_N) by incorporating the mean daily endogenous excretion for small (0.339mgN.g^{-1} dry wt.day^{-1}) and large (0.201mgN.g^{-1} dry wt.day^{-1}) fish. The regressions are:

for I_N

Small $E_N = 0.471 + 0.147\ I_N \text{mgN.g}^{-1}$dry wt.day^{-1} ($r^2=0.88$, n=6)

Large $E_N = 0.390 + 0.093\ I_N \text{mgN.g}^{-1}$dry wt.day^{-1} ($r^2=0.95$, n=7)

and for pI_N

Small $E_N = 0.485 + 0.152\ pI_N \text{mgN.g}^{-1}$ dry wt.day^{-1} ($r^2=0.84$, n=6)

Large $E_N = 0.381 + 0.101\ pI_N \text{mgN.g}^{-1}$ dry wt.day^{-1} ($r^2=0.95$, n=7)

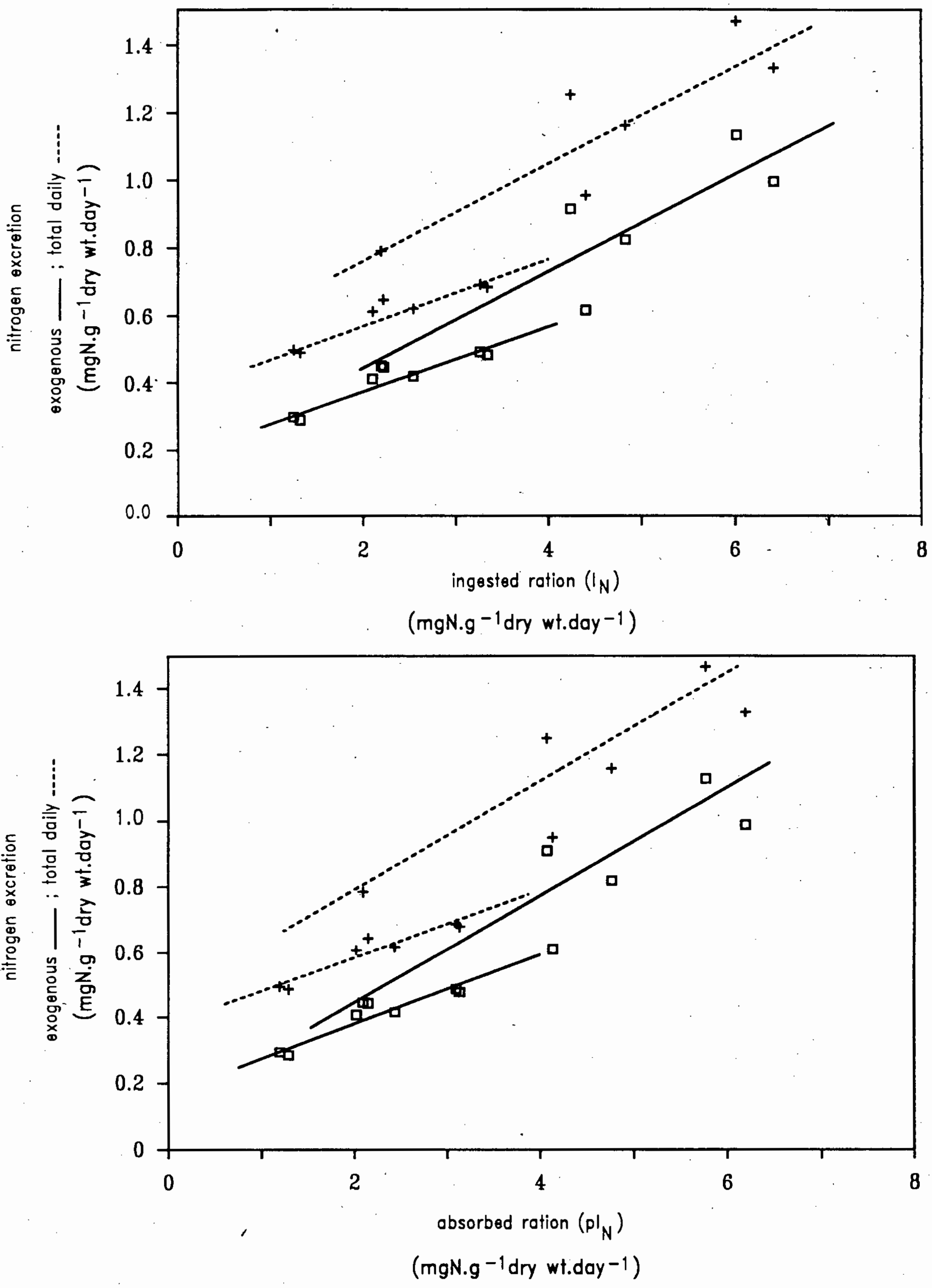

Fig. 2.7: Relationship between ingested (a) and absorbed (b) ration, and exogenous and total daily nitrogen excretion (solid lines – small fish; dotted line – large fish)

The latter relations can be used to calculate the maintenance ration for small and large fish.

Small $G_N = pI_N - E_N$

$= pI_N - (0.485 + 0.152\ pI_N)$

$= 0.848\ pI_N - 0.485 \text{mgN.g}^{-1} \text{ dry wt.day}^{-1}$

with $G_N = 0$, $pI_N = 0.572 \text{mgN.g}^{-1} \text{ dry wt.day}^{-1}$

Large $G_N = pI_N - (0.381 + 0.101\ pI_N)$

$= 0.899\ pI_N - 0.381 \text{mgN.g}^{-1} \text{ dry wt.day}^{-1}$

with $G_N = 0$, $pI_N = 0.424 \text{mgN.g}^{-1} \text{ dry wt.day}^{-1}$

Therefore for zero net growth i.e. no gain or loss in nitrogen, small (57g) and large (282g) white steenbras, over the experimental ranges tested (16°C and 20°C; FR1 and FR2), require an absorbed ration of 0.57mgN.g^{-1} dry wt.day^{-1} and 0.42mgN.g^{-1} dry wt.day^{-1}, respectively for basal maintenance. When expressed as mg.g^{-1} dry wt.day^{-1} the maintenance rations are 7.55mg.g^{-1} dry wt.day^{-1} (0.76% body wt.) for small fish and 5.99mg.g^{-1} dry wt.day^{-1} (0.60% body wt.) for large fish.

A significant positive linear relationship between nitrogen absorbed (pI_N) and nitrogen retained (rI_N) was obtained ($p < 0.001$) (Fig. 2.8), the linear relationship being:

$$rI_N = -0.064 + 0.838\ pI_N \quad (r^2=0.99,\ n=13).$$

The slope of the regression indicates the mean rate of retention or net growth efficiency (83.8%) for both small and large fish.

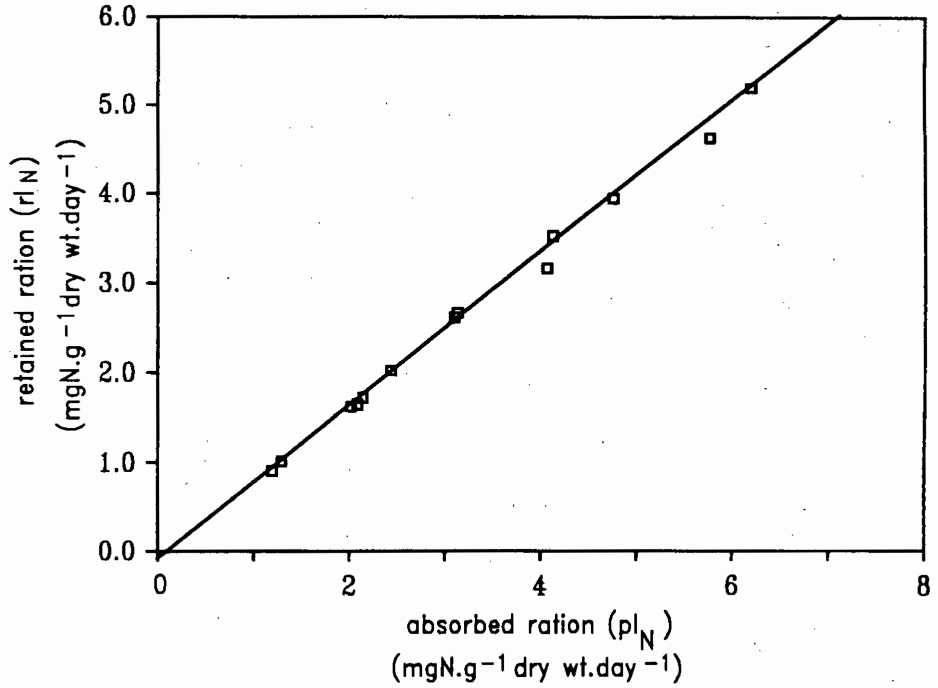

Fig. 2.8: Relationship between absorbed ration (pl_N) and retained ration (rl_N).

DISCUSSION

Biochemical compositon

The ash and energy content, respectively, of the fish, food and faeces for *L.lithognathus* in the present study ranged from 14-15% and 22-23kJ.g^{-1} for the fish, 9-11% and 19-21kJ.g^{-1} for the food, and 14-20% and 13-16kJ.g^{-1} for the faeces (Table 2.2). These values are similar to the ash and energy content of the fish, food and faeces obtained for African catfish (*Clarias gariepinus*) fed on commercial pellets (Henken *et al.*, 1986): 13-14% and 23-25kJ.g^{-1} for the fish, 10-13% and 21-23kJ.g^{-1} for the food, and 9-11% and 21-22kJ.g^{-1} for the faeces.

For *L.lithognathus* fed on a natural diet of white mussel (Cockroft & Du Preez, 1989) the composition of the faeces was very different from that obtained for *L.lithognathus* fed on commercial trout pellets in the present study. The ash content of the faeces for *L.lithognathus* on a diet of white mussel was considerably higher (82-86%) and the energy content lower (3-4kJ.g^{-1}).

Faecal elimination

Large variations in faecal production rates were observed for *L.lithognathus* feeding on a single diet. James *et al.* (1989a) found similar results for the Cape anchovy (*Engraulis capensis*) where faecal elimination rates were variable even when the same type of food was eaten. In the present study, bouts of faecal production appeared to occur when the fish were disturbed, at certain times of the day, resulting in irregular faecal production.

Relationships between faecal production and food consumption have been described both by linear relationships (Gerking, 1955a) and by power curves (Allen & Wootton, 1983; Cui & Wootton, 1988a). Cui & Lui (1990a) showed that a linear relationship existed with five of the six teleost species tested. The results of the present study demonstrated a stronger linear correlation ($r^2 = 0.95$) than power curve ($r^2 = 0.85$).

Absorption efficiency

The majority of workers have found that body weight, ration size and temperature do not significantly affect absorption efficiency in fish (Pandian & Marian, 1985). Similar results were obtained in the present study where no significant differences in absorption efficiencies in relation to these variables were found. Cockroft & Du Preez (1989) also found that for *L.lithognathus* there was no clear relationship between fish mass, temperature, and absorption efficiency.

By contrast the type of diet is an important factor affecting absorption efficiency (Kelso, 1972; Elliott, 1982; James *et al.*, 1989b; Cui & Lui, 1990a). Absorption efficiencies among fish fed on commercially prepared feeds are 80-90% (Beamish *et al.*, 1975). Few studies have been carried out with commercial diets commonly used in fish culture and high absorption efficiencies can be expected because the components of the food are finely ground, nutritionally balanced and contain a lower proportion of indigestible material (Knights, 1985). The carbon and nitrogen absorption efficiencies for white steenbras in this study when fed a prepared diet were higher (88-96% and 94-99% respectively) than those obtained from a natural diet of white mussel (47-87% and 83-88%) (Cockroft & Du Preez, 1989).

Absorption efficiencies give an indication of the efficiency with which an organism can utilize a certain type of food (Webb, 1978). From the high values obtained in this study, it is clear that *L.lithognathus* utilises the formulated diet very efficiently.

The biological value (BV) and net protein utilization (NPU) are frequently used in fish farming (Shepherd & Bromage, 1988) to determine the appropriate balance between nitrogen retention and growth (Knights, 1985). *L.lithognathus* retained 76-86% of the ingested ration (NPU) and 75-95% of the absorbed ration (BV) (Table 2.2) also indicating efficient utilisation of the diet.

The C:N ratio in the faeces generally increased during the course of the experiments (Figs. 2.1 and 2.1) resulting in the C:N ratio being higher in the faeces (16:1) than in the food (6:1) (Table 2.2). This could indicate that either nitrogen was being absorbed more efficiently than carbon or that nitrogen is a limiting factor.

Nitrogen excretion

A disadvantage in using large tanks for measuring excretion rates is the difficulty of measuring small changes in urea concentration. This problem was also experienced by James *et al* (1989b). Ammonia was produced in sufficient quantities to be measured accurately but since urea was excreted in small amounts, urea results could not be used for some experiments in the large tank. In these cases the proportions of ammonia and urea nitrogen excretion represented by Cockroft & Du Preez (1988) were used (see results).

L.lithognathus is primarily ammonotelic with urea and amino acids the secondary excretory products (Cockroft & DuPreez, 1989). In the present study it is clear that ammonia was the dominant form of nitrogen excreted, forming 77.93 ± 7.06% of the total dissolved nitrogen. These results are within the ranges obtained for *L.lithognathus* by Cockroft & DuPreez (1989) i.e. 75-95% ammonia, 0-10% urea and other DON 13%.

Peaks in ammonia excretion rates for various species of fish, within a few hours of feeding have been recorded by a number of workers (Brett & Zala, 1975; Elliott, 1976b; Rychly & Marina, 1977; Savitz *et al.*, 1977; Paulson, 1980; Durbin & Durbin, 1981; Jobling; 1981; Tatrai, 1981; Sullivan & Smith, 1982; Davenport & Sayer, 1986; Sayer, 1988) and is primarily related to food intake and not metabolic rate (Brett & Zala, 1975; Lied & Braaten, 1984; Ramarine *et al.*, 1987).

Large variations in the timing of the peaks, and subsequent return to prefeeding, levels occur. In the present study, ammonia excretion rates peaked 4-6h and about 22h after feeding, returning to prefeeding levels after 28-36h, depending on fish size and ration level (Figs. 2.5 and 2.6). Cockroft & Du Preez (1989) found that ammonia excretion rates for *L.lithognathus* peaked 5-7h after feeding, which is similar to the present study, but returned to prefeeding levels 10h sooner (within 18h). This is similar to sockeye salmon, *Oncorhynchus nerka*, where maximal ammonium excretion rates occurred 4h after feeding and returned to basal rate within 16h (Brett & Zala, 1975). In contrast, for juvenile Atlantic cod, *Gadus morhua*, the peak occurred 6.5-27.0h after feeding and declined to prefeeding levels after 4 days (Ramarine *et al.*, 1987). Such variations result from differing experimental conditions with respect to fish size, ration level, diet composition and temperature.

Urea excretion is typically highly variable (Sayer, 1988), the present study being no exception. Mean exogenous urea excretion for small fish was 15 $\pm 29\mu gN.g^{-1}$ dry $wt.h^{-1}$ and for large fish it was 10 $\pm$ $7\mu gN.g^{-1}$ dry $wt.h^{-1}$). Other workers have found lower and more constant levels (Olson & Fromm, 1971; Brett & Zala, 1975; Cockroft & Du Preez, 1989). For example, Brett & Zala (1975) found that urea excretion by *O.nerka* averaged 2.2 $\pm$ $0.2\mu gN.g^{-1}$ dry $wt.h^{-1}$. In high density cultures, such as are employed in the rearing of coho and chinook salmon, large fluctuations in the production of urea have been shown to occur (Burrows, 1964; McLean & Fraser, 1974, cited in Pandian, 1987). The pattern of urea excretion observed in the present study therefore may have been the result of using aggregations of fish.

Variable endogenous rates have been recorded (Rychly & Marina, 1977; Ramarine *et al.*, 1987) and have been shown to be size dependent (Gerking, 1955a; Tatrai, 1981). The total mean endogenous rates for *L.lithognathus* were size dependent, the rates being 1.6 times greater for small fish than larger ones (Table 2.4).

Cockroft & Du Preez (1989) found that nitrogen excretion for *L.lithognathus* was influenced by fish mass, meal size and temperature, excretion rates increasing with decreasing fish mass, and with increasing meal size and temperature. The present study found that fish size and ration size affected nitrogen excretion, but that temperature had no effect.

Nitrogen excretion rates are known to be temperature dependent (Elliott, 1976; Jobling, 1981; Du Preez & Cockroft, 1988a,b; Cockroft & Du Preez, 1989). The present study showed no change in excretion rates at the two temperatures for all the experiments (Figs. 2.5 and 2.6). Temperature independence in excretion rates has been shown to occur in bluegill sunfish, *Lepomis macrochirus* (Savitz, 1969), rainbow trout, *Salmo gairdeni* (Paulson, 1980), and juvenile bream, *Abramis brama* (Tatrai & Penczak, 1985). Savitz (1969) and Paulson (1980) explained this as thermal compensation, a rapid physiological adjustment of fish subjected to extreme seasonal temperature variation (different thermal regimes). This explanation seems appropriate for *L.lithognathus* in the present study since juveniles of this species occupy estuaries and are probably subject to rapid temperature fluctuations. The discrepency between the results obtained by Cockroft & Du Preez (1989) and this study could possibly be due to the fact that in their study the fish were captured in a surf zone environment where there may have been less temperature variation than in estuaries. It is thus possible that such fish might take longer to acclimate metabolically to a new thermal regime.

A constant proportion of the ingested and absorbed rations were excreted by *L.lithognathus* (small - 14.7% I_N and 15.2% pI_N; large - 9.3% I_N and 10.1% pI_N, respectively). Similar relationships have been determined for other species (Gerking, 1955a; Savitz *et al.*, 1977; Durbin & Durbin, 1983; James *et al.*, 1989). Small and large *L.lithognathus* respectively, retained 85.3% and 90.7% of the ingested ration and 84.8% and 89.9% of the absorbed ration, which is very high compared to the 41.5%R_N and 47.8%pR_N found for Cape anchovy (*Engraulis capensis*) by James *et al.* (1989b). Results were also high compared to the 38.5% R_N and 34.5% pR_N found for Atlantic menhaden (*Brevoortia tyrannus*) by Durbin & Durbin (1981b), but closer to approximately 60% for largemouth bass, *Micropterus salmoides* (Savitz *et al.*, 1977).

Small *L.lithognathus* require a low maintenance ration of 0.57mgN.g^{-1} dry wt.day^{-1}, whilst large fish require an even lower ration of 0.42mgN.g^{-1} dry wt.day^{-1}. These rates are similar to those found for *B.tyrannus* (Durbin & Durbin, 1981b) (0.70 mgN.g^{-1} dry wt.day^{-1}) which like *L.lithognathus* are characterized by low activity levels. *E.capensis* has much higher activity levels and therefore requires a higher maintenance ration of 2.17mgN.g^{-1}dry wt.day^{-1} (James *et al.*, 1989b).

The relationship between nitrogen absorbed and retained (Fig. 2.8) can be used to indicate the net growth efficiency (K_2) of *L.lithognathus*. The net growth efficiency is that fraction of the absorbed ration in excess of maintenance available for growth (Brett & Groves, 1979). The slope obtained for *L.lithognathus* in the present study was 0.838 which gives a net growth efficiency of 83.8%. Similar results were obtained by other workers (Gerking, 1955a, 1971; Savitz *et al.*, 1977; Jobling, 1981; Ramarine *et al.*, 1987) where the rate of retention equals the slope of the regression.

Jobling (1981) extrapolated the line of the relationship between nitrogen absorbed and retained, taking the negative Y-intercept as the estimate of endogenous excretion (food intake = 0) and the X-intercept as an estimate of maintenance requirements (growth =

0). Using this method for the regression in Fig. 2.8, an endogenous excretion rate of 0.064mgN.g^{-1} dry wt.day^{-1} and a maintenance requirement of 0.078mgN.g^{-1} dry wt.day^{-1} was obtained. These are much lower than the values measured for endogenous excretion (mean for small and large fish - 0.27mgN.g^{-1} dry wt.day^{-1}) and for maintenance ration (mean for small and large fish - 0.50mgN.g^{-1} dry wt.day^{-1}). Therefore the above method of extrapolation does not hold in the present study.

Examples of values given from the literature of the relative proportions of the different nitrogen losses by a number of fish fed natural and formulated diets is shown in Table 2.6. Much of the variability can be attributed to the differing conditions under which estimations of exogenous and endogenous losses have been made.

TABLE 2.6: The percentage contribution of faecal and nonfaecal nitrogen losses from the ingested ration.

Species	Diet	Faecal	Nonfaecal	Faecal + Nonfaecal	Source
L. lithognathus white steenbras	formulated	1.17–6.08	13.89–23.63	18.79–26.97	present study
Onycorhynchus nerca sockeye salmon	formulated	3	27	30	Brett & Groves (1979)
L.lithognathus	natural	4.04–6.98	2.16–3.94	10.57–12.06	Cockroft & Du Preez (1989)
Lichia amia leervis	natural	19.98–22.65	1.42–2.95	21.40–25.60	Du Preez & Cockroft (1988b)
Salmo trutta brown trout	natural	15–25	4–12	25–30	Elliott (1976)
6 teleost spp.	natural	7.39–10.96	3.61–5.51	10.53–16.74	Cui & Liu (1990)

Diet appears to affect nonfaecal loss in particular. When fed a formulated diet the percentage of nonfaecal loss is much greater (14-27%) than when fed a natural diet (1-6%).

Conclusions

Fish size and ration size affected nitrogen excretion rates but temperature had no affect. Maintenance rations of 7.55 mg.g^{-1} dry wt.day^{-1} (0.76% body wt.) and 5.59 mg.g^{-1} dry wt.day^{-1} (0.60% body wt.) were determined for small and large fish, respectively. The high absorption efficiencies and high nitrogen retention indicate that *L. lithognathus* utilizes the formulated diet very efficiently.

CHAPTER 3

OXYGEN CONSUMPTION

INTRODUCTION

A large proportion of the energy ingested by fish is channelled into metabolism (Brett & Groves, 1979; Cui & Wootton, 1988b; Du Preez *et al.*, 1990). Oxygen consumption provides an indirect measurement of metabolism, (Fry, 1957; Braaten, 1979; Pandian, 1987), reflecting the total energy expenditure for activity of a fish including feeding (Braaten, 1979).

The total energy of metabolism is represented by R in the energy budget equation ($C = F + U + R + G$) and consists of standard metabolism (R_s), active metabolism (R_a) and specific dynamic action (R_{SDA}) (Webb, 1978; Elliott, 1982).

Standard metabolic (R_s) rates are the minimum rates observed in resting, unfed fish (Brett & Groves, 1979; Priede, 1985), maintenance energy requirements being comparable to these rates (Kutty, 1981; Pandian, 1987). Standard rates are often not measurable since laboratory fish are rarely in this state. Minimum oxygen consumption rates in experimental fish therefore, are more an indication of routine metabolism (Knights, 1985). Routine rates occur when the fish display normal spontaneous activity (Brett & Groves, 1979; Kutty, 1981) and reflect the normal metabolic state in the natural environment (Diana, 1982; Kutty, 1981; Du Preez *et al.*, 1990).

Maximum sustained activity for a fish swimming steadily is known as active metabolism (Braaten, 1979). R_{SDA}, the energy losses associated with feeding (Pandian, 1987), and R_a are usually difficult to separate in feeding fish therefore this may be combined and called "feeding" metabolism (Cui & Wootton, 1988b).

Rate of food consumption, temperature and fish size are major factors affecting metabolic rate (Brett, 1979; Elliott, 1982: Soofiani & Hawkins, 1982; Cui & Wootton, 1988b). Metabolic rate has been shown to increase linearly with increased rate of food consumption (Brett, 1979; Soofiani & Hawkins, 1982; Cui & Wootton, 1988b) as well as with increasing temperature and decreasing fish size (Elliott, 1982; Brett & Groves, 1979).

Various types of respirometers have been used to measure oxygen consumption of fish in captivity. Continuous flow respirometers have the advantage that experiments can be performed over a long period (Marais *et al.*, 1976). The oxygen consumption of individual white steenbras, *Lithognathus lithognathus*, has been investigated in such a system (Du Preez *et al.*, 1986b). To determine the oxygen consumption of fish under culture conditions such respirometers would not be suitable as they are relatively small, using single fish. The alternative is closed-chamber systems which have the disadvantage that oxygen concentrations decrease and waste products accumulate (Fry, 1957, 1971; Beamish & Dickie, 1967) restricting the experimental time period. However, closed systems may be considerably larger allowing the measurement of oxygen consumption in groups of fish, thus simulating aquaculture conditions more closely.

This chapter examines the effects of fish size, ration size and temperature on the metabolism of *L. lithognathus* under culture conditions using groups of fish in a large, closed-system respirometer. Measurements of routine and feeding oxygen consumption rates were examined under different experimental conditions.

MATERIALS AND METHODS

The details of the experimental system and standard procedures are given in Chapter 2. In brief, a simple closed system respirometer was used where a 3mm thick transparent Lexan lid which fitted snugly to the sides of the tank, was lowered onto surface of the water. When the oxygen levels got below 2mg.l^{-1} the lid was removed and the water aerated.

Experiments were conducted to investigate oxygen consumption for small and large fish at 16°C and 20°C, and at FR1 and FR2. The fish were starved for 3-4 days before an experiment. Each experiment began at 09h00 with a measurement of prefeeding levels (routine oxygen consumption - the fish displayed normal spontaneous activity) for 2.5h. The fish were then fed to satiation over a 30min period (FR1 - low ration) and a 1h 30min period (FR2 - high ration). For FR2 the lid was removed at the end of the feeding period to take out any remaining food which was reweighed to calculate the total amount of food eaten. The lid was replaced as soon as possible.

Two main levels of oxygen consumption were distinguished: routine (R_r) and feeding (R_f). For the purpose of this study standard metabolism (R_s) is actually "low routine" (R_r) since the fish displayed normal spontaneous activity. Feeding rates comprised of an active period (R_a) during which the fish were engaged in feeding, and a postfeeding period during which respiration was elevated above routine rates as a result of the meal.

No measure of activity was taken but the maximum rate observed was taken to be a measure of active rates (R_a) which is part of feeding metabolism (R_f). Metabolic scope (scope for activity) was also calculated which is the difference between active and routine (Fry, 1957).

Water samples for the determination of oxygen content were collected every 30 min before and for every 30min for 2h after feeding, then every 1-2h thereafter over a period of 36h i.e. at the same time as the water samples for ammonia and urea were taken (Chapter 2). Three 125ml water samples were taken at each time interval from three different points in the tank and three 20ml subsamples from each were used to determine the dissolved oxygen content of the tank water using a modified Winkler technique (Parsons *et al.*, 1984). Half strength sodium thiosulphate solution (0.005N) was used to increase the titre volume. Precision of the method was ± 0.016mg $O_2.l^{-1}$.

To convert oxygen consumption (mgO_2) to equivalent carbon (mgC) and energy (kJ) units the following conversions were used:

$$\text{mg C utilized per day} = \text{mg } O_2 \text{ consumed per day } 12/32 \times RQ$$

(Parsons *et al.*, 1984).

$$\text{kJ utilized per day} = \text{mg } O_2 \text{ consumed per day} \times 0.0136\text{kJ}.$$

A respiratory quotient (RQ) of 0.90 and an energy equivalent of 0.0136kJ.mg O_2 (3.25cal.mg O_2) was used since these values are applicable to ammoniotelic, carnivorous fish whose principle source of energy is protein and lipid (Brett & Groves, 1979)

To test for significant differences of values for the different variables the Mann-Whitney nonparametric test was used (Zar, 1984).

RESULTS

A summary of the experimental trials is shown in Table 2.1 (Chapter 2). Since the fish were graded into groups of similar size it seems reasonable to assume that each of the fish in the group consumed an equal portion of the available ration and contributed equally to oxygen consumption during the experiments.

The oxygen consumption rates of small and large fish are shown in Table 3.1. These results suggest that the fish were well acclimatized to the experimental conditions as they showed no increased oxygen consumption due to stress, except for Expt. 2 where a higher than usual maximum (active) rate was measured.

TABLE 3.1: Oxygen consumption rates in terms of routine (R_r), mean feeding (R_f mean) and active (R_a), and mean values ($\pm$ 1SD) for small and large fish.

Expt.	Oxygen consumption			Metabolic scope
	R_r	R_f mean	R_a	
		(mg O_2 .g wet wt.$^{-1}$hr^{-1})		(mg O_2 .g wet wt.$^{-1}$hr^{-1})
SMALL				
4	0.049	0.103	0.206	0.157
6	0.054	0.116	0.245	0.191
8	0.055	0.124	0.223	0.168
10	0.082	0.145	0.273	0.191
12	0.070	0.129	0.266	0.196
14	0.055	0.105	0.294	0.239
mean	0.061 (0.013)	0.120 (0.016)	0.251 (0.033)	0.190 (0.028)
LARGE				
2	0.030	0.090	0.273	0.233
3	0.030	0.072	0.111	0.071
5	0.030	0.077	0.121	0.084
7	0.032	0.078	0.116	0.060
9	0.040	0.080	0.127	0.087
11	0.030	0.087	0.143	0.095
13	0.053	0.077	0.127	0.079
mean	0.035 (0.009)	0.080 (0.006)	0.145 (0.057)	0.080 (0.013)

Levels of oxygen consumption

Table 3.1 summarises the routine (minimum value), feeding (mean over 36h and includes R_a and R_f) and R_a (maximum value) levels of oxygen consumption measured for all the experiments. The mean routine metabolic rate was $0.061 \pm 0.013 mgO_2.g^{-1}$ wet $wt.h^{-1}$ and $0.035 \pm 0.009 mgO_2.g^{-1}$wet $wt.h^{-1}$ for small and large fish respectively, these being significantly different ($p < 0.05$). For small fish R_f and R_a were on average 2 times and 4 times routine rates, respectively. For large fish R_f and R_a were 2.5 times and 4.5 times routine rates, respectively. Metabolic scope was 2.4 times greater for small fish compared to large fish (Table 3.1).

Fish size, ration size and temperature

Respiration rates peaked 2-4h after feeding and declined to prefeeding levels within 36h for all experiments (Fig. 3.1). Nitrogen excretion rates were also shown to return to prefeeding levels within this time period (Figs. 2.1 and 2.2, Chapter 2).

Oxygen consumption rates were significantly different for small and large fish ($p < 0.05$), rates of small fish being on average 2 times the rates of large fish ($p < 0.05$) (Fig. 3.1, Table 3.1). Within the ranges measured, ration size and temperature did not significantly affect respiration rates ($p > 0.05$).

Energy losses associated with respiration

Table 3.2 summarises the carbon (R_c) and energy (R_e) losses in relation to the ingested ration (I_c, I_e). Respiration in excess of routine ('R) was calculated by subtracting the routine rates from the total oxygen consumption rates during the period of elevated respiration in response to feeding. The mean ingested ration for small

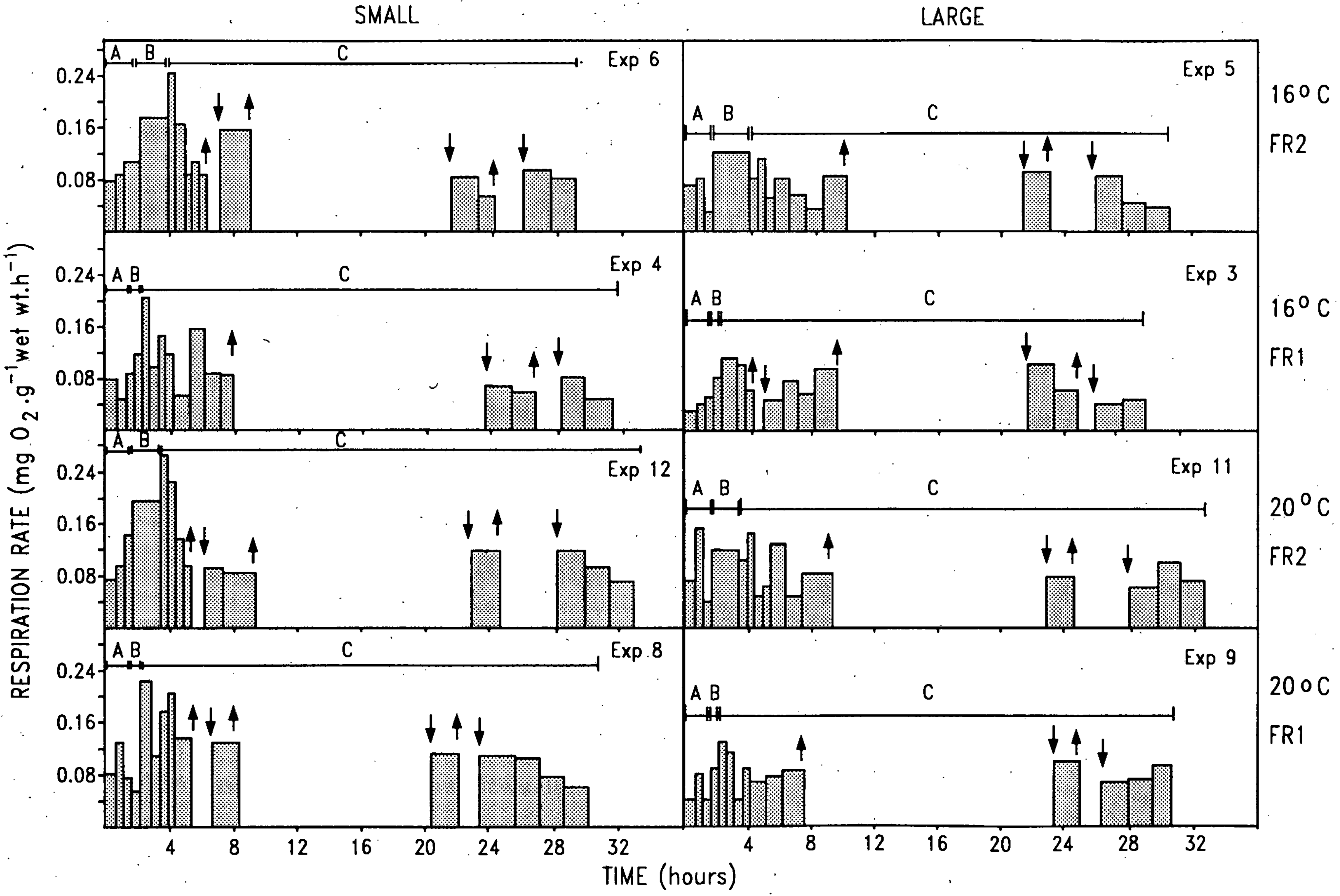

Fig. 3.1: Respiration rates for small and large *L. lithognathus* during prefeeding (A), feeding (B), and postfeeding (C) periods (arrows indicate where the lid was removed ↑ and replaced ↓)

TABLE 3.2: Respiration in excess ('R) and including (R) routine due to feeding, and equivalent carbon (c) and energy (e) losses in relation to ingested ration (I_c, I_e). All values have been standardised against dry weight of fish per day.

Expt.	Ingested ration (I_c)			Respiration in excess of routine ('R)				Respiration including routine (R)			
	I	I_c	I_e	$'R_c$	$'R_c/I_c$	$'R_e$	$'R_e/I_e$	R_c	R_c/I_c	R_e	R_e/I_e
	mg dry wt. $g^{-1}day^{-1}$	mg C $g^{-1}day^{-1}$	$kJg^{-1}day^{-1}$	mg C g day^{-1}	%	$kJg^{-1}day^{-1}$	%	mg C g day^{-1}	%	$kJg^{-1}day^{-1}$	%
small											
4	28.85	12.84	0.58	0.483	3.77	0.020	3.37	2.121	16.52	0.086	14.83
6	63.53	28.27	1.27	1.203	4.26	0.048	3.82	2.841	10.05	0.114	8.98
8	57.99	25.81	1.16	1.267	4.91	0.051	4.40	2.905	11.26	0.117	10.09
10	55.77	24.84	1.12	1.481	5.96	0.060	5.31	3.119	12.56	0.126	11.25
12	84.43	37.57	1.69	1.247	3.32	0.050	2.97	2.885	7.68	0.116	6.86
14	17.12	35.24	1.59	0.659	1.87	0.027	1.66	2.297	6.52	0.093	5.85
large											
2	17.27	7.69	0.35	1.014	13.19	0.041	11.65	1.954	25.41	0.079	22.57
3	16.37	7.29	0.33	0.966	13.26	0.039	11.79	1.906	26.15	0.077	23.33
5	33.35	14.84	0.67	1.037	7.00	0.042	6.23	1.977	27.12	0.080	11.94
7	27.43	12.22	0.55	1.106	9.05	0.045	8.10	2.046	13.79	0.083	15.09
9	29.19	12.99	0.59	1.163	8.96	0.047	7.93	2.103	17.21	0.085	14.41
11	43.87	19.52	0.88	1.119	5.75	0.045	5.11	2.059	15.85	0.083	9.43
13	42.76	19.05	0.86	0.446	2.35	0.018	2.08	1.386	7.10	0.056	6.51

fish in terms of carbon and energy was 27.43 ± 8.80mgC.g^{-1} dry wt.day^{-1} and 1.24 ± 0.040kJ.g^{-1} dry wt.day $^{-1}$ respectively. For large fish these values were half that of the small fish, 13.37 ± 4.88mgC.g^{-1} dry wt.day^{-1} and 0.60 ± 0.22kJ.g^{-1} dry wt.day^{-1} respectively. Respiration in excess of routine due to feeding, in terms of carbon and energy, contributed approximately 2-6% and 2-14% for small and large fish, respectively (Table 3.2). The greater losses in carbon for large fish compared to small fish are due to more carbon being respired during the postfeeding phase in large fish (Fig. 3.2). Absolute respiration rates are greater in small fish compared to large fish. However, when carbon losses are expressed as a percentage of the ingested ration, the relative losses are greater in large fish since the ration size was smaller. It appears that the least losses in carbon occur at FR2 and 20°C but there was no significant difference in carbon losses with ration size ($p > 0.05$).

By incorporating the routine metabolic rates, total metabolic costs, in terms of carbon and energy, can be calculated (Table 3.2). Total respiratory losses as a percentage of R_I for small fish were 10.77 ± 3.60% for carbon and 9.64 ± 3.23% for energy losses. For large fish losses amounted to 18.95 ± 7.53% for carbon and 14.75 ± 6.31% for energy. These differences between small and large fish were not significant ($p > 0.05$).

There were no significant differences in carbon and energy losses associated with feeding for ration size and temperature ($p > 0.05$).

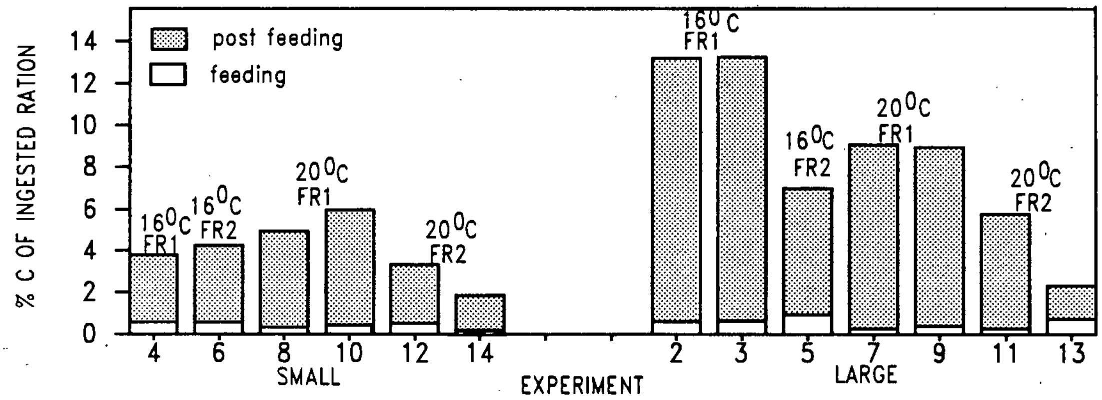

Fig. 3.2: The percentage carbon losses in relation to the ingested ration, for feeding and post feeding periods.

DISCUSSION

Increased oxygen consumption in the initial stages of experiments due to stress and handling has been reported by a number of workers (Caulton, 1978; Marais, 1978; Kutty, 1981; Du Preez *et al.*, 1986b; Ross & McKinney, 1988; Van Waversveld *et al.*, 1989). Such a response only occurred in Expt. 2 where the fish were stressed due to disturbance. A high faecal production peak also occurred during this experiment (Fig. 2.2, Chapter 2).

Increments in oxygen consumption due to stress have potential benefit in assessing the relative energy costs inherent in the stress response and can provide information to avoid or minimise stress (Knights, 1985). The maximum metabolic expansibility (ratio of maximum to standard metabolism) (Beamish, 1964; Brett, 1972) also known as metabolic scope, expresses the capacity for increase of metabolism above standard (Kutty, 1981). The value of $0.273 mgO_2.g^{-1}.h^{-1}$ from Expt. 2 gives an indication of the expansibility (6.8:1) or stress response. The metabolic scope for hatchery-reared rainbow trout, *Salmo gairdneri*, was shown to range from $0.348\text{-}0.498 mgO_2.g^{-1}.h^{-1}$ (5-20°C) (Dickson & Kramer, 1971). Accurate measurements of activity for *L. lithognathus* are required to determine the actual metabolic scope under culture conditions.

Levels of oxygen consumption

From the literature it appears that there is no clear distinction in the usual "standard", "routine" and "active" definitions of metabolic rates used. The metabolic rate of starved fish or the minimum rate observed is often regarded as an approximation of standard metabolism (Beamish, 1964; Brett & Zala, 1975; Elliott, 1976; Brett & Groves, 1979; Du Preez *et al.*, 1986a,b; Du Preez, 1987a; Cui & Wootton, 1988b).

Braaten (1979) stated that metabolism calculated from food-growth experiments at maintenance levels appear to be similar to routine metabolism and that the term "routine" can range from close to standard to half active metabolic rates. The lowest oxygen consumption rates measured in the present study were considered to be routine because the fish were spontaneously active throughout the experimental period. Brett & Groves (1979) reported a mean value for standard metabolism (from 34 species, 365 records of temperate fish) to be 0.089 ± $0.034 mgO_2.g^{-1}.h^{-1}$ with a range of 0.026-$0.229 mgO_2.g^{-1}.h^{-1}$. The routine values obtained for *L.lithognathus* in this study were probably close to standard metabolism since these values are within the lower part of this range. Machiels & Henken (1986) defined metabolism of African catfish (*Clarias gariepinus*) by expressing the total metabolic rate as the sum of fasting metabolic rate (routine) and feeding metabolic rate (metabolic expenditure due to feeding and digestion).

Knights (1985) reported feeding metabolic rates of eels, *Anguilla anguilla*, to be only 1.2-1.3 times the routine rates (prefeeding). For *L.lithognathus* feeding rates (R_f) was 2-3 times routine rates (Table 3.1). The maximum rates (R_a) were 4-4.5 times the routine rates. Feeding and active rates have been reported to be 2-6 times and 9-12 times standard metabolism, respectively (Brett, 1964; Muir & Niimi, 1972). Many active rates are probably feeding rates (Pandian, 1987), and when fed on maximum rations (satiation) feeding rates correspond closely to active rates (Soofiani & Hawkins, 1982). This is the case for *L.lithognathus* in the present study since the fish were fed satiation rations. The active rates were 4-4.5 times the standard rates which is within the feeding range obtained by other workers.

Metabolic scope (scope for activity) is a measure of the energy available for growth and activity (Beamish & Dickie, 1967; Kerr, 1971). Numerous factors may influence standard or active rates or both and therefore affect scope for activity (Fry, 1957). Fish size was the only factor which affected the metabolic scope for *L.lithognathus*, metabolic scope being 2-3 times greater in small fish compared to large fish.

Determination of metabolic scope in a farming situation is useful for comparative purposes in relation to higher levels of metabolism due to feeding activity and stress (Knights, 1985).

The oxygen consumption rates for *L. lithognathus* observed in this study were considerably lower than those obtained by Du Preez *et al.* (1986b) for *L. lithognathus* (Table 3.3). When temperature, fish size and ration size are similar the main difference is the method and diet. Du Preez *et al.* (1986b) used individual fish in the respirometer as opposed to groups of fish in the present study. Brook trout, *Salvelinis fontivialis*, showed a reduction in respiration rates when measured in groups as opposed to individual fish (Job, 1955). This was attributed to the lesser activity characteristic of a group of trout. Van Waversveld *et al.* (1989), in a review on heat production of fish, similarly obtained the lowest heat production on groups of fish. Groups effects are the most likely reason for the discrepancy between the present study and Du Preez *et al.* (1986b).

TABLE 3.3: Comparison of oxygen consumption rates for *L. lithognathus*. Routine, mean and active are defined as they are used in the present study.

Method, diet & ration (% body wt.)	Acclimation & starvation	size (g)	temp (°C)	Oxygen consumption ($mg\,O_2.g^{-1}$ wet wt.h^{-1}) Routine	Mean	Active	Reference
closed/groups	6–8 weeks/	57	20	0.052	0.119	0.264	present study
pellets/4–12%	3–4 days	282		0.048	0.072	0.128	
continuous flow/	8 weeks/	57	20	*0.240	0.317	0.430	Du Preez *et al.*
single/natural/	24–162 hrs	282		0.161	0.199	0.249	(1986b)
4–8%							

* Values calculated from regressions of body mass and oxygen consumption (Du Preez *et al.*, , 1986b)

Other factors which reduce oxygen consumption rates are long-term monitoring (acclimatization period) and fasting period before the start of the experiment (Van Waversveld *et al.*, 1989). In Du Preez's study the oxygen consumption had stabilised within 12h and so no effects of handling were observed, even though the fish were acclimated for 8 days. The acclimation and starvation periods in the present study were more than sufficient to achieve the lowest rates.

Fish size, ration size and temperature

The relationship between body size and metabolism of fish has been established by many previous workers and is described by log-linear relationship (Paloheimo & Dickie, 1966). Fish size was the only variable to significantly affect the metabolic rates observed for *L.lithognathus* in this study. Metabolic rates for small fish ranged from 1.1-2.5 times metabolic rates of large fish (Table 3.1).

Metabolic rate has been shown to increase linearly as food intake increases (Brett & Groves, 1979; Soofiani & Hawkins, 1982). Oxygen consumption rates were marginally greater at FR2 for small and large fish (Fig. 3.1) but not significantly different ($p > 0.05$).

The majority of studies have shown that respiration rates increase with increasing temperature (Beamish, 1964; Elliott, 1982; Brett & Groves, 1979; Paul, 1986; Paul *et al.*, 1988; Du Preez *et al.*, 1986a,b; Du Preez, 1987a). However, some studies have reported temperature independence in oxygen consumption rates over certain temperature ranges. Tilapia, *Sarotherodon mossambicus*, showed a distinct suppression in the pattern of oxygen consumption over the thermal range of 28-37°C (Job, 1969; Caulton, 1978). Temperature did not have a significant effect on oxygen consumption of American plaice, *Hippoglossoides platessoides*, due to seasonal temperature fluctuations which result in metabolic compensation (McKinnon, 1973). Marais (1978) similarly found that one of three mullet species, *Liza richardsoni*, which is more southerly distributed, showed a zone of relatively constant metabolic rate which was explained by metabolic compensation.

There was no significant influence of temperature on respiration rates for *L.lithognathus* in the present study. Du Preez *et al.* (1986b), however, did find a significant increase in oxygen consumption with increasing temperature. In the present study nitrogen excretion rates were also shown to be temperature independent (Chapter 2). *L.lithognathus* like *L.richardsoni* is found in estuaries (Marais, 1981; Beckley, 1984) and thus experience considerable temperature fluctuations in its natural environment. The lack of a temperature effect in the present study probably indicates a similar capability for metabolic compensation.

Energy losses associated with feeding

It is apparent that diet and subsequent digestion rates influence the oxygen consumption pattern following feeding. In the present study *L.lithognathus* was fed a commercial pelleted diet which is more easily digested and absorbed compared to the natural diet of white mussel which Du Preez *et al.* (1986b) fed *L.lithognathus*. When fed pellets, the peak in oxygen consumption occurred 2-4h after feeding and returned to prefeeding levels within 36h. With the mussel diet the peak occurred 10h after feeding and slowly decreased to prefeeding levels within 40h (Du Preez *et al.*, 1986b).

The heat increment following a meal (SDA) is not clearly understood but is largely as a result of deamination of proteins (Beamish *et al.*, 1975). As previously explained, SDA was included in the feeding metabolism (R_f) for the purpose of the present study. SDA can represent high energy costs due to elevated rates of protein synthesis and turnover following feeding (Jobling, 1981). The percentage of the ingested energy which goes to SDA varies from 2.2-45% (most 5-16%) (Beamish, 1974; Pierce & Wissing, 1974; Caulton, 1978; Brett & Groves, 1979; Vahl & Davenport, 1979; Elliott, 1982; Soofiani & Hawkins, 1982). Du Preez *et al.* (1986b) obtained a mean value of 17.6 ± 8.8% for *L.lithognathus* (52-1583g) as the percentage of ingested energy attributed to feeding. In the present study the energy losses associated with feeding was 5.73 ± 3.31% of the ingested energy (31-380g). This value is less than half the value obtained by Du Preez *et al.* (1986b).

The difference in these values can be attributed to diet. When fish are fed high energy commercial pellets in a laboratory situation foraging activity is minimal and less energy has to be expended in feeding (Pierce & Wissing, 1974; Staples & Nomura, 1976). Brett & Zala (1985) noted that on a moist pelleted diet fed to salmon, SDA energy costs were low since the diet was easily digested and absorbed and the need for amino acid interconversions and catabolism is low. Knights (1985) reported that SDA may be a less important source of energy loss in culture than laboratory studies suggest and he obtained a value for R_f to be 2% of the ingested ration for eels, *Anguilla anguilla*. Since *L.lithognathus* was kept under culture conditions in the present study, low energy costs associated with feeding would be expected.

Conclusions

The results of the present study indicate that the ration size and temperature ranges used in the experiments had no significant affect on the metabolism of *L.lithognathus* under culture conditions. It is likely that lower or higher ration sizes and temperatures would result in significant changes to the metabolic response of *L.lithognathus* and should be measured in future studies.

The use of a large respirometer with groups of fish to measure the oxygen consumption of fish under culture conditions is advisable since the fish are subjected to less stress and these conditions simulate aquaculture conditions more closely.

CHAPTER 4

LONG-TERM GROWTH AND FEEDING

INTRODUCTION

Growth rate is an important parameter in the cultivation of fish species (Haug *et al.*, 1989) and is primarily affected by temperature, fish size and ration size (Brett *et al.*, 1969; Brett & Shelbourn, 1975; Elliott, 1975b,c; Brett, 1979; Braaten, 1979; Du Preez, 1987b; Shepherd & Bromage, 1988).

Weight gain, food conversion efficiency, condition factor and body composition are useful indicators of adequacy of diet, ration level and general "well-being" of fish under culture conditions (Cho *et al.*, 1982; Klaoudatas & Apostopoulos, 1986; Shepherd & Bromage, 1988; Bolger & Connolly, 1989).

Food conversion efficiency is important in fish culture (Brown, 1957) and shows the capacity to convert food into flesh (Brett & Groves, 1979). Optimal conversion ratios should be determined because feed costs form a major proportion of production costs (Priede & Secombes, 1988).

To assess the suitability of white steenbras, *Lithognathus lithognathus* under culture conditions, the effects of feeding level, body size, and temperature on growth rate, food conversion, condition factor and proximate body composition were determined.

MATERIALS AND METHODS

Capture, transport and maintenance of experimental fish

Juveniles of *L.lithognathus* are abundant in estuaries (Mehl, 1973; Day *et al.*, 1981; Wallace *et al.*, 1984;) and so sufficient numbers are available for experimental purposes. Juvenile fish (20-450g) were captured at the Breede River (34°43'S; 20°51'E) and Heuningnes River (34°43'S; 20°07'E) estuaries using a 50 by 20m

(12mm mesh) seine net. The fish were transported to the laboratory in aerated seawater in 200l plastic drums.

By altering salinity and water temperature of transport water, stress levels and mortality rates can be reduced (Alexis *et al.*, 1984; Barton & Schreck, 1987). Capture and handling stress on two local estuarine fish species (*Rhabdosargus holubi* and *Liza richardsoni*) was reduced by lowering the temperature and salinity of the seawater (Kabalin, 1989). In the present study, the salinity (12 ‰) and temperature (18 ± 1 °C) of the transport water was lowered which helped minimize mortality and resulted in 80% survival of the captured fish.

The fish were maintained in 3000l portapools supplied with a continuous flow of 5μm filtered seawater at ambient temperature (16 ± 1°C). The fish adapted to laboratory conditions and were feeding well within 10 days.

Handling and transportation of the fish caused a loss of scales resulting in bacterial infections. Injured fish were removed from the main tanks and kept in separate tanks where they were treated with tetracyline (an antibiotic) at doses of 1.6mg.l^{-1}. Most of the fish recovered successfully.

The fish were fed initially on a natural diet of white mussels (*Donax serra*) and were then weaned onto an artificial diet of commercial trout pellets (Meadow Feeds Mill, Table 4.1). They were fed once a day and were well acclimated (4-10 months) before the experiments were conducted.

Experiment 1 - Fish size and feeding frequency

The effects of fish size and feeding frequency on food intake and growth at 16°C was investigated to determine optimum levels of feed intake. The fish were sorted into "small" (20 - 50g) and "large" (200 - 450g) size classes. Groups of 30 small fish and 8 large fish per treatment were kept in 3000l portapools which were divided into three

TABLE 4.1: Proximate composition of the formulated diet

Ingredients	(%)
Wheat bran	14
Fish meal	48
Gluten	8
Bread flour	13
Vegetable acid oil	1
Vitamen premix [1]	11
Mineral premix [2]	5

Chemical analysis	(%)
Moisture	8
Crude protein	45
Crude fat	8
Carbohydrate	25
Ash	14
Energy (kJ.g $^{-1}$)	20.05

---

[1] Vit A (8000 000IU); D3 (200 000IU); E (250 000IU); K (20g); B1 (21g); B2 (50g); B6 (28g); B9 (8g); B12 (20mg); choline chloride (1725g); niacin (200g); D–calcium pantothenate (100g); Vit K (20g); biotin (500mg); inositol (350g); Vit C (750g).

[2] Mn (18g); Cu (5g); Fe (150g); Zn (20g); Co (5g); I (1g); Magnesium (900g); KCL (250g); AlSo4 (1g).

separate compartments with anchovy mesh partitions. The following feeding regimes (FR) were used:

FR1 - fed to satiation once a day
FR2 - fed to satiation twice a day
FR3 - fed to satiation three times a day

The fish were considered to have been fed to satiation when voluntary cessation of feeding had occurred (Braaten, 1979). This usually occurred within 15min of the commencement of feeding. Each feeding regime was done in duplicate. The fish were starved for 12h prior to being weighed (g) and measured (SL, mm) every two weeks for 62 days. The amount of food consumed each day for all groups was recorded.

Feed conversion ratio (FCR) and gross conversion efficiency (GCE) was based on "dry" feed to wet fish weight using the following formulas (Priede & Secombe, 1988):

$$\text{FCR} = \text{ration/specific growth rate}$$

$$\text{GCE} = \text{specific growth rate/ration} \times 100$$

Where ration and specific growth rate are % wet body weight per day. To convert % wet body wt. to % dry body wt. for the ration, the wet:dry wt. regression was used (note Chapter 4, results).

Specific growth rate (SGR) (the percent daily increase in weight, Priede & Secombe, 1988) was calculated in the following way:

$$\text{SGR} = \ln W_1 - \ln W_o/t\ (\text{days}) \times 100$$

Where W_o = initial weight, W_1 = final weight, t = days.

Condition factor (K) is based on the relationship between length and weight and assumes a heavier fish of a given length is in better condition (Bolger & Connolly, 1989). The equation used to calculate this was:

$$K = \text{wt (g)}/\text{length (cm)}^b \times 1000$$

where b = 2.17, which was determined from length-weight regression, $Y = 2.17X - 208.31$ ($r^2 = 0.93$, $n=241$), for the size range 26-437g.

Experiment 2 - Temperature

This experiment examined the influence of temperature at FR1 and FR2 on growth and body composition. Fish in the size range 40-110g were placed into groups of 17 fish with each group consisting of a similar range of fish size. Each treatment was duplicated and fed under the following feeding regimes:

FR1 - at 16 ± 1°C and 20 ± 1°C
FR2 - at 16 ± 1°C and 20 ± 1°C

The fish were kept in 200l fibreglass tanks supplied with $5\mu m$ filtered seawater.

The fish were weighed and measured every two weeks for 56 days. For proximate analysis a sample of 8 fish was taken for analysis at the start of the experiment and at the end, a sample of six fish from each pool was taken. A similar method was employed by Miglavis & Jobling (1989). The fish were killed by a blow to the head and a section of white muscle below the dorsal fin (2-8g) cut out, frozen in liquid nitrogen and stored at -80°C until analysed.

Samples were freeze dried (-50°C, 0.005atm) to constant weight and ground to a fine powder with a pestle and mortar. A subsample (15-20mg) of tissue was homogenised with distilled water in a 1mg.ml^{-1} concentration. This homogenate was then used for the determination of protein and lipid content.

Protein was determined by the Folin-Ciocalteau method (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as a standard. Lipid was determined by the method of Marsh & Weinstein (1966) with a chloroform:methanol (2:1) extraction using tripalmatin as a standard. The above methods are a modification of the microanalytical scheme from Holland & Gabbot (1971) and Holland & Hannant (1973). All analyses were carried out in triplicate.

Moisture was determined by drying subsamples at 60°C for 48h and ash determined by weighing the residue after combustion at 550°C overnight. Energy values were obtained by a Bomb Calorimeter (Model CP500) and expressed as in kJ.g^{-1} dry wt. of sample.

The protein and lipid content of the food was determined by the same methods as the fish tissue, and carbohydrate content was determined by the method of Folin & Malmros (1929) using glucose as a standard.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test significant differences among treatments ($p < 0.01$; $p < 0.05$ significance levels) with respect to condition factor, specific growth rate, food conversion and proximate body composition.

RESULTS

Experiment 1 - Fish size and feeding frequency

Food intake

Food intake for both small and large fish, over the experimental period, showed considerable daily variation (Fig. 4.1). Food intake increased with feeding level and fish size and ranged from 0.41g.fish^{-1}.day^{-1} at FR1 to 1.03g.fish^{-1}.day^{-1} at FR3 for small fish, and 0.39g.fish^{-1} .day^{-1} at FR1 to 1.25g.fish^{-1}.day^{-1} at FR for large fish. There was no clear increase or decrease in food intake with time which was possibly due to the erratic feeding pattern of the fish where a period of intense feeding was generally followed by one of reduced feeding.

Ration size (% dry body wt.) decreased with increasing fish size ranging from 4.50-11.52% body wt. and 1.67-4.00% for small and large fish, respectively.

Growth performance

All groups of small and large fish gained weight over the experimental period, the greatest increase occurring at FR1 for small fish and at FR2 for large fish (Fig. 4.2). Table 4.2 summarises the mean growth performance of *L.lithognathus* over the 62 days. The mean initial weight for small fish was 28 ± 2g and for large fish 250 ± 23g. The condition factor (K) was significantly greater for large fish compared to small fish ($p < 0.05$) and was significantly greater at FR2 for small fish ($p < 0.05$). Over the experimental period, condition factor ranged from 1.19-1.35 for small fish and 2.00-2.19 for large fish the highest values occurring at FR2 for small fish and at FR3 for large fish.

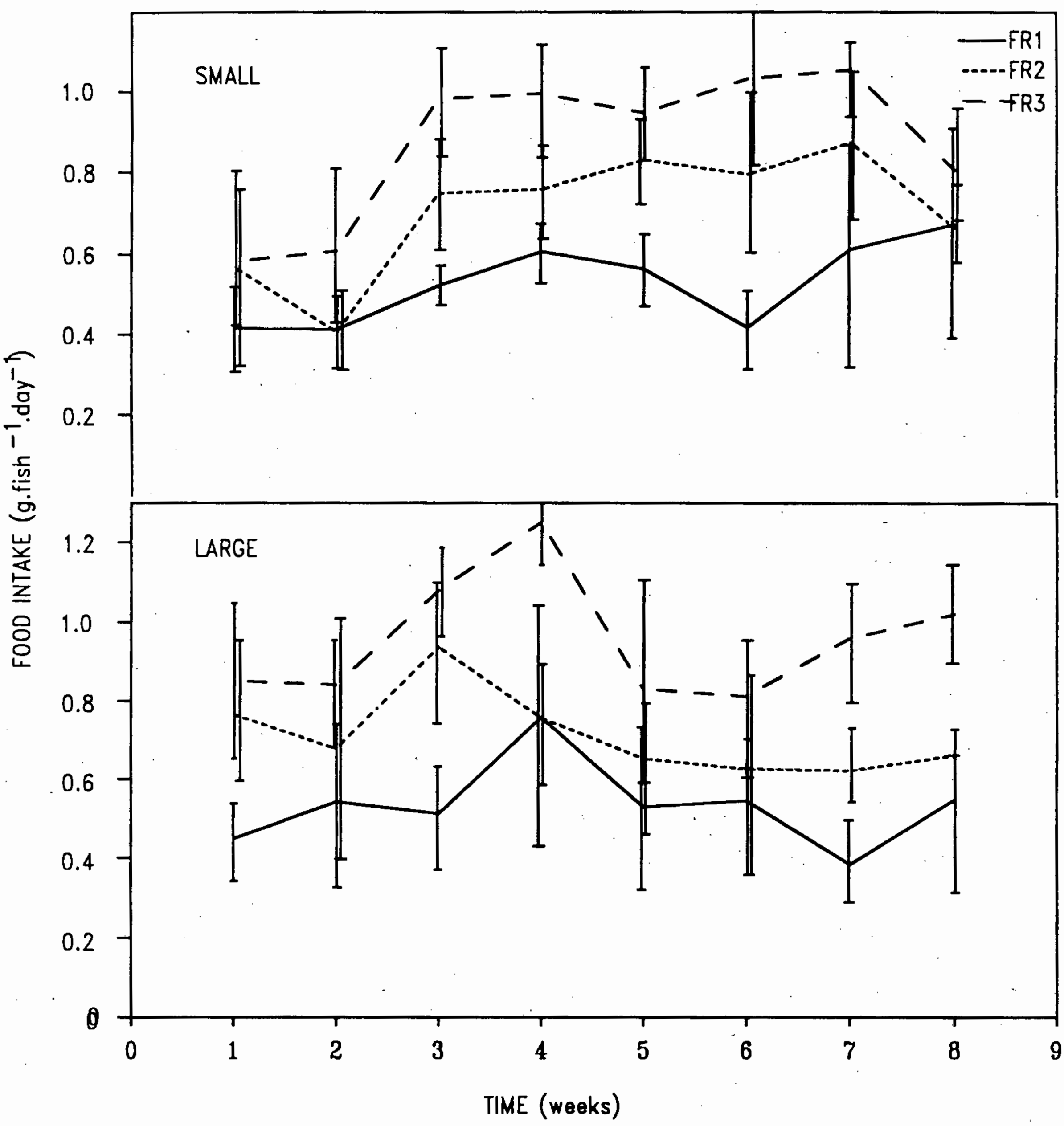

Fig. 4.1: Mean food intake for small and large *L.lithognathus* at the different feeding levels.

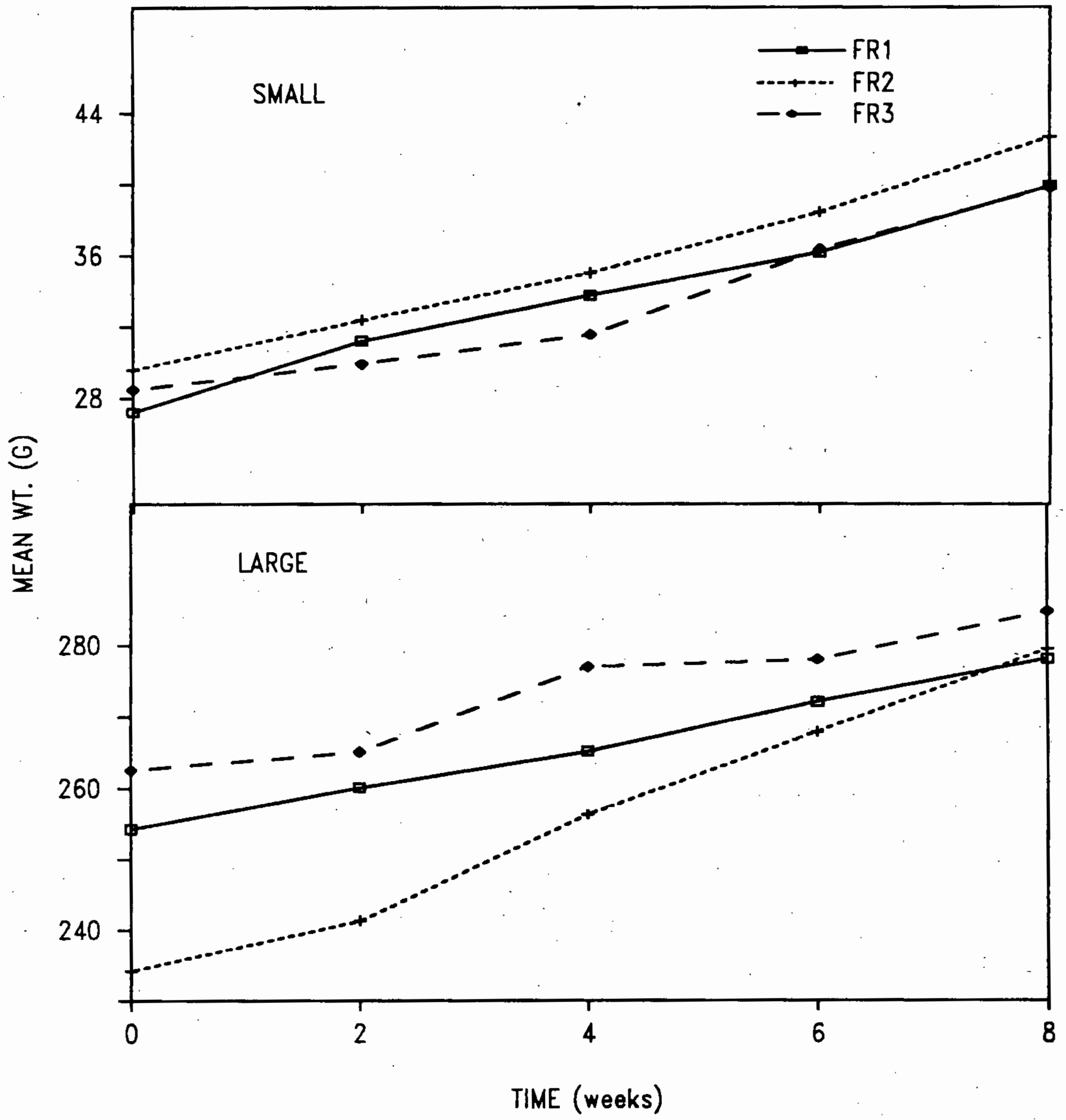

Fig. 4.2: Mean weight increase of small and large *L.lithognathus* at the different feeding levels

TABLE 4.2: Mean growth performance at *L.lithognathus* at 16^{0}C over the 62 days (± 1SD)^{1}

Parameter	small			large		
	FR1	FR2	FR4	FR1	FR2	FR4
Initial body wt. (g)	27.21 (4.17)	29.55 (5.63)	28.47 (4.52)	254.23 (60.10)	234.25 (62.10)	262.58 (41.25)
Final body wt. (g)	39.94 (8.06)	42.68 (7.59)	39.83 (7.35)	278.23 (61.07)	279.61 (52.10)	284.99 (61.34)
Initial length (mm)	101.97 (6.13)	104.40 (6.54)	102.62 (5.88)	222.88 (13.13)	215.44 (16.86)	219.06 16.34)
Final length (mm)	116.52 (7.68)	118.43 (7.04)	115.03 (5.16)	229.21 (17.00)	230.00 (20.90)	232.43 (16.36)
Condition factor (K)^{2}	1.29 (0.12)^{a}	1.34 (0.10)^{b}	1.29 (0.10)^{a}	2.10 (0.10)^{c}	2.10 (0.12)^{c}	2.09 (0.14)^{c}
SGR (% body wt.day-1)^{3}	0.62 (0.09)^{a}	0.59 (0.06)^{a}	0.60 (0.09)^{a}	0.15 (0.09)^{b}	0.29 (0.06)^{c}	0.13 (0.10)^{b}
Ration (% body wt.day^{-1})	1.59 (0.17)	1.93 (0.30)	2.66 (0.61)	0.57 (0.06)	0.87 (0.04)	1.14 (0.04)
FCR^{4}	2.65 (0.46)^{a}	3.25 (0.30)^{b}	4.06 (0.59)^{c}	3.53 (0.66)^{b}	2.82 (0.66)^{a}	16.07 (15.13)^{d}
GCE^{5}	38.70 (5.71)^{a}	31.02 (2.71)^{b}	25.08 (3.22)^{c}	28.69 (5.47)^{c}	37.04 (9.24)^{a}	13.10 (11.72)^{d}
Survival	100	100	100	100	100	100

[1] Values with the same superscipts are not significantly different (p = 0.05)

[2] Condition factor (K) = W/L^{b} X 1000

[3] SGR = $\ln W_1 - \ln W_0 / t$ (days) x 100

[4] FCR = ration/SGR

[5] CGE = SGR/ration X 100

The specific growth rate (SGR) for small fish was not significantly affected by feeding level ($p > 0.05$) but for large fish, specific growth rate was significantly greater at FR2 ($p < 0.05$) (Table 4.2). The best growth rates were at FR1 and FR2 for small and large fish, respectively. Specific growth rates were 2-4 times greater in small fish compared to large fish.

Food conversion ratio (FCR) and gross conversion efficiency (GCE) were significantly different at the different feeding regimes, with the optimum values at FR1 (FCR 2.65; GCE 38.7%) for small fish and at FR2 (FCR 2.82; GCE 37.04%) for large fish (Table 4.2). Survival was 100% for all treatments.

Ration size, food conversion, condition factor and specific growth rates varied between each weighing period but no trend with respect to ration was evident (Figs. 4.3 and 4.4).

Experiment 2 - Temperature

Food intake

Mean food intake showed a similar pattern to Expt. 1 with large day to day variations (Fig. 4.5). Food intake increased with feeding level and temperature and ranged from 0.44 $g.fish^{-1}.day^{-1}$ at FR1 and 16°C to 1.05 $g.fish^{-1}.day^{-1}$ at FR2 and 20°C (Fig. 4.5).

Ration size increased with increased feeding level and at the higher temperature, ranging from 1.80-4.26 % body wt.

Growth performance

All groups of fish showed an increase in weight over the experimental period, the greatest increase occurring at 20°C for both FR1 and FR2 (Fig. 4.6).

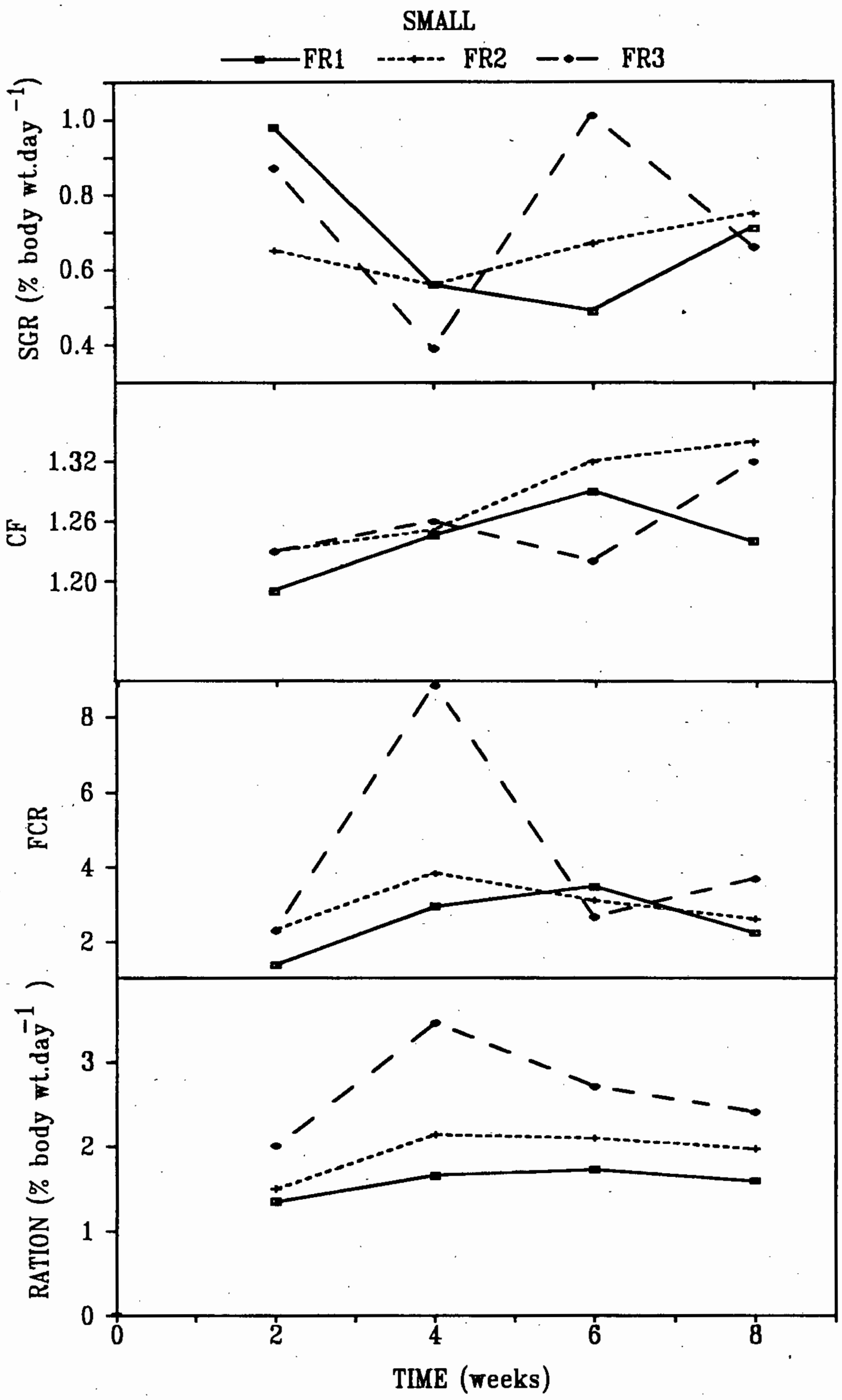

Fig. 4.3: Ration, food conversion (FCR), condition factor (K) and specific growth rate (SGR) for small *L.lithognathus* at each weighing

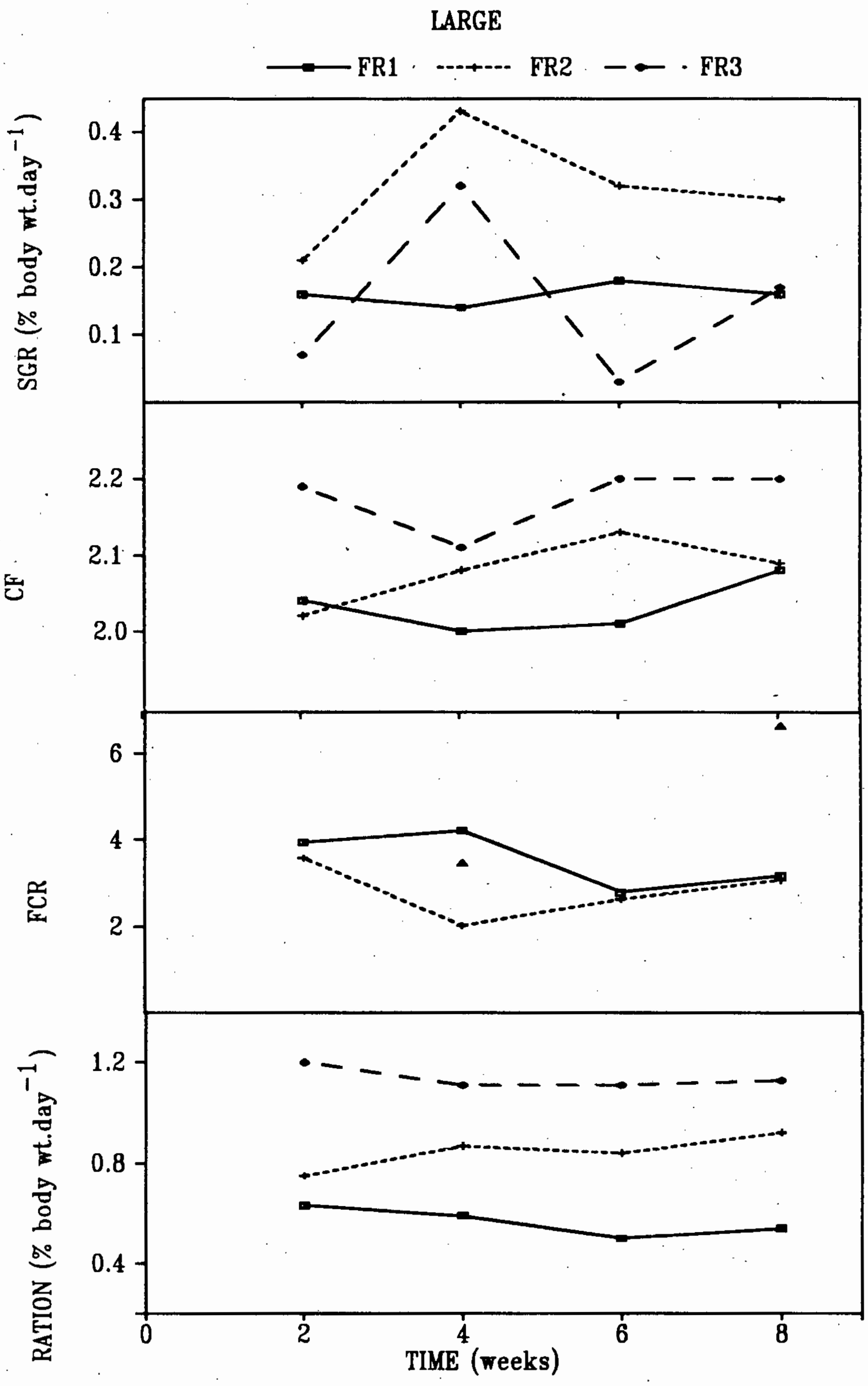

Fig. 4.4: Ration, food conversion (FCR), condition factor (K) and specific growth rate (SGR) for large *L. lithognathus* at each weighing

Table 4.3 summarises the mean growth performance of *L. lithognathus* subjected to the different treatments. The mean initial weight was 75g. The condition factor was significantly affected by feeding regime ($p < 0.05$), the highest value occurred at FR2 and 16°C and ranging from 1.60-1.78.

Specific growth rates were significantly greater at the higher temperature ($p < 0.05$). The greatest growth rate occurred at FR1 and 20°C (Table 4.3).

Food conversion was optimal at the lowest ration (FR1 16°C) with FCR increasing (GCE decreasing) significantly with ration size ($p < 0.05$) (Table 4.3). The fish showed reduced survival at the higher temperature but was never less than 90%.

Ration, food conversion, condition factor and specific growth rate varied at each of the weighings with no trend occurring with respect to ration levels (Fig. 4.6).

Proximate body composition

Table 4.4 summarises the initial and final proximate body composition (% dry body wt.) of the fish subjected to the different feeding regimes. Body moisture, protein, ash and energy were not significantly affected by feeding level or temperature ($p > 0.05$) and the combined effect of these variables made the differences even less apparent. Lipid levels increased significantly with feeding level ($p < 0.05$).

Moisture, protein, ash and energy content of final body composition did not change significantly from the initial sample ($p > 0.05$). Lipid levels increased significantly ($p < 0.05$) from the initial sample being greatest at FR2 and 20°C (Table 4.4).

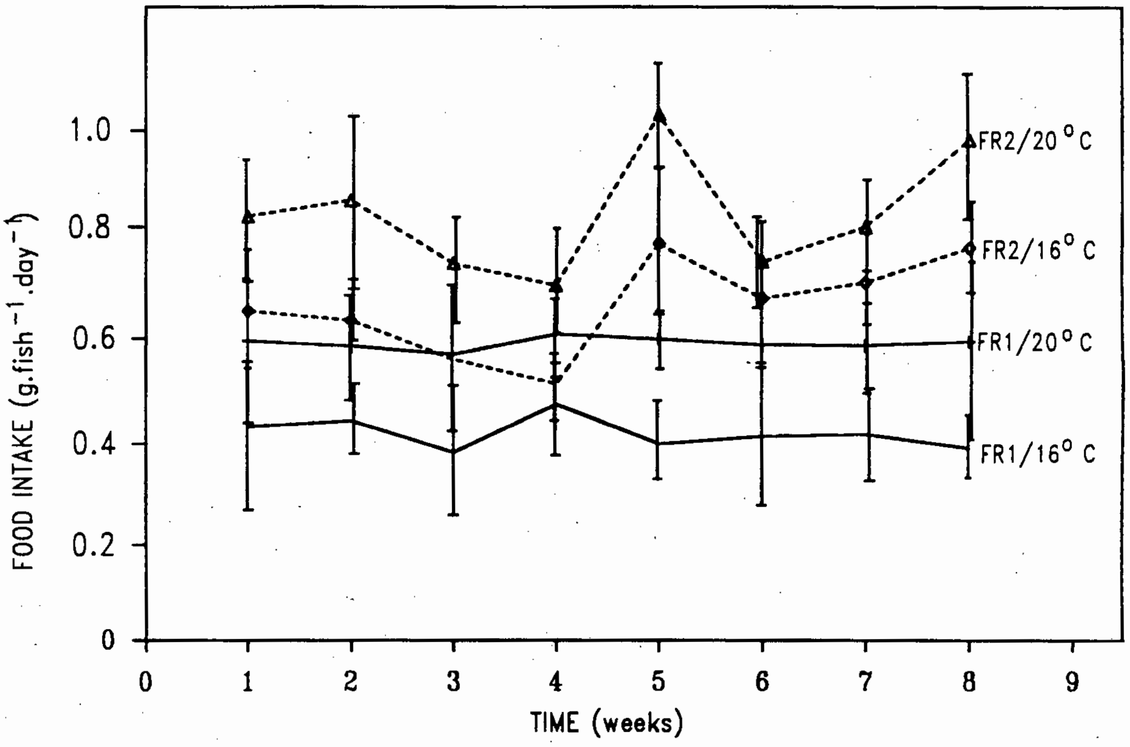

Fig. 4.5: Mean food intake for 74g *L.lithognathus* at the different feeding levels.

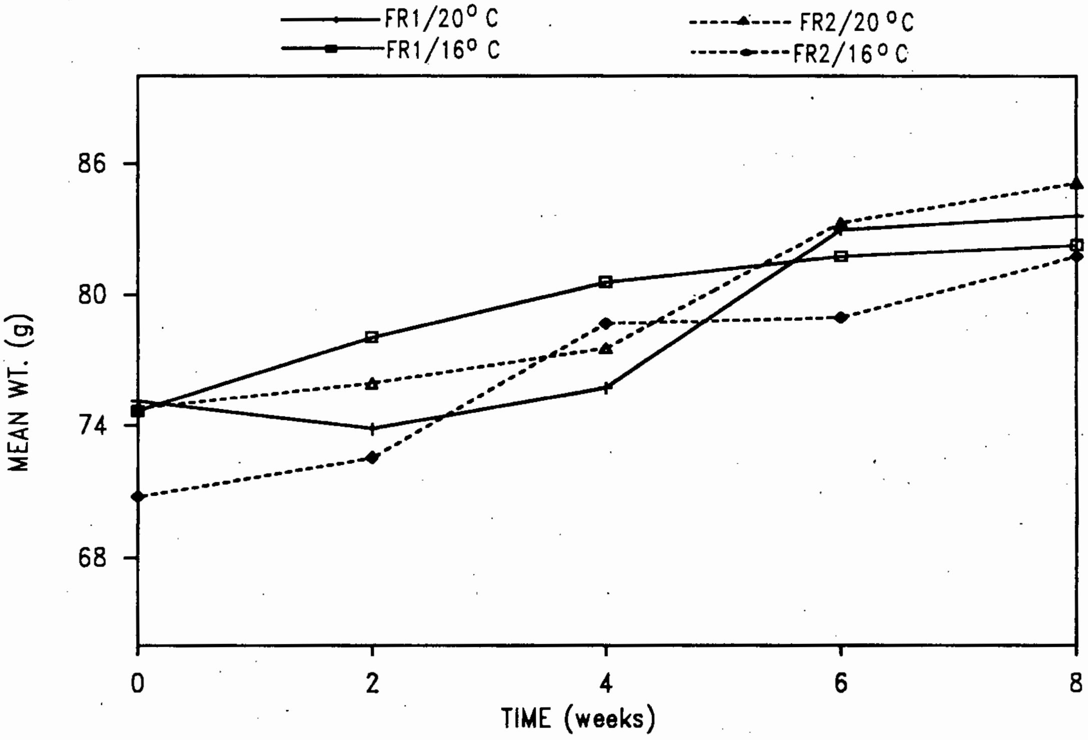

Fig. 4.6: Mean weight increase of 74g *L.lithognathus* at the different feeding regimes.

TABLE 4.3: Mean growth performance of *L.lithognathus* over 56 days (± 1SD)[1]

Parameter	FR1		FR2	
	16 °C	20 °C	16 °C	20 °C
Initial body wt. (g)	74.68 (16.61)	75.13 (12.05)	70.77 (10.55)	74.80 (16.57)
Final body wt. (g)	82.32 (18.91)	83.64 (19.06)	81.77 (14.15)	85.13 (18.44)
Initial length (mm)	139.62 (9.70)	140.82 (7.53)	134.29 (7.07)	139.15 (9.76)
Final length (mm)	145.64 (12.79)	145.60 (10.74)	141.40 (7.46)	145.40 (13.26)
Condition factor (K)[2]	1.64 (0.16)^{a}	1.69 (0.15)^{b}	1.79 (0.18)^{c}	1.60 (0.19)^{d}
SGR (% body wt.day-1)[3]	0.30 (0.09)^{a}	0.46 (1.72)^{b}	0.28 (0.10)^{a}	0.44 (0.26)^{b}
Ration (% body wt.day^{-1})	0.59 (0.09)	0.85 (0.13)	0.91 (0.10)	1.44 (0.10)
FCR[4]	3.16 (1.24)^{a}	3.47 (1.82)^{a}	4.81 (2.36)^{b}	16.07 (15.13)^{c}
GCE[5]	35.87 (15.63)^{a}	30.87 (9.95)^{a}	24.25 (8.45)^{b}	16.13 (12.28)^{c}
Survival	97	94	100	91

[1] Values with the same superscipts are not significantly different ($p = 0.05$)

[2] Condition factor (K) = W/L^{b} X 1000

[3] SGR = $\ln W_1 - \ln W_0 / t$ (days) x 100

[4] FCR = ration/SGR

[5] CGE = SGR/ration X 100

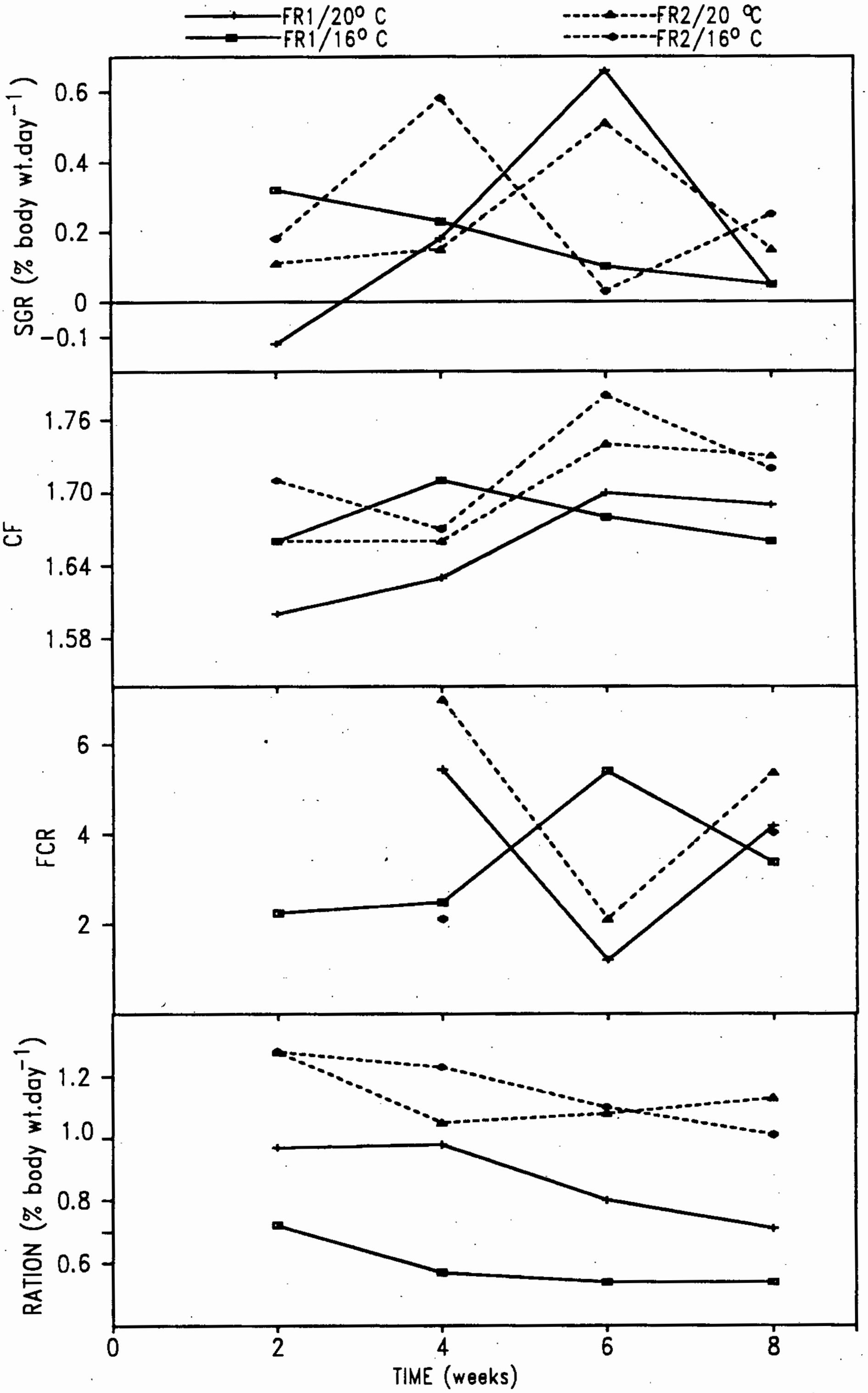

Fig. 4.7: Ration, food conversion (FCR), condition factor (K) and specific growth rate (SGR) for *L.lithognathus* at each weighing.

TABLE 4.4: Proximate body composition of ***L.lithognathus*** at the different feeding regimes (± SE) (protein, lipid and ash expressed as % dry wt.)[1]

Composition	Initial	Final			
		FR1		FR2	
		16 °C	20 °C	16 °C	20 °C
Moisture (%)	72.57 (2.11)^{a}	72.79 (1.84)^{a}	73.66 (2.26)^{a}	73.91 (0.75)^{a}	73.98 (0.86)^{a}
Protein (%)	44.89 (9.69)^{a}	43.06 (8.79)^{a}	44.61 (11.39)^{a}	45.25 (14.72)^{a}	43.52 (7.63)^{a}
Lipid (%)	15.01 (1.67)	20.02 (1.23)	19.10 (1.09)	21.30 (1.73)	22.56 (1.89)
Ash (%)	7.71 (2.79)^{a}	9.09 (1.91)^{a}	8.97 (4.25)^{a}	10.07 (2.16)^{a}	8.60 (2.52)^{a}
Energy (kJ.g^{-1})	22.52 (0.60)^{a}	23.21 (0.86)^{a}	23.22 (0.71)^{a}	22.15 (0.65)^{a}	22.42 (0.51)^{a}

[1] Values with the same superscipts are not significantly different (p = 0.05)

DISCUSSION

Food intake

The daily food intake (g.fish^{-1}.day^{-1}) of *L.lithognathus* in the present study showed considerable variation with no distinct feeding pattern (Fig. 4.7). A day or two of intense feeding was followed by reduced food intake. Daily variations in food intake has been observed for a number of fish species (Brown, 1957; Pandian, 1970; Niimi & Beamish, 1977; Elliott, 1975b; De Silva & Balbontin, 1974; Du Preez, 1987b) and is due to changes in appetite from day to day (Brown, 1957) and the rate of food processing (Brett, 1971). For *L.lithognathus*, Du Preez (1987b) observed a similar feeding pattern to the present study when his fish were fed on white mussel.

In Expt.1 food intake (g.fish^{-1}.day^{-1}) was greater for large fish (250g) compared to small fish (28g) but when expressed as % dry body weight, the ration size was greater for small fish. Ration size increased with feeding level, the ration size for small fish (5.29-8.86% body wt.) being significantly greater ($p < 0.05$) than that of large fish (1.90-3.80% body wt.). Increased food intake occurs with increased feeding frequency but there is a limit to the amount of food that can be eaten (Elliott, 1975b; Grayton & Beamish, 1977). In the present study the fish were being overfed at the highest feeding levels (FR3 at 16°C; FR2 at 20°C) and were satisfied after one or two feedings a day at 16°C in both Expt.1 and 2. Du Preez (1987b) for *L.lithognathus* and Grayton & Beamish (1977) for rainbow trout similarly found that the fish were satisfied with just two feedings a day.

It is possible, that in the present study, food intake, particularly for the large fish, was overestimated since pelleted food is susceptible to breaking up in the water or in the mouths of fish (Talbot, 1985) with subsequent loss of material. Priede & Secombe (1988) said that feeding fish continuously to satiation is not efficient in farmed species.

The literature is inconsistent in the use of the term "ration size" . It can be expressed as % body weight, using either wet or dry weight, making comparisons very difficult. Elliott (1975b) cautioned that when expressing food intake as a percentage body weight, comparisons can only be made between fish of similar size and similar food moisture content. To compare the food intake of *L.lithognathus* in the present study to Du Preez's study a general comparison is made. Du Preez (1987) expressed the food intake of *L.lithognathus* (15°C, 150-797g) as % wet body mass (wet food:wet fish wt.) and obtained values of 2.43-6.18% body wt. at FR1 and 4.07-8.86% body wt. at FR2. For *L.lithognathus* (16°C, 28-250g), in the present study, food intake (dry food:dry fish wt.) ranged from 4.50-11.52% body wt. which is similar to Du Preez's study.

Food intake is known to increase with temperature up to an optimum, after which it decreases (Warren & Davis, 1967; Niimi & Beamish, 1974; Elliott, 1975a; Brett & Groves, 1979; Priede & Secombe, 1988). In the present study ration size increased at the higher temperature (Table 4.3). It is interesting to note that for a 74g fish at 20°C (2.83 % body wt., Expt.2) the ration size was similar to a 250g fish at 16°C (2.90 % body wt., Expt.1).

Growth

The relationship between growth and ration is curvilinear where growth rate increases linearly with ration to an optimum ration. With further increase in ration, the curve flexes further, reaching a plateau at maximum growth rate (Brett, 1979; Brett & Groves, 1979; Priede & Secombe, 1988). A few workers have found that specific growth rate decreased at high rations (Brett *et al.*, 1969; De Silva & Balbontin, 1974; Huisman, 1976). In the present study *L.lithognathus* showed a slight decline in specific growth rate with increasing ration level at both temperatures (Tables 4.2 and 4.3) but the decline was not significant. This further supports the fact that the fish were being fed on excess rations.

Specific growth rates increase with increasing temperature up to a temperature where an optimum growth is reached, and decreases thereafter (Elliott, 1975b,c; Brett & Groves, 1979). The specific growth rates of *L.lithognathus* in the present study were higher at 20°C in Expt.2 (Table 4.3). At 16°C the growth rates were on average 0.60 $\pm$ 0.02%.day^{-1} for 28g fish, 0.29 $\pm$ 0.01%.day^{-1} for a 74g fish and 0.19 $\pm$ 0.09%.day^{-1} for 250g fish. At 20°C for the 74g fish the growth was 0.45%.day^{-1}. Du Preez (1987b) reported lower specific growth rates for *L.lithognathus*: individual fish at 20°C (17-726g fish) mean rate was 0.19 $\pm$ 0.12%.day^{-1} and for a group of fish at 15°C (151-797g fish) 0.22 $\pm$ 0.17%.day^{-1}. For commercially grown Atlantic salmon and rainbow trout in sea cages the specific growth rates for 30-150g fish (8-14°C) were 1.3-2.2%.day^{-1} and for 150-600g fish the range was 0.2-1.7%.day^{-1} (Priede & Secombe, 1988).

Efficiency of food utilization

Condition factor, food conversion and body composition are useful indicators of adequacy of diet, ration level and general "well-being" of the fish under culture conditions (Cho *et al*, 1982; Kladaoudatas & Apostopoulos, 1986; Shepherd & Bromage, 1988; Bolger & Connolly, 1989).

In the present study condition factor and food conversion for *L.lithognathus* in both Expt.1 and 2 were optimal at the lowest ration level, except for large fish at FR2 and 16°C in Expt.1 (Tables 4.2 and 4.3).

Measuring condition (K) of fish is standard practice in fisheries biology (Bolger & Connolly, 1989). A K value of less than one indicates poor condition, whilst a value greater than 1 indicates well fed fish in good condition. The condition factors for *L.lithognathus*, in the present study, were all greater than 1 indicating that the fish were in good condition and feeding well.

Both overfeeding and underfeeding can lead to inefficient food utilization (Jackson, 1988). Food conversion gives an indication of the efficiency of food utilization. The food conversion ratio (FCR) the normal measure of conversion used in commercial practice (Priede & Secombe, 1988) whilst in scientific literature, conversion efficiency (GCE) is used (Parker, 1987). A FCR of less than 2 indicates good utilization of food and is often found when fish are fed on a formulated diet (Parker, 1987; Shepherd & Bromage, 1988). However, in such cases, FCR can range from 1-4 (Bardach *et al.*, 1972).

L.lithognathus, in the present study, showed inefficient use of the food at the high feeding levels (FR3 and 16°C; FR1, FR2 and 20°C) with a mean of 21.30 ± 8.16%. Mean conversion efficiencies at the lower rations were 30.77 ± 6.40% at FR1 and 16°C, and 34.42 ± 5.16% at FR2 and 16°C. Young herring, *Clupea harengus*, showed higher conversion efficiencies at the low ration (5-30%) compared to the satiation ration (5-13%) (De Silva & Balbontin, 1974). For sockeye salmon, *Onycorhynchus nerka*, a mean of 25.0 ± 2.5% conversion efficiency was obtained (Brett & Shelbourn, 1975).

The conversion efficiencies for *L.lithognathus* fed on white mussel (Du Preez, 1987b) were very low (3.0 ± 0.3%) compared to the present study. This was probably due to the different diets since conversion efficiencies for fish fed commercial pelleted diets are higher than those fed on natural diets (Knights, 1985).

Proximate body composition

Proximate composition of fish is a consequence of growth rate and thus environmental quality (Brett *et al.*, 1969; Niimi & Beamish, 1972; Elliott, 1976a). The proximate composition of *L.lithognathus* was not significantly affected by feeding level or temperature in terms of moisture, protein, ash and energy. Lipid levels increased with increasing ration level. Farmed and laboratory reared cod (*Gadus morhua*) which are well provided with food show lipid accumulation (Jobling, 1988). Other studies have also shown that only body lipid (and perhaps moisture) are affected by feeding level (Grayton & Beamish, 1977; Storebakken & Austreng, 1987; Parazo, 1990).

Starved or restricted feeding results in a reduction of percentage lipid and increased water content (Elliott, 1976a; Miglavs & Jobling, 1989). The reduced lipid levels in cases of food deprivation are due to the protein-sparing effect where lipids and carbohydrates are used for energy, conserving protein gains (Shepherd & Bromage, 1988). The results of the present study indicate that for *L. lithognathus*, the protein-sparing effect is evident since protein levels remained constant.

Conclusions

It appears that *L. lithognathus* has the capacity to ingest food, far in excess of its energy requirements. At these rations the fish shows low food conversion efficiency. When fed on the rations at 16°C the best food conversion was produced suggesting that this may have been the optimum feeding level. Although specific growth rates were higher at the higher temperature, the food intake was excessive and wastage of food occurred. For *L. lithognathus* under culture conditions the results of the study indicate that feeding regimes should be maintained at a relatively low level.

CHAPTER 5

CARBON AND NITROGEN BUDGETS AND THE DEVELOPMENT OF A GROWTH MODEL

INTRODUCTION

Many workers involved in fish growth studies, from an aquaculture point of view, have expressed the bioenergetics of fish growth in various forms of balanced energy equations. The basic energy equation is: $C = F + U + R + G$, where C = Energy consumed, F = Faecal energy loss, U = Urinary energy loss (nonfaecal), R = Respiratory energy loss, and G = Growth in term of energy. In fish cultivation a knowledge of the efficiency with which food is converted into growth is of considerable importance (Birkett, 1969).

Numerous energy budgets have been constructed for fish (Brafield, 1985). Some related to farmed species are: sockeye salmon, *Oncorhynchus nerka*, (Brett *et al.*, 1969, 1971; Brett, 1964, 1973; Brett & Zala, 1975); largemouth bass, *Micropterus salmoides*, (Niimi & Beamish, 1974); brown trout, *Salmo trutta*, (Elliott, 1976b,c, 1982); Atlantic salmon, *Salmo salar*, (Braaten, 1976); rainbow trout, *Salmo gairdeni* (Staples & Nomura, 1976; Cho *et al.*, 1982); grey mullet, *Rhinomugil corsula*, *Mugil cephalis* and *Liza macrolepis* (Kutty, 1981); European eels, *Anguila anguilla*, (Knights, 1985).

Recent studies on energy budgets of teleost species have been done by Cui & Liu (1990a,b,c,d) and Du Preez *et al.* (1990). The use of energetic models to predict growth has been done by Stewart *et al.* (1983) for lake trout (*Salvelinus namaycush*), Kitchell *et al.* (1977) for yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*), Machiels & Henken (1986) and Machiels & Van Dam (1987) for African catfish (*Clarias gariepinus*), Preall & Ringler (1989) for brown trout (*Salmo trutta*), and Cui & Liu (1989) for European minnow (*Phoxinus phoxinus*).

Growth models using carbon and nitrogen as currencies for the energy budget have been used for by Durbin & Durbin (1983c) and James *et al.* (1989c) for planktivorous fish. The present chapter uses the same approach to predict the growth of white steenbras (*L. lithognathus*) for a range of ration sizes under culture conditions.

BUDGETS

The Mann-Whitney non-parametric test was used to test significant differences between two groups of values at the 0.05 significance level (Zar, 1984)

The budgets are based on experimental data collected from white steenbras of small (57 ± 14g) and large (282 ± 48g) sizes using the data from nitrogen excretion (Chapter 2) and respiration (Chapter 3) experiments.

CARBON BUDGET

The budget is based upon the equation of Durbin & Durbin (1983) as follows:

$$G_C = I_C - R_C - E_C - F_C \qquad (1)$$

where G_C = growth, I_C = ingested ration, and R_C, E_C and F_C are losses through respiration, excretion and defaecation, respectively. The budget was calculated for all of the experiments, and a mean determined for small and large fish. The following calculations are based on one example, experiment 4.

Ingested ration (I_C)

From Table 3.2, $I_C = 12.84\text{mgC.g}^{-1}\text{ dry wt.day}^{-1}$ (2)

Absorbed ration (pI_C)

When faecal losses (F_C) are subtracted from the ingested ration (I_C) a measure of the absorbed ration (pI_C) is obtained. The carbon absorption efficiency, which was determined in Chapter 2 (Table 2.3), p is 0.899.

Since $pI_C = I_C - F_C$, Eqn. 1 then becomes:

$$G_C = pI_C - R_C - E_C \text{ mgC.g}^{-1} \text{ dry wt.day}^{-1} \qquad (3)$$

therefore

$$pI_C = 11.54 \text{mgC.g}^{-1} \text{ dry wt.day}^{-1} \qquad (4)$$

Daily carbon losses

Respiration (R_C)

Respiration represents the major source of carbon expenditure during routine (nonfeeding) metabolism and feeding.

The total daily carbon losse through respiration (Table 3.2, Chapter 3) was:

$$R_C = 2.121 \text{mgC.g}^{-1} \text{ dry wt.}^{-1} \qquad (5)$$

Total daily excretion (E_C)

Cockroft & Du Preez (1989) and the present study both showed that for *L.lithognathus* the organic excretion was mainly in the form of urea (7%) and other DON (12%). Other DON consists of creatinine, creatine, amino acids and uric acid (Elliott, 1976).

For the purposes of this model the organic excretion will include urea, amino acids (glycine) and creatinine. The C:N ratio of these compounds are 0.5C:1N for urea, 1.33C:1N for creatinine, and 2C:1N for glycine. Therefore for every 1g of organic nitrogen excreted, 12/14 X 0.5g C (urea) + 12/14 X 1.67g C (mixture of glycine and creatine) are lost.

Total organic nitrogen excretion amounted to 1.86g C for every 1g N excreted. This is equivalent to a loss of 0.19 X 1.86g C which is 0.353g C for every 1g N excreted.

Total daily losses through nitrogen excretion are the sum of endogenous and exogenous excretion (Chapter 2). For *L.lithognathus* the total daily nitrogen excretion (E_N) is linearly related to nitrogen in the ingested ration (I_N) (Chapter 2) where:

Small: $E_N = 0.471 + 0.147\ I_N\ \mathrm{mgN.g^{-1}\ dry\ wt.day^{-1}}$ (6)

Large: $E_N = 0.390 + 0.093\ I_N\ \mathrm{mgN.g^{-1}\ dry\ wt.day^{-1}}$ (7)

In terms of carbon:

Small: $E_C = 0.166 + 0.052\ I_N\ \mathrm{mgN.g^{-1}\ dry\ wt.day^{-1}}$ (8)

Large: $E_C = 0.138 + 0.033\ I_N\ \mathrm{mgN.g^{-1}\ dry\ wt.day^{-1}}$ (9)

The C:N ratio of the pelleted diet was 6:1. therefore:

Small: $E_C = 0.166 + 0.009 I_C\ \mathrm{mgC.g^{-1}\ dry\ wt.day^{-1}}$ (10)

Large: $E_C = 0.138 + 0.006 I_C\ \mathrm{mgC.g^{-1}\ dry\ wt.day^{-1}}$ (11)

To calculate E_C, I_C was substituted into these equations. Table 5.1 summarises the mean carbon budget from all the experiments, for small and large fish. Where $K1_C$ and $K2_C$ are the gross and net growth efficiencies, respectively: $K1_C = G_C/I_C$ and $K2_C = G_C/pI_C$.

TABLE 5.1: The mean carbon budgets for small and large fish (± 1SD).

Entity	Small	Large
	(mgC.g^{-1} dry wt.day^{-1})	
I_C	27.43 ± 8.80	13.37 ± 4.88
pI_C	25.16 ± 8.29	12.12 ± 4.23
F_C	2.27 ± 0.90	1.25 ± 0.69
R_C	2.69 ± 0.39	1.92 ± 0.24
E_C	0.41 ± 0.08	0.22 ± 0.03
G_C	22.05 ± 8.08	9.98 ± 4.30
$K1_C$	0.79 ± 0.05	0.74 ± 0.06
$K2_C$	0.86 ± 0.04	0.80 ± 0.08

Growth (G_C)

To calculate growth in carbon the values calculated above for each component in the budget are substituted into Eqn.1.

NITROGEN BUDGET

The nitrogen budgets were based on the general equation:

$$G_N = I_N - E_N - F_N \text{ mgN.g}^{-1} \text{ dry wt.day}^{-1} \qquad (12)$$

simplified to

$$G_N = pI_N - E_N \text{ mgN.g}^{-1} \text{ dry wt.day}^{-1} \qquad (13)$$

Experiment 4 is used as an example for the following calculations.

Ingested ration (I_N)

The nitrogen content of the food is 7.58% N (Table 2.2), therefore

$I_N = 2.19 \text{mgN.g}^{-1} \text{ dry wt.day}^{-1}$

Absorbed ration (pI_N)

Nitrogen absorption efficiency (Table 2.4, Chapter 4), $p = 0.957$, therefore

$pI_N = 2.10 \text{mgN.g}^{-1} \text{ dry wt.day}^{-1}$

Daily nitrogen losses

Excretion (E_N)

Eqn's 6 and 7 describe the relation between total daily nitrogen excretion (E_N) and the ingested ration (I_N). Substituting I_N into these equations:

$E_N = 0.793 \text{ mgN.g}^{-1} \text{ dry wt.day}^{-1}$

Growth (G_N)

To calculate growth in term of nitrogen, the values calculated above for each component in the budget are substituted into Eqn.13.

Table 5.2 summarises the mean nitrogen budget for all the experiments, for small and large fish.

TABLE 5.2: The mean nitrogen budget for small and large fish (± 1SD).

Entity	Small	Large
	(mgN.g^{-1} dry wt.day^{-1})	
I_N	4.69 ± 1.50	2.28 ± 0.83
pI_N	4.50 ± 1.46	2.19 ± 0.78
F_N	0.17 ± 0.08	0.08 ± 0.06
E_N	1.16 ± 0.22	0.60 ± 0.08
G_N	3.35 ± 1.24	1.59 ± 0.70
$K1_N$	0.70 ± 0.06	0.68 ± 0.07
$K2_N$	0.73 ± 0.05	0.70 ± 0.08

RESULTS

Specific growth rates

At a mean ingested ration of 27.43 mgC.g^{-1} dry wt.day^{-1} for small fish (Table 5.1) growth in term of carbon (G_C) becomes 22.05 mgC.g^{-1} dry wt.day^{-1}. Expressed as a specific growth rate (% body wt. increase per day) using the percent carbon content of the fish (Table 2.2, Chapter 2), this becomes 4.5%.day^{-1}. For large fish growth in carbon is 2.0%.day^{-1} at a ration of 13.37 mgC.g^{-1} dry wt.day^{-1}.

The same calculation can be done in terms of nitrogen (Table 5.2). At a ration of 4.69 mgN.g^{-1} dry wt.day^{-1} small fish grow at a rate of 3.4%.day^{-1}, and for large fish at a ration of 2.28 mgN.g^{-1} dry wt.day^{-1} the specific growth rate is 1.6%.day^{-1}.

These calculations predicted from the budgets show that the specific growth rate of small fish was significantly greater than that of large fish ($p < 0.05$). The specific growth rate in terms of carbon and nitrogen increases linearly with increasing ration size (Fig.5.1) within the range of rations used in the energetics experiments (0-100 mg.g^{-1} dry wt.day^{-1}).

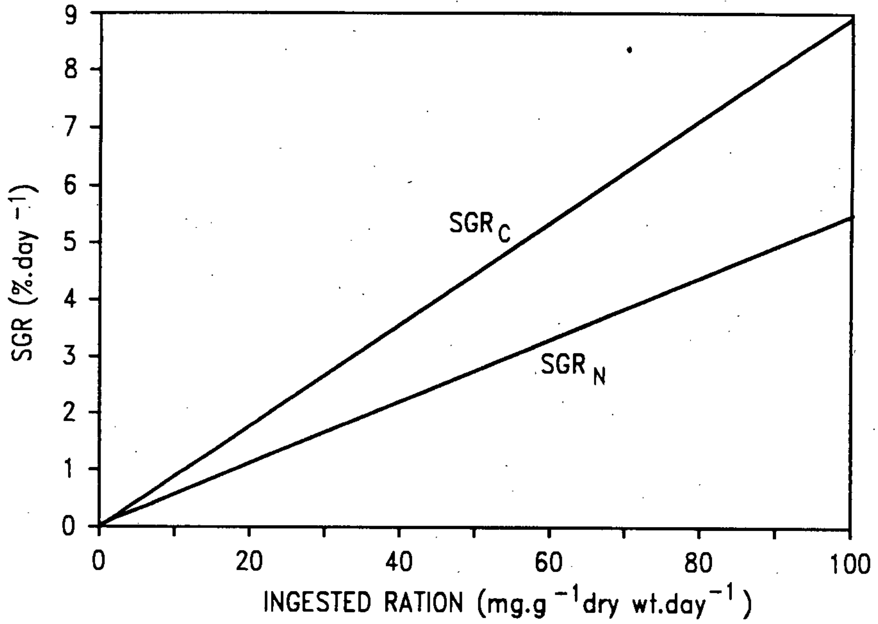

Fig. 5.1: Specific growth rate in term of carbon (SGR_C) and nitrogen.(SGR_N) for the ration range used in the models.

Carbon and nitrogen budgets

Using the carbon and nitrogen budgets summarised in Tables 5.1 and 5.2, and an ingested ration of 0-100mg.g^{-1} dry wt.day^{-1}, the models in Figs. 5.2 and 5.3 were derived. These models represent the carbon and nitrogen losses for small and large fish due to defaecation (A_1, A_2), carbon and nitrogen expenditure due to respiration and excretion (B_1, B_2), net carbon and nitrogen intake (C_1, C_2), and finally growth in carbon and nitrogen (D_1, D_2).

Carbon and nitrogen intake

Since the mean absorption efficiencies for small and large fish were very similar (Table 3.3, Chapter 3) the carbon and nitrogen losses due to defaecation were the same (A_1, A_2). With increasing ration there is a slight decrease in the absorbed ration.

Carbon and nitrogen expenditure

Large fish respired more carbon as a percentage of the ingested ration compared to small fish (Fig.4.2, Chapter 4) resulting in greater carbon expenditure (R_C) (1.8 times) (Fig.5.2, B_2). Carbon expenditure due to excretion (E_C) was 1.4 times greater for small fish compared to large (Fig. 5.2, B_1). When respired and excreted carbon expenditure are added the scope for growth (shaded area) is not significantly greater ($p > 0.05$, 0.4 times) for small fish (Fig. 5.2, C_1).

In terms of nitrogen expenditure, losses only occur via excretion (E_N), this being relatively greater for small fish (Fig. 5.3, B_1). Hence, there is less scope for growth in small fish (Fig.5.3, C_1).

The intersection of the lines (Figs. 5.2 and 5.3, C_1, C_1) represents pI_c and (R_C +) or pI_N and E_N and indicates the minimum ingested ration at which *L.lithognathus* can attain its daily maintenance ration. In terms of carbon this is approximately 2.5mg.g^{-1} dry wt.day^{-1} and 1.5mg.g^{-1} dry wt.day^{-1} for small and large fish, respectively. In terms of nitrogen it is approximately 9mg.g^{-1} dry wt.day^{-1} and 5mg.g^{-1} dry wt.day^{-1} for small and large fish, respectively. This is close to the maintenance ration calculated by the regressions (E_N and I_N/pI_N) in Chapter 2 (7.55mg.g^{-1} dry wt.day^{-1} - small; 5.59mg.g^{-1} dry wt.day^{-1} - large)

Growth rate and growth efficiency

Growth (G) in terms of carbon was significantly greater ($p < 0.05$) greater for small fish compared to large, and growth in terms of nitrogen was significantly greater ($p < 0.05$) for large fish compared to small fish (Figs. 5.2 and 5.3, D_1 and D_2). Where the line intercepts at zero this corresponds to the maintenance ration, below which negative growth occurs.

Gross growth efficiency (K_{1C}, K_{1C}) increases asymptotically with increasing ration

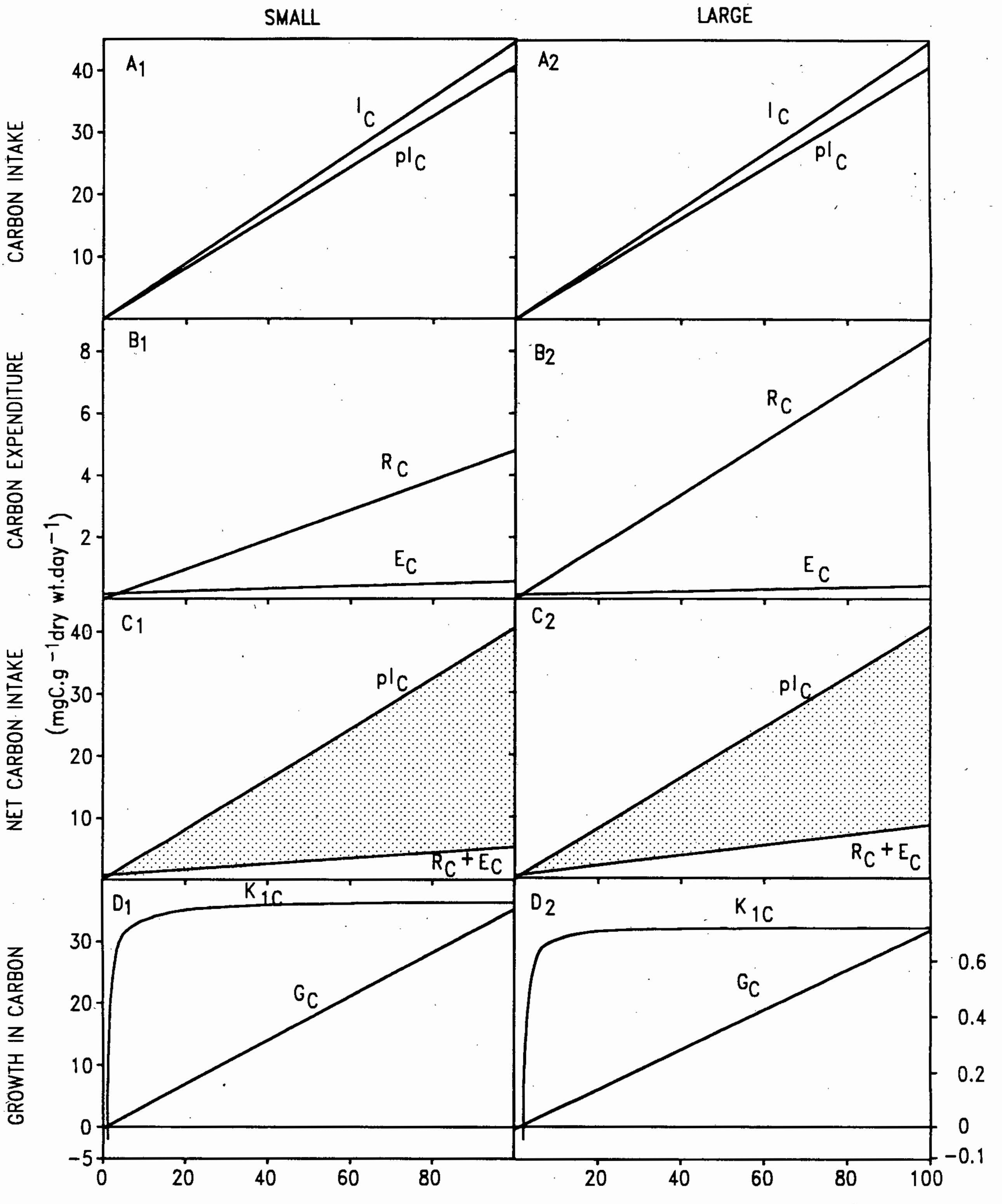

Fig. 5.2: Carbon budget models derived for small and large *L. lithognathus* for a range of ration sizes in relation to ingested ration (I_C), absorbed ration (pI_C), respiration (R_C), excretion (E_C), growth (G_C) and growth efficiency (K_{1C}) (shaded area reprecents scope for growth)

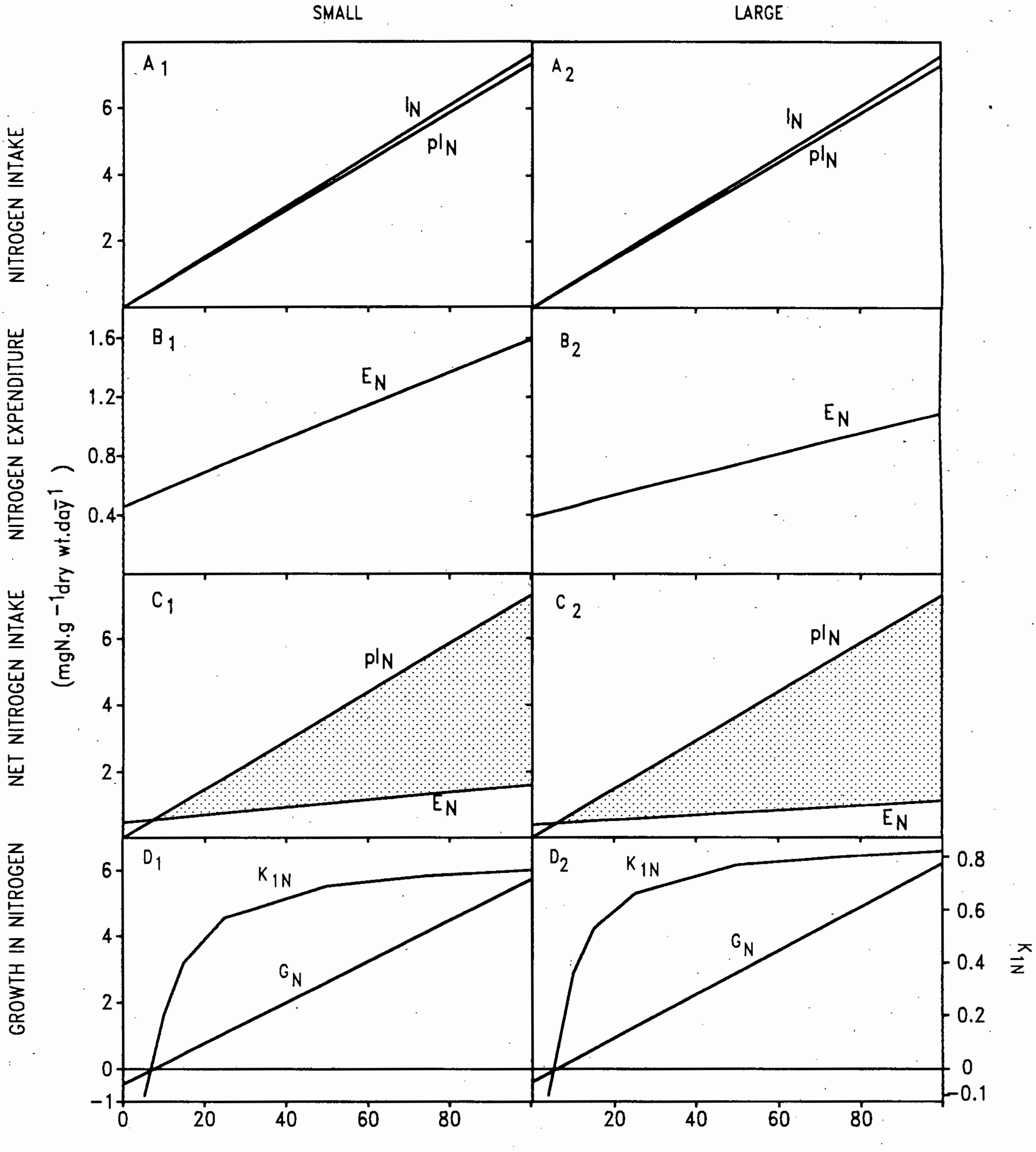

Fig. 5.3: Nitrogen budget models derived for small and large *L. lithognathus* for a range of ration sizes, in relation to ingested ration (I_N), absorbed ration (pI_N), excretion (E_N) growth (G_N) and growth efficiency (K_{1N}) (shaded area represents scope for growth)

(Figs. 5.2 and 5.3, D_1 and D_2). The gross growth efficiency in terms of carbon was 7-8% greater in small fish compared to large fish. In terms of nitrogen, gross growth efficiency was 7-16% greater in large fish, the percentage difference increasing as ration size decreased i.e. at a ration of 10mg.g^{-1} dry wt.day^{-1} K_{1N} is 36% for large fish and 20% for small fish, this difference being 7% different at 100mg.g^{-1} dry wt.day^{-1}.

DISCUSSION

Specific growth rates

The specific growth rates (SGR) in terms of carbon and nitrogen were up to 9%.day^{-1} at 100mg.g^{-1} dry wt.day^{-1} when derived from the models (Fig. 5.1). The specific growth rate determined for *L. lithognathus* in the long-term experiments (Tables 2.2 and 2.3, Chapter 2) were much lower ranging from 0.30-0.62%.day^{-1} for small fish (73-28g) and -0.01-0.29%.day^{-1} for large fish (260g), at 16°C. At 20°C a specific growth rate of 0.46 %. day^{-1} for a 74g fish was obtained. The rations in the long-term experiments, where specific growth rate and food conversion (FCR) were optimal, were 52.95mg.g^{-1} dry wt.day^{-1} (5.29% body wt.) for a 28g fish at FR1, 19.65mg.g^{-1} dry wt.day^{-1} (1.97% body wt.) for a 74g fish at FR1, and 28.97mg.g^{-1} dry wt.day^{-1} (2.90% body wt.) for a 250g fish at FR2 (all at 16°C). These rations are similar to the food intake in the energetics experiments when fed to satiation (Chapters 2 and 3).

It was mentioned in Chapter 4 that the food intake was possibly overestimated due to loss of food during feeding. If this was the case, then food intake was overestimated which would mean that ration sizes would be in the lower part of the range used in the models. Also, it is unlikely that high ration levels could be maintained on a daily basis since food intake varied considerably from day to day (Figs. 4.1 and 4.3, Chapter 4). Cui & Wootton (1989) noted that when minnows were fed at maximum rations in a short-term study the fish are "over growing" relative to the average growth rate over a long period. This suggested that a regulatory mechanism was involved in long-term growth.

Carbon and nitrogen budgets

Carbon and nitrogen intake

If the absorption efficiency changes with different ration sizes, the proportion of energy available for metabolism and growth will also change (Durbin & Durbin, 1983). Pandian & Marian (1985) found that fish size and ration size does not affect absorption efficiency, although a few workers (Elliott, 1976b; Solomon & Brafield, 1972) have found that absorption efficiency decreases slightly at higher rations.

For *L. lithognathus*, carbon and nitrogen absorption efficiencies were not significantly affected by ration size (Chapter 2). If this is the case then absorption efficiency will remain the same at increasing rations and not affect energy available for growth. The carbon and nitrogen models show a increase in absorption efficiency (pI) with ingested ration (I) with a slight decline in pI at the highest rations (Figs. 5.2 and 5.3, A_1 and A_2).

Carbon and nitrogen expenditure

The models derived from the carbon and nitrogen budgets demonstrate that the difference between small and large fish is due to respired and excreted expenditure. Chapter 3 showed that although weight specific respiration rates per gram for small fish were greater than in large fish, relative losses in carbon were greater in large fish when expressed as a percentage of the ingested ration. This resulted in greater carbon expenditure for large fish compared to small (Fig. 5.2, B_2).

Nitrogen excretion was greater in small fish compared to large fish (Chapter 2), particularly endogenous, resulting in greater total daily losses in nitrogen for small fish (Fig. 5.3, C_1).

The range between the maintenance ration and maximum ration is the scope for growth which is the ration range within which growth can occur (Elliott, 1975b). From the models for *L. lithognathus* it is clear that carbon and nitrogen expenditure due to respiration and excretion determine the scope for growth. Scope for growth is known to decrease with fish size (Brett, 1979).

In the present study, the scope for growth in terms of carbon was greater for small fish compared to large fish. In terms of nitrogen, however, scope for growth was less for small fish compared to large fish (Figs. 5.2 and 5.3, C_1 and C_2). This indicates that the small fish have greater nitrogen requirements and the diet for these fish should be supplemented. Dietary protein requirements are known to be higher for small fish than for large fish (Pandian, 1987; Shepherd & Bromage, 1988).

The maintenance protein requirement for bluegill sunfish (*Lepomis marochinus*) was found to decrease with increasing fish size: 0.36 mgN.g^{-1} dry wt.day^{-1} for a 14g fish and 0.26 mgN.g^{-1} dry wt.day^{-1} for a 85g fish (Gerking, 1971). Similarly for *L. lithognathus* in the present study, the maintenance protein requirement was greater for a 57g fish (0.57 mgN.g^{-1} dry wt.day^{-1}) compared to a 282g fish (0.42 mgN.g^{-1} dry wt.day^{-1}). Note that the maintenance protein requirements for *L. lithognathus* are similar to *L. macrochinus* in the study of Gerking (1971).

When expressed as mg.g^{-1} dry wt.day^{-1} the maintenance rations are 7.55mg.g^{-1} dry wt.day^{-1} and 5.99mg.g^{-1} dry wt.day^{-1} for small and large fish, respectively (Chapter 2).

Growth and growth efficiency

Most experimental studies have found that the relationship between growth rate and ration is curvilinear where growth rate increases linearly to a point where the ration of growth to ration is optimal (Brett, 1979; Brett & Groves, 1979; Priede & Secombe, 1988). With a further increase in ration the growth-ration curve flexes further, reaching a plateau at maximum growth rate (Brett, 1979). Gross growth efficiency (K_1) is a function of ration level, and it reflects the fraction of the ration that is retained for growth (Brett & Groves, 1979). As a consequence of the growth-ration relationship, growth efficiency increases from zero at the maintenance ration to a maximum at the optimum ration but declines with a further increase in ration (Elliott, 1975b; Brett, 1979; Brett & Groves, 1979).

In the carbon and nitrogen models developed for *L. lithognathus* the growth-ration relationship did not reach an optimum ration that was less than maximum. Gross growth efficiency increased to a maximum but stayed at that level with increasing ration size (Figs. 5.2 and 5.3, D_1 and D_2). The long-term growth experiments for *L. lithognathus* showed that specific growth rates decreased at high feeding levels (Tables 4.2 and 4.3, Chapter 4) suggesting that the maximum growth level was reached.

The failure of bioenergetic models to predict a curvilinear growth-ration relationship was also found by Kitchell *et al.* (1977) for yellow perch, *Perca flavescens*, Stewert *et al.* (1983) for lake trout, *Salvelinus namaycush*, and Cui & Wootton (1989) for European minnows, *Phoxinus phoxinus*. The bioenergetic model obtained for *L. lithognathus* predicted realistic growth at low rations but unrealistic growth at higher rations. A similar result was found for African catfish, *Clarias gariepinus*, when growth was predicted from a bioenergetics model (Machiels & Henken, 1986; Machiels & von Dam, 1987).

Cui & Wootton (1989) noted that when minnows were fed at maximum rations in a short-term study, the fish were "over-growing" relative to the average growth rate over a long period. Also, as the model overestimated growth at high rations, it was inevitable that the bioenergetics model grossly overestimated long-term growth by fish fed maximum rations. This explanation would apply to the present study particularly as the fish were being over fed at the high rations.

The studies of Kitchell *et al.* (1977) and Stewert *et al.* (1983) extrapolated from existing information which introduces errors in the estimates of the different parameters.

The gross efficiency of protein utilization ($mgN.g^{-1}$ dry $wt.day^{-1}$) for bluegill sunfish was 39% for a 14g fish. The gross efficiencies for white steenbras in the models in terms of carbon were high: 76-79% and 69-71% for small and large fish, respectively. In terms of nitrogen the lower part of the range is realistic: 20-75% for small fish and 36-82% for large fish. The greater efficiency in nitrogen for large fish seems unusual since efficiency is know to decrease with increasing size (Gerking, 1971; Elliott, 1975b; Brett & Groves, 1979). Higher efficiencies would be expected for fish in culture feeding on high quality commercial diets as was shown by the maximum efficiency of 66% for eels in seawater (Knights, 1985). This is closer to the ranges in the present study for *L. lithognathus* under culture conditions.

Comparison of energy budgets

The energy losses associated with respiration were very similar to the carbon losses (Table 3.2, Chapter 3) therefore an energy budget is analogous to the carbon budget. When expressed as a percentage of the food consumed the values for each of the energy budget components ($C = F + U + R + G$) for *L. lithognathus* under culture conditions are: $100 = 9 + 2 + 12 + 77$. Brett & Groves (1979) presented a general budget for carnivorous fish fed on natural diets to be: $100 = 7 + 20 + 44 + 29$. Du Preez *et al.*

(1990) derived a general budget for teleosts fed on natural diets to be: 100 = 10 + 4 + 44 + 42, and the budget for *L.lithognathus* was: 100 = 6 + 4 + 48 + 42 (for 15°C and 20°C). In this study a high proportion of energy was allocated to metabolism particularly R_{SDA} (14-23%). This discrepency, which was discussed in Chapter 3, is possibly due to groups effects and diet. Lower respiration rates have been found to occur in groups of fish as opposed to single fish (Job, 1955; Van Waversveld *et al.*, 1989) and the majority of respiration studies have used individual fish. Commercial diets are more easily digested hence feeding metabolism is less costly (Knights, 1985).

Conclusions

Although the models developed for *L.lithognathus* under culture conditions overestimated growth at higher rations, growth predictions at lower rations can be made. One might ask whether these models would be applicable in a practical farm situation. A apt quote by Elliott (1982) can answer this "all mathematical models are approximations to reality. If they were accurate descriptions of reality, they would cease to models !"

The results of the carbon and nitrogen budgets for *L.lithognathus* are useful in that they gave estimates of maintenance requirements and showed the relative proportions of carbon and nitrogen lost via respiration and excretion.

CHAPTER 6

CONCLUSIONS

The determination of the bioenergetics of *L.lithognathus* under culture conditions using a large respirometer has provided invaluable information which can be used in a practical farming situation. *L.lithognathus* showed efficient utilization of a commercial pelleted diet. This diet produced high absorption efficiencies, net protein utilization, food conversion and condition factors. The results suggested that feeding regimes should be maintained at relatively low levels to avoid overfeeding and food wastage. Food conversion was less efficient at high rations.

Faecal production and nitrogen excretion were relatively low suggesting that deterioration of water quality should not pose a problem at the stocking density used. The high nitrogen retention indicated that a large proportion of the nitrogen in the diet was being utilized.

The low metabolic rates measured for *L.lithognathus* indicated that a small proportion of energy was lost via respiration.

The growth models, derived from the carbon and nitrogen budgets, are useful in estimating growth only at low feeding levels. As this is, however, the regime that is likely to be used in practice, the models are still considered useful.

The use of a large respirometer to measure the bioenergetics of fish under culture conditions is advisable because the conditions in such an apparatus are similar to those found on fish farms. A disadvantage of such a system is the difficulty in measuring small changes in nitrogen excretion but as Knights (1985) pointed out, relative differences in metabolism, growth and efficiencies are generally of greater importance in aquaculture than absolutely accurate data.

Specific growth rates of *L.lithognathus* were found to be relatively slow at 16°C, but increased at 20°C. However, survival was slightly reduced at the higher temperature. Specific growth rates may be improved at higher temperatures by adopting low feeding rations and careful management of water quality.

In terms of actual practice in farming white steenbras the availability of juveniles, total time to market size and cost of feed versus value of product are important factors. As was mentioned in the introduction *L.lithognathus* was selected on economic and biological criteria. Large numbers of juvenile white steenbras are found in local estuaries. A marketable size would be 300-400g which could be grown in approximately 2-4 years (Bennett, unpubl.). The cost of feed could be minimised since optimum FCR, condition factor and SGR were obtained on the lowest feeding levels. White steenbras is only available seasonally and it is in much demand from restaurants, and commands a retail price of about R11.00 per kg.

In conclusion, the results of the present study indicated that *L.lithognathus* would be a suitable candidate for aquaculture provided the following research questions are answered:

1. Determination of mass production of larvae under controlled conditions i.e. production of broodstock.
2. Acquisition of suitable coastal areas for culture.
3. Detailed market research to evaluate target markets and price.
4. Development of a local feed based on low cost components.
5. Research into dietary protein requirements of different fish sizes, to improve growth rate.
6. Investigate diseases associated with increased water temperature.
7. Determination of the contribution of activity and feeding metabolism to the energy budget.
8. Research into alternative feeding regimes at higher temperatures to improve food conversion.

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