UNIVERSITY OF STIRLING

Protein intake, ammonia excretion and growth of *Oreochromis spilurus* in sea water.

by

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ABSTRACT

The effect of protein intake on ammonia excretion and growth rates and the related effects of ammonia levels on growth rate were studied in the tilapia species Oreochromis spilurus, in sea water. The effect of protein intake for stock in the size range of 30 - 300 g BW, was determined by manipulating dietary protein level (P:E ratio) and feeding rate.

The results over a protein level range of 20 - 43.35% and feeding rate of 0.5 - 6% BW/D show that protein intake has a significant effect on both growth and ammonia excretion rates. It was possible to draw a linear relationship between growth (SGR) and ammonia excretion rate at each P:E ratio level. Variation in the P:E ratio leads to variation in the slope of this relationship which reflects mainly the different contribution of protein energy and non-protein energy to growth and ammonia output. The P:E ratio appears to be significant to growth only at low feeding rate (sub-satiation), while it is relatively unimportant at medium and high feeding rates (close to satiation). However, P:E ratio is important for ammonia output at all feeding rates.

Optimum growth for small fish in terms of PCR and PER can be achieved by feeding at sub-satiation (2% BW/D) with a high protein diet (43.35%). This growth was associated with high ammonia output (21% of N intake), mainly because of the limited non-protein energy supply with this feeding regime. This suggests that high PER values obtained from nutrition studies do not necessarily mean high protein utilization for growth and subsequently low protein catabolism and low ammonia output. It suggests, in fact, that a more efficient growth can be obtained by protein metabolism rather than carbohydrate metabolism.

For low ammonia output low P:E ratio diet fed at high ration is recommended mainly to allow protein saving by the carbohydrates. It was not possible to define one particular protein intake as an optimum for growth, PCR and ammonia output. However, optimum growth can be achieved with low ammonia output by feeding low P:E ratio diet at high feeding rate, but at poorer PCR and PER.

The negative effect of ambient ammonia on growth rate was found to be significant. If un-ionized ammonia levels are increased from 0.00 to 0.30 mg/L, optimum growth can only be achieved at higher protein intakes. At higher ammonia levels optimum growth cannot be achieved at any protein intake. Ammonia appears to

reduce food consumption, therefore, it would be advisable to provide all nutrient requirements concentrated in small rations. Furthermore, ammonia stress seems to reduce feed utilisation efficiency, therefore, it would be advisable to use a very efficient energy source, such as protein, to balance this reduction. Therefore, under conditions where ammonia output is important for controlling ambient ammonia (eg. where water supply is restricted) feeding a high protein diet at low ration seems to offer the optimum strategy.

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CHAPTER 1: Introduction

1. Introduction

In aquaculture, good fish growth rate and food conversion are usually considered amongst the most important factors affecting the success of the operation. Although these are clearly required it may well be that in circumstances such as intensive aquaculture, the relationships between food intake and growth rate, and the "feedback" effects of waste output (related to feed regime) on growth rate may lead to different aims for optimising efficiency. It is the purpose of this study to attempt to identify some of these relationships for the case of the tilapia [redacted] grown in intensive conditions in sea water.

In animal husbandry optimum nutrient requirements are usually defined according to their growth responses and food utilisation efficiencies. Other response parameters frequently considered include body composition and the general health status of the stock. In aquaculture most of the nutrient requirements are selected on these criteria also (Lovell, 1985).

Metabolism of food results in a variety of waste materials. The output of these materials has received considerable attention from the metabolic and energetic points of view. Measurements of output are usually used to explain food metabolism in order to define the best

nutrient intake for growth and food utilisation. This approach is generally adequate for terrestial animals whose wastes are produced on land, and therefore once excreted are seldom expected to have a negative growth effect.

However, in aquaculture, metabolic activities result in a range of solid and soluble wastes being produced in water (Muir, 1982) which in turn may have a direct effect on the physiological processes of the stock (Alabaster, 1982). Unlike terrestial animals where ad. libitum feeding is relatively easy, fish feeding strategies are also complicated by the aquatic environment due to the rapid deterioration of uneaten food in water (Tacon and Cowey, 1985). In nutrition studies optimum nutrient requirements are usually defined at (or assumed to be at) optimum environmental conditions. However, in commercial operations the fish might (for economic reasons) have to live in sub-optimal conditions, when in view of the likely reduction in appetite and feed utilisation one may well question the value of requirements obtained in this manner.

In this respect Brett (1979) stated that growing, unlike such activities as swimming or respiring, is inseparably coupled with a powerful "biotic" factor, so that any "abiotic" factor will tend to interact between

feeding and growth. Thus, fish growth in the fish farm will depend on the balance between food and water quality and in turn, the economics of the farm will depend on the management of these two factors.

This food-growth-water quality relationship characterises and separates aquaculture from other animal husbandry enterprises. Nevertheless, in spite of this relationship most nutrient requirements are still defined on their nutritional responses only. This might be acceptable for systems where water supply is less limiting such as floating cages, but for pond and tank cultivation there is clearly a need to take greater account of the more complete relationship between food and growth and water quality. Studies on food, growth and water quality can in turn provide valuable information such as water and space requirements.

The study of this complete relationship is complicated by the complex nature of the many factors of feed composition, growth parameters and the variety of the excreted waste materials. In view of this complexity it was decided to define the main factors, as the simpler components of protein, ammonia output, and growth.

2. Protein Intake and Growth

The most heavily demanded nutrient by fish for good growth is protein. Protein has a significant contribution on fish growth rate because it is required for structural and functional purposes (NRC, 1983; Cowey, and Sargent 1979; Tacon and Cowey, 1985). Fish, like other animals, lack the ability to synthesize amino acids from simple inorganic materials and must depend on dietary amino acid or amino acid precursors that can be converted into amino acids. Dietary protein consumed is digested or hydrolyzed to release free amino acids that are then used to synthesize new body protein. Since proteins are continuously being used by the animal, either to build new tissues (as during growth and reproduction) or to repair worn tissues, a regular intake of protein or amino acids is required.

If adequate protein is not provided in the diet, there is a rapid reduction or cessation of growth and loss of weight because the animal withdraws protein from some tissues to maintain the functions of more vital ones. On the other hand, if too much protein is supplied, proportionally less will be used to make new protein and the rest will be metabolized to produce energy.

The distinguishing characteristic of protein from other nutrients in fish rises from the fact that the dietary amino acids can be used both as an energy source and as a precursors for lipid and carbohydrate synthesis (Watanabe, 1986). With respect to their primary sources of energy, fish appear to be different from mammals, in which dietary carbohydrate is accummulated in the glycogen pool, while an excess of carbohydrate is converted into neutral lipid. In fish however, carbohydrate is hardly converted either to lipid or to glycogen, while protein can be converted to both (Watanabe, 1986). Therefore, protein appears to be the primary source of energy in fish.

3. Protein Intake and Feed Requirements

As protein can be utilized by fish as a nutrient and as an energy source (NRC, 1983; Cowey and Sargent, 1979; Tacon and Cowey, 1985) overall requirements are, therefore, high. The optimal dietary protein level required for maximal growth in farmed fishes is reported to be 50-300% higher than that of terrestial farm animals (Cowey, 1975). Thus fish normally require levels between 25-50% protein in their diets (Tacon and Cowey, 1985). As protein is relatively expensive this will increase the cost of formulating these diets, and so it is important to make sure of good protein utilization.

It has been commonly reported that fish eat to meet their energy demand (Cowey and Sargent, 1979), and thus feeding rate is regulated mainly by the energy content of the diet. However, recent literature shows that feeding rate is affected also by the protein content of the diet. For example, Ogino (1980) found an inverse relationship between dietary protein requirement and feeding rate in carp. Similarly, Wange et. al. (1983) found a negative relationship between feeding rate and the protein content in feeding O.niloticus. Thus, Tacon and Cowey (1985) recommended that optimum nutrient requirements cannot be defined unless a series of different feeding levels are tested so as to elecit a maximum growth response.

4. Protein Intake and Water Requirements

The effect of protein intake is not only limited to the fish growth rate, but extends to affect the water requirement in the fish farm. The major nitrogenous byproduct of protein metabolism is ammonia (Forster and Goldstein, 1969). Generally ammonia constitutes about 60-90% of total nitrogen excreted in fishes (Brett and Groves, 1979). Biochemically, ammonia arises from catabolism of protein and amino acids, primarily via the action of amino acids oxidases trans - and de-aminase (Kormanik and Cameron, 1981). The primary site of ammonia production is probably the liver, but the necessary enzymes have also been located in the kidneys,

gills and skeletal muscle tissue (Randall and Wright, 1987).

Ammonia excretion is influenced by many factors of which protein intake is the most important. Paulson (1980) found that nitrogen consumption was by far the most important factor influencing ammonia excretion.

Ammonia is known to be a toxic compound to fish (Alabaster and Lloyd, 1980; Smart, 1981; Colt and Armstrong, 1981). Spotte (1979) reported that ammonia is the most lethal form of inorganic nitrogen produced in aquarium water, while ammonia at sub-lethal concentrations results in a reduction in fish growth rate. Russo and Thurston (1977) reported that ammonia may uncouple oxidative phosphorylation, and reduced growth may result in the inability of the animal to convert food energy to ATP. Corticosteroid hormones are also released proportionally to sub-lethal ammonia exposure. These hormones cause a negative nitrogen balance by deaminating amino acids, which are then not available for protein synthesis essential for growth.

Because of this relationship between ammonia and fish growth ammonia has been considered by many aquaculturalists as an important parameter in defining the water requirement of the fish farm. Thus Speece

(1973) provided a mathematical formula for calculating the required water flow based on ammonia production rates. Similarly, in a recirculating systems water treatment filters can be sized according to ammonia production rates (Speece, 1973; Rogers and Klemetson, 1985).

While several cultural procedures such as continuous water exchange, biological filtration, ion-exchange, air bubbling, or chemical reaction with chlorine are available for controlling ammonia level it should also be possible to control ammonia level in the fish farm by manipulating protein intake and hence protein catabolism. Thus by optimising protein utilisation and minimising protein catabolism it may become possible to lower ammonia level in the fish farm, and so reduce water requirements.

5. [illegible]

We come to a protein-growth-ammonia relationship where protein is required for good fish growth, while protein metabolism results in the formation of ammonia. Ammonia is excreted by the fish and if accumulated in the culture system starts to affect fish growth rate. From an economic perspective, because of the high dietary protein requirement and the high formulating cost, it is

important to make sure of good protein utilization by the fish.

If protein is in excess of the requirements of the organism, or the constituent amino acids are poorly balanced in relation to growth needs, deamination occurs with excretion of nitrogen, mainly as ammonia and urea (Brett and Groves, 1979). Improper protein utilization by the fish will result not only in low utilization of a highly costly item, but will increase the production of ammonia. Because of the positive relationship between protein intake and growth rate (Cowey and Sargent, 1979; Tacon and Cowey, 1985) fish farmers are usually willing to increase protein in the diet to increase the fish growth rate.

However, the excreted ammonia must be diluted, removed or transferred to non-toxic compounds to permit good growth rate, where water management to reduce ammonia hold extra financial expense (Colt and Armstrong, 1981). Furthermore, the functional relationship between growth and ammonia must be known because it will probably be impossible to operate in the "no effects" levels because of economic considerations (Colt and Armstrong, 1981).

In this research two important parameters in the defining of nutritional and environmental requirements of O.mossambicus are to be studied. It is the intention to relate these parameters in order to formulate a functional relationship, with the aim of optimising farm design and management strategy. Thus protein intake could be designed to provide the desired results in terms of growth and ammonia production rates, while water quality management based on the tolerance of the fish to ammonia can be defined and maintained by adjusting the degree of flow rate, recirculation and/or filteration rate.

6. Strategy for Testing Species and System

In areas where freshwater supply is limited, food production is also often limited. In areas such as Kuwait and other Gulf countries, there is a strong desire to increase food production and as a result these countries seek any viable opportunities. Since sea water is abundantly available, marine fish farming of local and other suitable species has been recognised as a possible option.

The development of the culture of tilapia in sea water offers particular potential, due to simple hatchery techniques, relative robustness, and good growth performance. The tolerance of some tilapia species to

high salinity has been recognised since 1960's (Chervinski, 1961), though little research has been carried out to define their biological requirements in sea water. Species of tilapia such as: O.mossambicus, O.spilurus and the red hybrid tilapia are known to tolerate high salinities (see eg. Stickney, 1986). O.spilurus in particular has been recognised as a good species for mariculture (Al. Ahmad et. al., 1986).

The use of Oreochromis spilurus in aquaculture is relatively recent. There is thus very little information in the literature on its requirements for culture. The major reason for this has probably been its relatively slow growth rate and the presence of other faster growing tilapia species suitable for fresh waters. Balaria & Hatton (1979) reported that the maximum recorded size for O.spilurus is 1.0kg, whereas the maximum size recorded for O. niloticus and O.mossambicus, the most widely cultured tilapia species, is 2.5 and 1.7 kg respectively. However its high tolerance to salinity (Osborne, 1979) offers particular advantages. Thus, Al. Ahmad et. al. (1986) concluded that O.spilurus was the most suitable tilapia for sea water cultivation in Kuwait in comparison with O.aureus and red tilapia. The fish has been found to grow and spawn in sea water of salinity 39-40 ppt in Kuwait (Al.Ahmad, et. al., 1986). As a result O.spilurus has been selected in arid countries such as Kuwait

(Al.Ahmad, et. al.,1986) and Saudi Arabia (Osborne, 1979) for its sea water cultivation.

7. The Implications of Salinity

The use of sea water might be expected to result in a change in the biological requirements of O.spilurus. Brett (1979) reported that salinity as a "masking factor" will increase the maintenance metabolism of fish. As a result more energy will be directed towards maintenance and subsequently a lower fraction of the consumed energy will be directed towards other activities. This would probably result in a change in the energy partitioning and feed utilization. Furthermore, the higher maintenance metabolism probably will reduce the metabolic scope available for active and feeding metabolisms.

Priede (1985) noted that the metabolic scope of fish varies according to species and stage of development as well as being influenced by environmental variables, notably temperature. He added that it is evident that salinity, among other environment factors, may affect the metabolic scope so as to modify its basic relationships. He further noted that there is strong evidence that a number of species regulate their feeding activity simply to keep their metabolic rate within the bounds of the metabolic scope.

Any change in the feeding rate might also result in a change in the nutrient requirements of the fish (eg. Ogino, 1980). There are indications also that salinity affects the protein metabolism. Schmidt-Nielsen (1979) reported the involvement of the amino acids in cell volume regulation during salinity stress. As the salinity of the medium increases or decreases, corresponding changes occur in the intracellular amino acid concentrations in accordance with the maintenance of constant cell volume.

Similarly, salinity acclimation as it changes the mechanism of osmoregulation might affect the response of the fish to other environmental factors. For example, Lloyd and Orr (1969) suggested that any environmental factor that affects water balance (eg salinity) also affects the ammonia tolerance of fish.

Therefore, it seems very likely that with all these modifications to metabolism, nutritional requirement, and response to environmental factors, that the final growth rate will also change with salinity. As a result data available on the nutritional and environmental requirements for tilapia in freshwater conditions might not be applicable in sea water. Thus, if the relationships between protein, ammonia output and growth can be determined for O.spilurus in sea water, an

important step can be taken in developing commercial intensive tilapia culture potential in arid lands. The nature of the relationships developed may also improve understanding of similar approaches to aquaculture with different species, in different environments.

8. Literature Review

The protein-growth-ammonia relationship can be considered to include several sub-relationships such as those between protein intake and growth, protein intake and ammonia excretion, and environmental ammonia and growth. The following discussion, therefore, will be based on these main areas:

8.1 Protein Intake - Growth Relationship

The protein intake-growth relationship for fish has been the subject of many reviews; Cowey and Sargent (1972); Phillips (1969); Cowey and Sargent (1979); NRC (1983); and Tacon and Cowey (1985), in which a number of fundamental factors are identified:

I. Nutritional Factors:

(1) Amino acid composition and Protein source:

One of the main factors involved in the utilization of dietary protein is the amino acid pattern of the protein concerned and how nearly this matches the amino acid requirements of the fish (Cowey and Sargent, 1979).

In common with terrestrial animals, fish require the same ten indispensable or essential amino acids (EAA) within their diet (Tacon and Cowey, 1985). Millikin (1982) reported that dietary amino acid imbalances may result in higher dietary protein requirements than those normally needed, as well as antagonisms between certain amino acids.

Over nine different protein sources have been used for the determination of the dietary protein requirement in fishes (Tacon and Cowey, 1985). These range from purified proteins to whole protein composite used either alone or in combination with free amino acid supplements. However, these protein sources differ in their amino acid compositions and as a result different results were obtained. This subject has been reviewed recently by Tacon and Cowey (1985) who suggested a lipid-extracted fish meal or lipid-extracted fish muscle to be used as a standard reference protein because of the close similarity between the dietary EAA requirement of fishes and the EAA profile of fish muscle/carcasses.

(2) Feeding Regime:

Since "optimal growth" is generally used as the criterion for estimating dietary protein requirement, it is essential that food supply is not limiting (Tacon and Cowey, 1985). Ad. libitum feeding, a method normally

used for terrestrial animals, is complicated by the aquatic environment due to the rapid deterioration of uneaten food in water, and by the different feeding habits of individual fish species. Tacon and Cowey (1985) recently reviewed the type of feeding practices used in fish culture and concluded that the most common feeding method employed has been the use of a fixed feeding regime in which the feeding level has been set arbitarily. They claimed that in contrast to the preferred feeding method where fish are fed to satiation at an optimum feeding frequency, a fixed feeding regime directly influences the outcome of the observed dietary requirement. Thus, Ogino (1980) found a relationship between feeding level and dietary protein requirement. Therefore they recommended that a feeding programme should involve either different feeding levels or satiation feeding at an optimal feeding frequency.

(3) Energy Content of the Diet:

While proteins provide the enzymatic reactions and much of the structural apparatus of the cell, they are also a form of metabolizable energy (Cowey and Sargent, 1979). Under conditions when energy intake is inadequate, dietary protein will be used as an energy source. In fact, protein synthesis within the animal will only reflect the quality and quantity of dietary protein when a sufficient energy intake occurs. At high

energy intakes a proportion of amino acids will be deaminated and the carbon residue burned as energy. Although protein may serve as a source of energy for fish, approximately 16% is nitrogen that can not be used for energy (Phillips, 1969). Conversely an excessive energy intake of moderate dietary protein levels will lead to the accumulation of fat in the fish resulting in undesirable changes in carcass composition.

II. Environmental Factors:

(1) Temperature:

Millikin (1982) reported that there is an increase in dietary protein requirements with increase in the water temperature for striped bass. However, Slinger et. al. (1977) and Cho and Slinger (1978) found no effect of temperature on the dietary protein requirements for rainbow trout. Recently, Tacon and Cowey (1985) claimed that the weight of evidence is that increased water temperature does not lead to increased protein requirement.

(2) Salinity:

Zeitoun et. al. (1973) reported that there is an increase in dietary protein requirements with increase in the salinity from 10 to 20 ppt for rainbow trout. On the other hand, under similar acclimation conditions there was no change in dietary protein requirement for coho

salmon (Zeitoun et. al., 1974). Tacon and Cowey (1985) claimed that in view of the speculative method for arriving at dietary requirement from the dose-response (Zeitoun et. al., 1973), and the lack of information on the requirement of these fish species in full strength sea water (35 ppt), there are no firm data demonstrating tht the protein requirements of fish are elevated with increased salinity.

III. Physiological factors:

Fish Size:

Dietary protein requirement for any particular species changes with size. Millikin (1982) reported that dietary protein requirements generally decrease with increasing age or fish size.

IV. Dietary Protein Requirement of Tilapia:

Data available on dietary protein requirements for tilapia are restricted mainly to fry and juveniles (Jauncey and Ross, 1982). There are very few data available in the literature on the dietary protein requirements for large size tilapia. Jauncey and Ross (1982) recommended 50% protein, 40% protein and 30-35% protein for fry - 0.5g. 0.5-10g and 10-30g fish, respectively. They added that the optimum protein level for tilapias in excess of 35g has yet to be established but it is likely to be in the region of 25%.

8.2 Protein Intake - Ammonia Excretion Relationships:

Meade (1985) reported that although many factors affect ammonia toxicity, a subject which will be discussed in more detail in the next section, the effect of ammonia depends largely on exposure, which is a function of excretion or ammonia production by the fish themselves (assuming constant source water quality and rearing system exchange rates). Thus, he concluded that methods of predicting ammonia production are important. Ammonia excretion is influenced by many factors, of which protein intake is probably the most important. Paulson (1980) found that nitrogen consumption was by far the most important factor influencing ammonia excretion.

The relationship between protein intake and ammonia excretion has been studied for rainbow trout (Beamish and Thomas, 1984; Kaushik, 1980), European eel (Degani et. al., 1986; Knights, 1985), carp (Kaushik, 1980) and cod (Laid and Bratten, 1984). No study on this relationship is available yet for tilapia.

The subject of ammonia excretion itself is complicated by two factors. First, the fact that ammonia originates at two different sites. Some ammonia is produced from precursors in the liver, transported by the blood to the gills, and eliminated into the environment. Still another portion originates from deamination of

plasma amino acids in the gill tissue. Secondly, the form of ammonia excreted, whether NH_3 or NH_4^+ or both is not certain. Two theories exists for removal of ammonia at the gill; (i) active transport of NH_4^+, which takes place by ion exchange with a similarly charged species in the external environment; and (ii) passive transport (diffusion) of NH_3 (Spotte, 1979).

It is not intended in this study to clarify where or how ammonia is originated or excreted. The reason for this is once "ammonia" is excreted its presence in water will not be affected either by its origin or by its excreted form, but by the physical and chemical characteristics of the water. This will be discussed in more detail in the next section.

Besides protein intake there are many other factors that affect ammonia excretion rate. These factors can be classified as: nutritional, environmental, and physiological.

I. Nutritional Factors:

Ammonia production is directly related to the rate of protein catabolism (Vaarde, 1963) and, therefore, those factors affecting protein catabolism are likely to affect ammonia production.

(1) Protein Quality:

Forster and Goldstein (1969) reported that if the constituent amino acids of the protein are poorly balanced in relation to growth, deamination occurs with excretion of nitrogen, mainly as ammonia and urea across the gills. Furthermore, Spannhof et. al. (1985) reported that ammonia-nitrogen concentration in the blood of rainbow trout increases considerably after feeding on low-quality diets.

(2) Protein: Energy Ratio:

Atherton and Aitken (1970) found that the efficiency of nitrogen utilization depended on the fat content of the diet. Feeding a low fat diet a relatively large amount of ingested protein is deaminated and used as an energy source. But when a high fat diet is used, the percentage of the nitrogen intake used for growth greatly increases with a concomitant decrease of ammonia excretion. Rychly (1980) reported that nitrogen excretion, as measured by ammonia, increased with increasing protein and decreasing carbohydrate content of the diet. Kaushik and Teles (1985) found a decrease in ammonia excretion with increasing the digestible carbohydrates in the diet. Results obtained by Leid and Bratten (1984) suggested that the total amount of ammonia excretion depends on P:E ratio in Atlantic cod and there seems to be an optimum P:E ratio.

(3) Feeding Regime:

Meade (1985) quoted that not only diet composition but also feeding regime is a key in calculation of ammonia production. Because of its relationship to feeding, ammonia excretion rates fluctuate drastically. Brett and Zala (1975) showed that 4-4.5 hrs after feeding, ammonia excretion by Sockeye salmon increased to over 400% of pre-feeding level, where the pre-feeding level was equivalent to the constant excretion level for starved fish. Meade (1985) quoted also that daily feeding schedule, or distribution of food with time, has a major effect on peak concentrations of ammonia. Conversely, at a given daily diet amount, distribution schedule has little or no effet on total daily ammonia production. Parker and Davis (1981) quoted that ammonia production has been shown to be quantitatively related to amount of food eaten and temporally related to feeding time. Kaushik (1980) found that the differences in daily patterns of nitrogen excretion rates are not only due to feeding level but also to inherent adaptive mechanisms after a change in feeding rhythm.

II. Environmental Factors:

(1) Temperature:

Paulson (1980) reported that ammonia excretion rate is affected by water temperature. The relative contribution of the different food substrates to energy

production is also influenced by temperature adaptation. During acclimation to low temperatures, the relative capacity of the animal for lipid oxidation is increased, while carbohydrate oxidation is unchanged (Vaarde, 1983). Therefore, lower ammonia production at low temperature reflects the lower utilization of protein and the higher contribution of lipid as an energy source.

(2) Oxygen:

Kutty (1972) observed that a lowering of oxygen content in the environment did not decrease ammonia excretion in C. mossambicus. Thillart and Kesbeki (1978) also found ammonia excretion rate to be completely independent of oxygen availability in goldfish. (C. auratus). Thus, Vaarde, (1983) suggested the existence of a quantitatively important anaerobic ammonia-producing system in goldfish. He quoted also that body carbohydrate seems to be used as an anaerobic substrate, both during environmental oxygen deficiency and during tissue anoxia as a consequence of a severe work load.

(3) Salinity:

There is no data available on the effect of salinity on ammonia excretion. However, it has been noticed that salinity may affect the overall nitrogen partitioning and subsequently ammonia excretion. For example, Knights (1985) working with European eel (A

anguilla) found that urea nitrogen excretion is higher in fresh water than in sea water. He reported also that protein productive value (increase in body protein divided by protein intake) is slightly lower in fresh water (60-70%) than in sea water (70-80%) indicating lower nitrogen excretion in sea water. These results do, however, imply that protein retention is generally low in fresh water in eel.

(4) Ambient Ammonia:

Olson and Fromm (1971) quoted that ambient ammonia seems to affect the normal ammonia production rate. Exposure of fish to ammonia solutions generally raises the blood ammonia levels (Fromm and Gilette, 1968) either due to ammonia retention in the fish or by entry of ammonia from the external medium resulting in a reduction in overall ammonia excretion (Haywood, 1983).

III. Physiological Factors:

(1) Fish Size:

Paulson (1980) found that fish size affects ammonia excretion. Larger fish excrete slightly less ammonia than small fishes. This is probably due to changes in nitrogen budgets with age because of falling protein requirement for growth as fish mature (Knights, 1985).

(2) Fish Health:

Caulton (1978) reported that healthy T. randalli utilize lipid as a major energy source, whereas non-healthy fish (disease was not specified) tend to utilize protein as an energy source.

(3) Fish Activity:

Kutty (1981) working with O. mossambicus found out that short term and long term nitrogen excretion are higher at higher swimming speed than at low swimming speed. Furthermore, nitrogen (both ammonia nitrogen and total nitrogen) excretion increased markedly with the duration of exercise. This is mainly because at high fish activity rate a large part of energy consumption in fish is covered by protein catabolism (Vaarde, 1983). He added that under such an aerobic conditions, ammonia originates mainly in the liver by transdeamination and the hydrolysis of amino groups, while an additional quantity is formed in working skeletal muscles by purine nucleotide cycling.

(4) Data Available on Tilapia:

No data are available yet on the effect of protein (or food) intake on ammonia excretion for tilapia. However, there is information available on the ammonia excretion rate with reference to ambient oxygen level

(Kutty, 1972); hypoxia and recovery (Peer and Kutty, 1981), and fish activity (Kutty, 1981).

8.3 Environmental Ammonia - Growth Relationships

In intensive aquaculture some of the metabolic products that animals release into the water are directly toxic, whereas others become toxic through the activities of micro-organisms (Spotte, 1979). The main metabolic products are ammonia, urea, uric acid, and carbon dioxide. Of all these ammonia has special importance. Ammonia is known to be a toxic compound to fish (Alabaster and Lloyd, 1980; Smart, 1981; Colt and Armstrong, 1981; Haywood, 1983). Spotte (1979) reported that ammonia is the most lethal form of inorganic nitrogen produced in aquarium water. The toxity of ammonia to fish has been reviewed by Spotte (1979); Wickins (1981); Smart (1981); Colt and Armstrong (1981); Haywood (1983); and recently Meade (1985). There is a general agreement in these reviews on the adverse effect on fish growth rate at sub-lethal levels of ammonia.

The other organic nitrogenous wastes are not known to be directly toxic to fish or at least are apparently not toxic at the levels they are excreted. Thus, Colt and Armstrong (1981) reported that urea is non-toxic to aquatic animals at the concentrations present in culture

systems. However, Spotte (1979) discussing the nitrogen cycle in aquariums quoted that any nitrogenous organic matter can be indirectly toxic because it may potentially be broken down to ammonia. Furthermore, Colt and Armstrong (1981) reported that although urea is non-toxic to aquatic animals at the concentrations present in culture systems, it can be rapidly hydrolysed to ammonia and carbon dioxide. Generally, ammonia-nitrogen constitutes about 60-90% of total nitrogen excreted in fishes (Brett and Groves, 1979). On balance, however, it seems that ammonia sums up most of the adverse affect of the nitrogenous wastes in water.

Before explaining the nature of the ammonia-growth relationship it should be noted that ammonia exists in water in two forms; ionised (NH_4^+) and free or un-ionised (NH_3) ammonia. The relationship between the two forms is described in the following reaction: (Kormanik and Cameron, 1981) $NH_3 + H_2O \langle=\rangle NH_4^+ + OH^-$.

The hydrolysis of NH_4^+ in natural waters has a pK value of about 9, so that the percentage of NH_4^+ is always greater than the percentage of free ammonia. The hydrolysis of NH_4^+ is affected mainly by pH, temperature, and salinity, with pH exerting the greatest effect. An increase of one unit of pH causes the percentage of free ammonia to increase approximately 10 fold. Raising

temperature and decreasing salinity causes much smaller increases (Spotte, 1979). The importance of this differentiation is that each form of ammonia appears to exert a different level of toxicity. In earlier studies, only NH_3 was considered toxic (see, eg Tabata, 1962), but, recent papers have suggested that NH_4^+ is also toxic. Thus, Thurston et. al. (1981) concluded that NH_4^+ is toxic to a small but definable degree and that NH_3 is 300-400 times as toxic as NH_4^+. Furthermore, Willingham et. al. (1979) quoted that even if NH_4^+ is one to two orders of magnitude less toxic than NH_3, NH_4^+ is (usually) present at concentrations one to two orders of magnitude greater than NH_3. Thus, it might be expected that both forms of ammonia might exert an effect.

Factors Affecting Ammonia Toxicity:

I. Environmental Factors:

(1) pH:

This is perhaps the fundamental contributing factor (see eg, Bower & Bidwell, 1978). Generally a reduction in the pH of water results in a shift in the NH_3:NH_4^+ equilibrium towards the NH_4^+, resulting in a decrease of NH_3. Spotte (1979) quoted that with an increase in one pH unit the NH_3 fraction will increase 10-fold. However, as stated earlier, NH_3 contribution does not completely describe toxicity. Thus, Thurston et. al. (1981) noted that at least five recent studies indicated that the role

of pH in ammonia toxicity is more significant than that of controlling the equilibrium between NH_3 and NH_4^+. They concluded from their results in rainbow trout and fathead minows that NH_4^+ is toxic or that increased H^+ concentration increases NH_3 toxicity. Below 20mg/L of total ammonia the affect of NH_4^+ toxicity was negligible. From their results they speculated that NH_3 is 300-400 times as toxic as NH_4^+.

(2) Temperature:

A reduction in temperature reduces the fraction of NH_3 present and hence the overall toxicity (Haywood, 1983). Reduction in temperature has also been shown to affect the tolerance and susceptibility of fish to stress. However, reduced temperature was shown to increase the effect of ammonia toxicity, specially for values below the growth optimum (Colt and Tchobanoglous, 1978; Thurston and Russo, 1983). Haywood (1983) concluded that maximum tolerance of most species to ammonia is found between 10 and 20°C, most probably due to this being the optimal temperature range for most cold-water teleost fishes.

(3) Salinity:

Ammonia toxicity increases, in rainbow trout, as salinity either increases or decreases from a concentration roughly isotonic with blood (EIFAC, 1970).

Lloyd and Orr (1969) suggested that any environmental factor that affects water balance also affects ammonia tolerance. Tomasso et. al. (1980) found that an increase in environmental calcium increases tolerance to ammonia. Colt et. al. (1979) stated that there was no evidence that sublethal effects of ammonia were due solely to the NH_3 fraction, and speculated that, on the contrary, sublethal effects may be related to NH_4^+ and ambient Na^+ concentrations. The relationship between ammonia toxicity and salinity is not clear yet and more work is required to clarify the influence of Na^+, as well as temperature, pH, CO_2 and alkalinity on ammonia toxicity (Meade, 1985).

(4) DO and CO_2:

The affects of varying concentrations of both DO and CO_2 are obviously related (Haywood, 1983). Downing and Merkins (1955) demonstrated an inverse relation between toxicity of ammonia and DO concentration. Lloyd (1961) stated that as oxygen concentration of the water is reduced, the concentration of excreted CO_2 at the gill surface is also reduced and the pH value of the water at this surface rises, resulting in an apparent increase in ammonia toxicity. On the other hand depression of pH at the gill surface, from CO_2 excretion, may result in the actual ammonia exposure concentration in high pH water

being much lower than the ammonia concentration of the bulk water.

II. Physiological Factors:

(1) Fish Size:

Meade (1985) reported that the start-of-feeding life stage is the period of highest ammonia sensitivity, and noted that late alevins and new fry are much more sensitive to ammonia than eggs or developing alevins. Large rainbow trout, over 2kg, are more vulnerable than small trout, 20-300g, to acutely toxic ammonia levels (Thurston et. al., 1981).

(2) Acclimation and Stress:

Acclimation to low levels of ammonia increases resistence to lethal levels for rainbow trout, S. gairdneriì (Lloyd and Orr, 1969; Thurston, et. al., 1981); and O. aureus (Render and Stickney, 1979). However, Lloyd and Orr (1969) stated that the effect has only been observed to last for 2-3 days and is lost after 3 days. High swimming speeds or exertion, as well as handling stress, may affect resistence to ammonia toxicity (EIFAC, 1970).

III. Data Available on Tilapia:

The paper of Render and Stickney (1979) on O. aureus appears to be the only published literature

specifically on ammonia toxicity for tilapia. Other relevant studies are based on the culture of tilapia in recirculating systems (Otte and Rosenthal, 1979). None of the above studies was designed to evaluate long term effect of different ammonia levels.

9. General Protocool for Studying the Protei--Ammonia growth relationship:

To study this complex relationship within a single experimental procedure is difficult, because of the interrelationship between these three parameters and the complex effect of each parameter on the other two. In order to simplify the approach in studying this relationship it was decided to divide it first into simpler sub-relationships as follows: (i) Protein intake-growth relationship, (ii) protein intake-ammonia excretion relationship, and (iii) ammonia-growth relationship (Fig 1.1). Each relationship is to be studied for two fish sizes, corresponding to juvenille and adult sizes.

Studying the effect of protein intake on both growth and ammonia excretion is complex in that protein utilization is affected by many factors, the most important of which are Protein quality (amino acids profile); total energy level in the diet (protein:energy ratio); and feeding rate.

The subject of studying dietary protein requirement in fish was reviewed by Tacon and Cowey (1985) who suggested that:

(i) a lipid-extracted fish meal or lipid extracted fish muscle should be used as a standard reference protein. This is because of the close similarity between the dietary essential amino acids (EAA) requirement of fishes and the EAA profile of fish muscle/carcass.

(ii) Varying both protein and energy levels of the diet is an efficient procedure only ifmedium levels of both protein and non-protein energy sources are used in the diet. At very low and very high dietary protein levels variation in the metabolizable energy levels are not avoidable. This is particularly true if a low-digestible carbohydrate source is used to produce iso-caloric diet.

(iii) Dietary protein requirement cannot be made unless a series of different feeding levels are tested so as to elicit a maximum growth response. This is mainly because of the inverse relationship between dietary protein level and feeding rate.

In conclusion, these criteria were considered as the basic guideline for defining the optimum dietary protein requirement in this study.

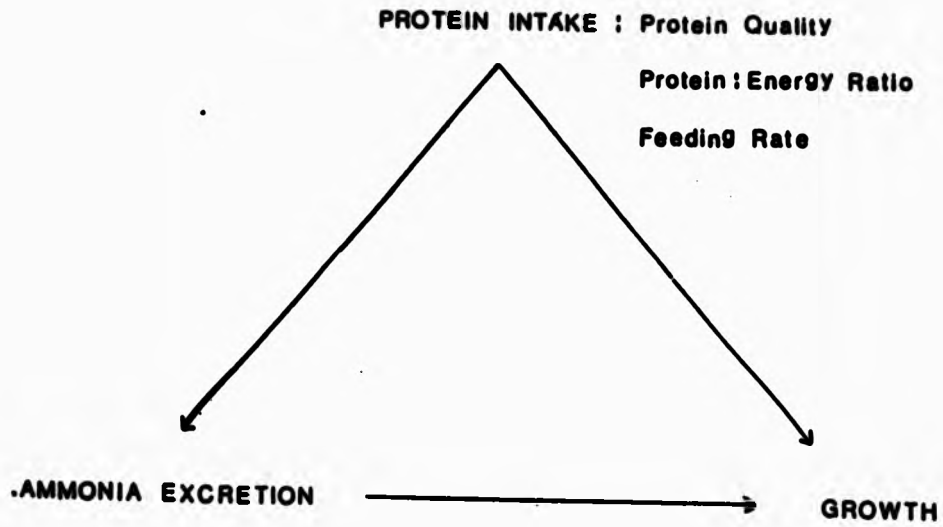


Figure 1.1 Relationship between protein intake, ammonia excretion and growth.

CHAPTER 2: General Materials and Methods

This chapter covers the basic methodologies and techniques used in this study. Any modifications on this standard technique, as required for certain experiments, will be mentioned later at the respective chapter.

1. Experimental Stocks

Fry of Oreochromis spilurus were originally imported from Kenya to Kuwait in 1982. Since then they have been bred and cultured in the Mariculture and Fisheries Department (MFD) of Kuwait Institute for Scientific Research (KISR). A sample from the original stock was analysed by the University of Stirling to check the purity of the strain. The amino acid profile indicated that the imported fish was Oreochromis spilurus spilurus (Kevin Hopkins, pers. comm).

For ammonia toxicity tests the large fish used during the summer of 1985 were drawn from the original imported stock. The small fish represented the F_1 generation which were spawned in 1984 by small spawners (about 100g bw) in their first spawning season.

The fish used in the growth and ammonia excretion studies were also from the F_1 generation of the original imported stock. Those fishes were spawned by large spawners (>200g bw) in 1985 during their second spawning

season. This F_1 stock was spawned by different groups of parents held in different tanks after which the fry were mixed together. Spawning of the broodstock was carried out only during the summer time (April - September) by collecting the eggs and the hatched fry from the mouths of the broodstock on a weekly basis. During the summer of 1986 this F_1 stock, which attained an average size of 30g, were used in both growth and ammonia excretion experiments. By the end of those two trials the same fish were collected together overwintered and redistributed in the summer of 1987 for use as large tilapia in both growth and ammonia excretion experiments.

(2) Acclimation to Sea Water:

As a general procedure broodstock were spawned and fry hatched in brackishwater of a salinity 3-5 ppt. Acclimation was carried out in $5m^3$ fibreglass tank by a batch water change, increasing salinity 5 ppt daily. During the acclimation period the 1,300 fish were kept in static water. Once the salinity reached full strength (38ppt) the fish were kept in running sea water at an average flow rate of 10l/min. The sea water was pumped from sea wells with the following characteristics: salinity = 38ppt; pH = 7.8 and dissolved oxygen = 4.5 ppm. Normally the fish were acclimated to sea water at 3-5 months age (5-30g bw).

During the holding period the fish were fed on Taiwanese origin tilapia feed (about 40% protein). This feed was readily accepted by tilapia in both brackish-and sea water as well as by the sea bream cultured at KISR.

(3) Holding System:

All experiments were performed in EVOS 5001 tanks. These fibreglass tanks have a dimension of 1m x 1m x 0.5m deep with a central sump connected to a 2" stand pipe. Sea water was pumped from a sea well. Each tank was supplied with ¾" PVC ball valve as a water inlet. Aeration was provided by a 5cm air stones at a rate of 1L/min. Air was supplied by a centralised blower which supplies air to the whole MFD station. Figure 2.1 shows the basic tank design used in this study. All pipes, valves, fittings used were made of PVC except aeration regulator valves and their reducers which were made from brass and steel, respectively. Flow rates, aeration rates, and stock densities were adjusted as required for the experimental conditions (described later).

The whole experimental area was situated in a large fibreglass-roofed greenhouse. All experimental tanks were covered by a cage nets to avoid fish jumping. Further shade nets were placed about 2m above the tanks to lower the bright sunlight during summer time. All experiments were performed during the summer season

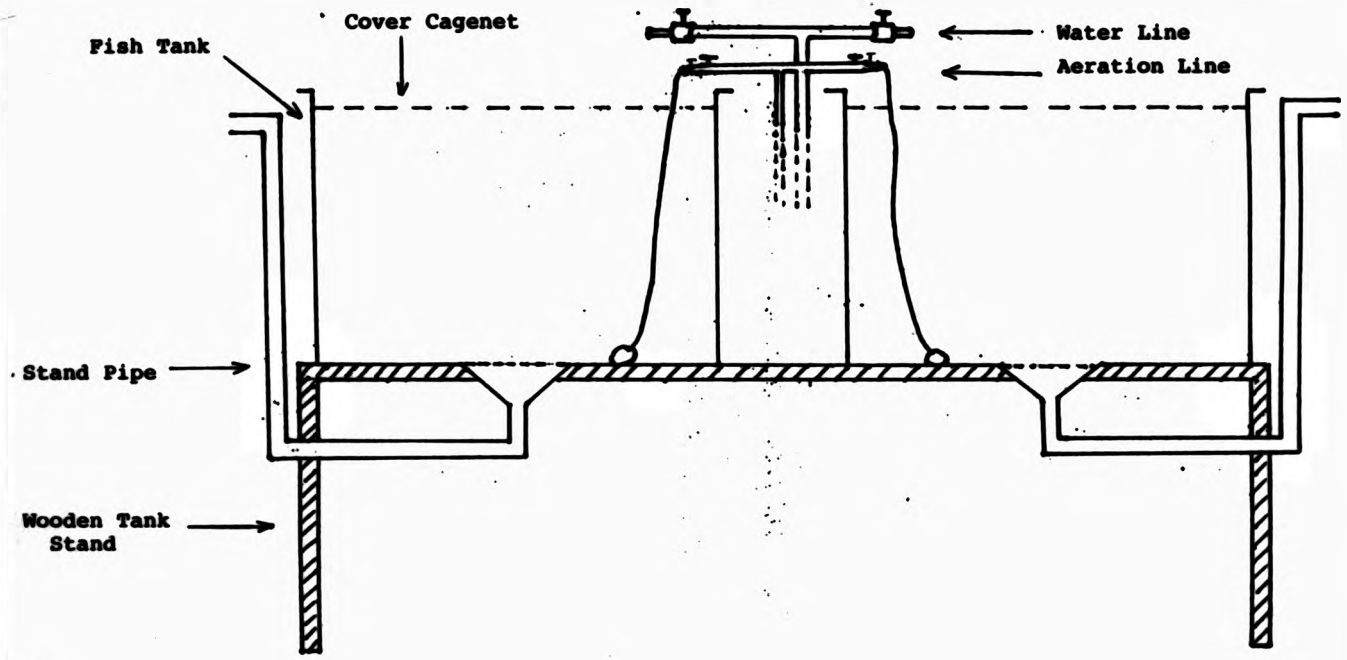


Fig 2.1 Basic tank design used in this study.

(April - September) when water temperature varies between 24-26°C. During the winter season the whole stock was kept in one 5m³ fibreglass tank with running sea water, at an average temperature of 19-21°C.

At winter holding periods the fish were fed on the Taiwanese tilapia pellets at a daily rate of 1.5-0.5% bw/d for small (20g) and large (100g) fish, respectively. During this period the fish were not handled at all.

(4) Feed Formulation and Presentation:

(i) Selection of Feed Ingredients:

White fish meal was used as a protein source in this study, as suggested by Tacon and Cowey (1985). A mixture of cod liver oil and corn oil was used to supply the necessary lipid. Although it has been reported that some tilapia, such as Tilapia Zilli, require only n-6 fatty acids characteristic of vegetable oils (Kanazawa, et. al., 1980; Watanabe, 1986), it was anticipated that in the sea water culture conditions of this study fatty acid requirements might change. Castell (1979) reported that the salt-water environment results in a more critical requirement for fatty acids of the n-3 series (typical of fish oil). Thus a mixture of vegetable oil and fish oil is used, as recommended by NRC (1983) for warm-water teleosts.

Carbohydrates were supplied from dextrin, which substitutes fish meal to produce iso-calorific diets, and starch. Vitamins and saline water mineral mixtures suggested by Jauncey and Ross (1982) for tilapia were used. Wheat middlings was used as a bulking agent for the vitamin mixture.

(ii) Selection of Ingredient Level (%):

Balarin and Haller (1982) suggested the use of protein levels between 25 to 35% for fattening of tilapia in tank culture systems. Similarly, Jauncey and Ross (1982) recommended 30% protein for tilapia larger than 35g. Therefore a 30-35% protein was selected as a median level. For 30g O. spilurus 25, 35 and 45% protein diets were tested, while for 200g fish 20, 30 and 40% protein diets were used.

Tilapia do not appear to utilise high levels of dietary lipids as effectively as salmonids or carp (Jauncey and Ross, 1982). Those authors suggested levels between 10-6% as optimal for maximising protein utilization for growth. Therefore, 10% lipid was used for small O. spilurus, whereas a 6% lipid was applied for the larger fish.

It appears that there is no specific requirement for dietary carbohydrate, as fish are capable of

synthesizing carbohydrates from both dietary protein and dietary lipid sources (Jauncey and Ross, 1982). Therefore, dextrin was used to replace protein component of the fish meal in an attempt to produce iso-calorific diets. Thus, the P:E ratio of the diets tested ranged from 51.4 to 89.9mg protein/Kcal. The diet composition used with small and large O. spilurus are shown in tables 1 and 2, respectively.

(5) Feed Preparation:

All feed ingredients, except vitamins, minerals and oil, were mixed first in a bowl mixer (Bear varimixer - R100) of 50kg capacity for 20 min. For mixing vitamins, minerals and oil a small amount (approx 1kg) of this dry mix was added to each component separately. These three batches were then separately mixed using a double cone mixer (Brook Crompton Parkinson Motors) for 15 min. After that they were added slowly to the main batch of dry mixed ingredients in the bowl mixer and mixed again for 20 min. Water was then added slowly until a stiff dough is formed (approx 2% water). The dough was then minced in an electrical mincer (Herbert - 4812), using a 2mm die for small fish diets and a 4mm die for the large fish. The spaghetti like mixed feed was dried in an oven at 60°C overnight, and then broken by hand, sieved and packed as pellets in plastic bags. A total of 25kg of each diet was

prepared. The feeds were stored all the time in a cold-room at -4°C until use.

(6) Feed and Fish Proximate Analysis:

Feed and fish samples were analysed at the beginning of each growth trial. For feed analysis random samples from the pellets were collected and then minced in an electrical mincer. The powder mix was then dried overnightin an electrical oven at 60°C. The feed samples were then kept in plastic bags inside a domestic fridge until analysis.

Fish samples for proximate body analysis were first frozen in a domestic deep freeze (-4°C). Within one week the frozen fish were chopped into small pieces and minced in an electrical mincer with a 2mm die. The minced fish meat was then dried in a freeze-dryer (New Brunswick Scientific V-13). The dry fish samples were then kept in plastic bags inside a domestic fridge until analysis.

Crude protein was measured by Macro-Kjeldahl procedure using open Kjeldahl apparatus "Labcono" (model 21232-05). The sample was digested first with concentrated H_2SO_4 in the presence of a catalyst (Na_2SO_4:$CuSO_4$ mixed at a ratio of 10:1). The sample was then distilled for about 2 hrs in the presence of zinc

and NaOH. The evaporated ammonia was then collected in a 200ml of 0.1N boric acid. Finally titration was carried out with 0.1N HCl. Crude protein percent was calculated as: 14.007 x 100 x 0.1 x 6.25 x ml of HCl/sample weight in mg.

Crude lipid was measured by extraction with ether (Labconc goldfish fat extraction apparatus - 35001). Lipid percent was calculated as: (Final beaker weight - Initial beaker weight/initial beaker weight) x 100. Crude ash was measured by heating at 360°C overnight, and its percentage was calculated as: (Final sample weight - Initial sample weight/initial sample weight) x 100. Gross energy was calculated as: 5.65, 9.45 and 4.10 Kcal/g for protein, lipid and carbohydrate, respectively.

(7) Digestibility Tests:

Due to the problems of quantitative faeces collection, most digestibility studies on fish have used an indirect method which utilizes an indicator or reference substance in the diet. Ideally, indicators should be totally indigestible and be evacuated at the same rate as the other gut contents. Chromic oxide (Cr_2O_3, which is used in this study, is the most commonly used external indicator substance for fish digestibility trials (Talbot, 1985).

By the end of the growth experiments of small and large fish, the fish were kept in the tanks for a further 10 days during which the faeces were collected on two occasions for chromic oxide analysis. For faeces collection the fish were anaesthetized first with 5 ppm quinaldine and then faeces were stripped out by hand. The two batches of faeces were then mixed together. For large O. spilurus, because of small fecal matter collected, the faeces produced by each diet for all feeding rates were grouped together. Cho and Kaushik (1985) reported that digestibility coefficients are little affected by biotic and abiotic factors and these coefficient values of individual ingredients can be assumed to be additive so that the final content of digestible protein and energy in the diets can be predicted.

The collected fecal matter was dried in the oven at 60°C for overnight, and stored in a plastic bag in the fridge until analysis. The total nitrogen and chromic oxide analysis of the faeces were carried out by the central analytical laboratory of KISR. For total nitrogen the sample was oxidized at 950°C in a CHN analyser, model CHN - Rapid, Haraeus. The librated NO_2 was detected by a thermal conductivity detector which gives signals proportional to the nitrogen oxidized.

For chromic oxide analysis the samples were first digested by nitric and percloric acids for about 4 hrs. The developed colour was then measured by a spectrophotometer at 260nm.

Table 1 The Composition of Diets Used with small *O. spilurus*

% of Protein	25	35	45
White fish meal	39.28	55.00	70.00
Mineral mix#	2.83	2.15	1.48
Vitamin mix#	2.00	2.00	2.00
Carboxymethylcellulose	3.00	3.00	3.00
Chromic oxide	0.50	0.50	0.50
Corn oil	4.00	4.00	4.00
Cod liver oil	4.04	2.67	1.36
Starch	4.47	4.26	4.05
Dextrin	32.00	22.00	12.00
Cellulose	7.88	4.42	1.61
Total	100.00	100.00	100.00

Proximate Analysis of Experimental Diets

Analysed Components	25	35	45
Crude Protein (%)	24.94	34.44	43.35
Ether Extract (%)	7.98	8.17	8.17
Ash (%)	1.52	4.82	9.39
Carbohydrate* (%)	65.56	52.57	39.09
P:E ratio (mg protein/Kcal)	51.4	70.7	89.9
Gross Energy (Kcal/g)	4.85	4.87	4.82

* By difference

As specified by Jauncey and Ross (1982)

Table 2 The Composition of Diets Used with Large *O. spilurus*

% of Protein	20	30	40
White fish meal	30.77	46.15	61.54
Mineral mix#	3.17	2.49	1.82
Vitamin mix#	2.00	2.00	2.00
Carboxymethylcellulose	3.00	3.00	3.00
Chromic oxide	0.50	0.50	0.50
Corn oil	4.00	4.00	4.00
Cod liver oil	3.38	2.08	0.77
Starch	4.58	4.37	4.16
Dextrin	37.00	27.00	17.00
Cellulose	11.60	8.41	5.21
Total	100.00	100.00	100.00

Proximate Analysis of Exprimental Diets:

Analysed Components	20	30	40
Crude Protein (%)	20.00	30.00	41.25
Ether Extract (%)	6.4	6.6	6.5
Ash (%)	8.6	12.0	14.7
Carbohydrate* (%)	65.00	51.40	37.55
P:E ratio (mg protein/Kcal)	45.5	67.7	92.1
Gross energy (Kcal/g)	4.40	4.43	4.48

* By difference

As specified by Jauncey and Ross (1982)

(8) Fish Handling and Measurements:

As a general procedure before any handling the fish were anesthetised first with 5 ppm quinaldine. Fish measurements were taken on a wet weight basis with an electrical balance (Sartorius - 5762 MP6E). For total weight measurements the fish were lifted by hand net and placed in a tared bucket with water. In ammonia toxicity trials only initial and final total weight were measured, whereas in growth trials the small fish were weighed every 2 weeks, and the large fish weighed every 3 weeks. In ammonia excretion tests the fish were weighed 10 days before the ammonia sampling day and then 3 days after ammonia measurements. During all other holding and overwintering periods the fish were not handled.

9. Feed Evaluation:

For feed evaluation the following parameters were calculated as follows:

(i) Specific growth rate (SGR):[(ln final body weight - ln initial body weight)/times in days] x 100.

(ii) Food conversion ratio (FCR):food intake divided by increase in body weight.

(iii) Protein efficiency ratio (PER):increase in body weight divided by protein intake.

(iv) Protein productive value (PPV):increase in body protein divided by protein intake.

(v) Nitrogen efficiency by growth and carcass analysis:

Nitrogen efficiency = (nitrogen retention/nitrogen absorption) x 100

where, Nitrogen absorption = Nitrogen consumption - Faecal nitrogen

and, Nitrogen retention = Final mean body nitrogen - Initial mean body nitrogen.

(vi) Nitrogen efficiency by nitrogen - balance method:

Nitrogen efficiency = (nitrogen retention/nitrogen absorption) x 100

where, Nitrogen absorption = Nitrogen consumption - Faecal nitrogen

and, Nitrogen retention = Nitrogen absorption - Nitrogen excretion (ammonia alone or ammonia + urea nitrogen).

10. Water Quality Analysis:

(i) Glass-ware cleaning:

All sampling, reaction and reagent bottles were cleaned initially with an acid mixture, to avoid contamination from previous usage, as suggested by Boyd (1979). This mixture was composed of 120g $Na_2Cr_2O_7$ in 1l of tap water and 1.6l conc. H_2SO_4, and was used for 15-20 min. The bottles were then rinsed and flushed with clean tap water. Between measurements the glass-ware was cleaned first with liquid detergent, then

washed with tap water, and finally rinsed with deionised water and dried in air.

(ii) Water Sampling:

In ammonia excretion and toxicity tests the water samples were collected by siphoning the water with a small hose from the fish tank to the sampling bottles. The water was allowed to overflow in the bottle to avoid air bubble formation. Normally, two 500ml sampling bottles were collected from each tank at each measurement period; one for oxygen measurement and the other for measuring ammonia and, when possible, urea. The same procedure was used for collecting water samples from the water reservoir in ammonia excretion tests.

In ammonia toxicity trials dilution water was required by the analytical procedure. The water used for dilution was collected by boat from open sea. The water was filtered first by 0.45mm Wheaton filter paper and then stored in dark plastic container. The water was allowed to age for at least one month before use.

(iii) Sample Preservation:

Oxygen measurements were performed directly and thus no preservation was used. For ammonia and urea measurements the water samples were first filtered with 0.45mm Wheaton filtered papers, and preserved with two

small drops of conc. H_2SO_4 per 500ml sampling bottle. Water samples were then placed in a refrigerator at 4°C until analysis. Experience gained in NFD laboratory indicated that variation in the water samples preserved in this way is about ±5% (Maria Boshkash, pers. comm.). Ammonia analysis was usually performed within one week of collection. This form of preservation was used only in the ammonia excretion experiment with small O. spilurus. In all other ammonia measurements no preservation was used (see later).

(iv) Water Analysis:

The sea water was pumped from a sea well with an ambient salinity, pH and oxygen levels of about 38ppt, 7.8 and 4.5ppm, respectively.

As a general rule 3 water replicates were measured for each parameter at each measurement time.

(a) Oxygen:

For oxygen measurements BOD(DO) probe connected to a YSI oxygen analyser model 701 was used. Water stirring was provided by a separate magnetic electrical stirrer. This method was found to be faster and give more stable readings than using the stirrer attached to the BOD probe itself. For calibration 1l of sea water was aerated until saturation (15-20 min). The temperature of this water sample was measured and the

saturation oxygen level was taken from a standard table. Calibration was carried out every 6hr during the 24hr sampling period in the ammonia excretion tests. For other tests calibration was performed prior to water samples measurements.

(b) Ammonia:

For ammonia analysis three methods were used. The method of Strickland and Parsons (1972) for ammonia determination in sea water was used in the ammonia toxicity trials. This method was used mainly because of its rapid reaction time. Second, the method of Grasshoff (1976) was used in the determination of ammonia excretion rate of small O. spilurus. This method although taking a longer time is more sensitive than the first, and was required to detect the small differences in the ammonia excretion rates. Although the water samples for this trial were preserved with acid, pH adjustment was not needed, as experience gained in the NFD laboratory indicated that at a starting pH of 2.5 the complete formation of the indophenol colour takes a longer time. About 16-18hrs were needed after which the colour becomes stable for at least 10 hrs.

In both procedures the ammonia samples were measured with a two-beam spectrophotometer (Bausch & Lomb, Spectronic 2000) at 630 nm using 1cm cuvettes.

Acidified sea water was used in the reference cell. The spectrophotometer was set on a concentration mode and calibrated to 0.00 concentration with a sea water blank and to 1.00 with a 1.00 mg NH_2N/l standard solution. The standard stock solution (1.00mg/ml) was prepared by dissolving dry NH_4Cl salt in a de-ionized water. The standard solutions were prepared freshly on the day of measurement by serial dilution of ammonium chloride stock solution (1.00mg NH_2N/ml) with filtered, aged sea water. The water samples were measured directly as a mg NH_2N/ml. The spectrophotometer was recalibrated every 2 hrs during measurements. The unionized ammonia level was calculated from standard tables (Bower and Bidwell, 1978) at the respective pH, temperature, and salinity.

The third method used for ammonia analysis was that carried out by autoanalyser (Skalar - 5100, Holland). This method was used only to measure the ammonia excretion rate of large fish. This method is based also on the development of indophenol colour. The method was that of the manual; using the following cycle: air:3sec; sample:60 sec; washing:120 sec; at a water bath temperature of 76°C. The photocell was adjusted at 630 nm and the absorbances were measured from the height of the peaks plotted bygraphic plotter. The calibration curve was developed with serial dilutions of a standard ammonium chloride solution, and

ammonia concentrations were calculated from the calibration curve. The instrument was calibrated every 2hrs during measurements.

(c) Urea:

For urea analysis the method of Koravskaya (1973) for determination of urea in sea water was used. The method is based on the reaction of urea in 10ml of sea water with 2ml diacetyl-monoxime in the presence of 0.5ml antipyrine to form a yellow colour. The reaction was performed in 15ml covered glass test tubes. After the addition of the chemicals the test tubes were placed in a boiling water bath for 1hr after which the colour is measured at 540 nm in 5cm cuvette using a Bausch & Lomb Spectronic 2000 spectrophotometer. A calibration curve was made from urea standard stock solution at each measurement day. The spectrophotometer was calibrated every 2 hrs. Standards, blanks and other measurements were prepared using the same general procedures as for the ammonia analysis.

(d) PH:

A pH meter Orion Research - 7108/digital ionalyzer was used. The meter was calibrated with a normal buffer solution of pH=7.0 before measurement.

11. Statistical Analysis:

For ammonia toxicity tests no replication was performed. Therefore, a regression analysis was calculated first, from which the ANOVA test was calculated (Zar, 1984). For growth experiments 3 replicates were performed with small fish, and only 2 replicates were carried out with larger ones. ANOVA tests were carried out as a two-way factorial analysis using SPSS/PC computer programmes. then the orthogonal contrast polynomial ANOVA was calculated. For regression analysis the Hewllet-Packard programmes for polynomial regressions were used.

For digestibility, ammonia, urea, and oxygen measurements the experiments were analysed as two-way factorial design without replication as described by Zar (1984). For regression analysis Hewllet-Packard computer programmes for polynomial regressions were used. The Student-Newmans-Keulss (SNK) test was used to differentiate between experimental treatment whereas covariance analysis withTuckay's test were used to differentiate between regression slopes.

CHAPTER 3: The Effect of Protein Intake on the Growth of *O.spilurus* in sea water.

Introduction

The quantitative protein requirement for tilapia has been studied for more than one species, including: O. aureus (Davis and Stickney, 1978; Winfree and Stickney, 1981), T. Zilli (Mazid et. al., 1979), O. niloticus (Santiago et. al., 1982; Vange et. al., 1985a and 1985b); O. mossambicus (Jauncey, 1982), and "Red tilapia" (Hepher et. al., 1983). However, no data are available yet on the dietary protein requirement for O. spilurus, probably because it has not until recently been considered as an important species for aquaculture. Because of its high tolerance to sea water (Al-Amoudi, 1987, Al-Ahmad et. al., 1986) it has now been recognised as a potential species for mariculture.

Almost all of the available data on dietary protein requirement for tilapias are based on freshwater culture conditions. It is possible that salinity acclimation, as a masking factor elevating maintenance metabolism (Brett, 1979) would result in a change in dietary requirements. Furthermore, several studies (Zeitoun et. al., 1973; Lall and Bishop, 1976; Smith and Thorpe, 1976; Macleod, 1977) suggest that some fishes like rainbow trout living in plasma-hyperosmotic seawater have a higher protein demand than those living in fresh water.

Dietary protein requirement for any particular species changes with size. Millikin (1982) reported that these generally decrease with increasing age or fish size. Most of the available data on dietary protein requirement for tilapia are based on fry or juveniles, while few quantitative data have been defined for large tilapias (Viola and Arieli, 1982).

Dietary protein requirements are normally expressed in terms of a fixed dietary percentage or as a ratio of protein to dietary energy. However, Ogino (1980) found an inverse relationship between feeding level and dietary protein requirement. As a result, Tacon and Cowey (1985) suggested that dietary nutrient (protein) requirements should be expressed in terms of feed intake (grams or kilojoules of nutrient required per kilogram body weight per day).

Thus, this experiment was designed to study the combined affect of protein concentration and feeding level.

Materials and Methods:

Basic experimental methodology is described in Chapter 2. Two experiments were performed, the first for 8 weeks on small O. mpilurus with an initial size ranging from 27.46 to 28.98g bw. Ten feeding regimes

were tested, three dietary protein levels (25, 35 and 45%) each fed at three feeding rates (2, 4 and 6% bw/d). Starved fish served as a control. In all feeding regimes 20 fish were stocked in each tank, at 3 replicates per treatment giving a total of 30 tanks. All fish were stocked in the experimental tanks 2 weeks before starting the experiment. All fish were sampled for total weight every 2 weeks, except the starved fish which were measured only at the beginning and end of the experiment.

The second experiment was performed 12 months later, using the same stock, for 12 weeks, with initial fish size ranging from 255.82 to 282.38g bw. Ten feeding regimes were tested, three dietary protein levels (20, 30 and 40%) each fed at three feeding rates (0.5, 1.75 and 3.0% bw/d). Starved fish served as a control. In all feeding regimes 10 fish were stocked in each tank, at 2 replicates per treatment giving a total of 20 tanks. All fish were stocked in the experimental tank 3 weeks before starting the experiment. All fish were measured for total weight every 3 weeks, except the starved fish which were measured only at the beginning and end of the experiment. Both sizes were fed 3 times a day at 8, 12 and 16hrs.

Table 3.1, summarises the experimental feeding regimes for the two sizes. The amount of food fed was adjusted at every measurement time, i.e. every two weeks with small fish and every 3 weeks with large fish. A continuous water change was maintained throughout the two experiments at a flow rate of 5l/min/tank. Temperature fluctuates in both experiments between 23.8 to 26.3°C. Oxygen was provided by aeration, with a minimum recorded level of 4.2mg/l.

Results:

(1) **Survival Rate:**

A summary of results is shown in Table 3.2. The survival rate in experiment 1 ranges from 91.65 to 100%. Some fish escaped from treatment 7 during the biweekly measurements and this is the reason for the differences in survival rates shown in Table 3.2.

Major mortalities occurred during the second experiment on large O. spilurus. These mortalities were continuous during the whole period of the experiment. By the end of the experiment the survival rate ranged from 40% to 95%.

(2) **Growth v/s time:**

The change in mean body weight of small fish is shown in Fig. 3.1. All fed fish showed positive growth

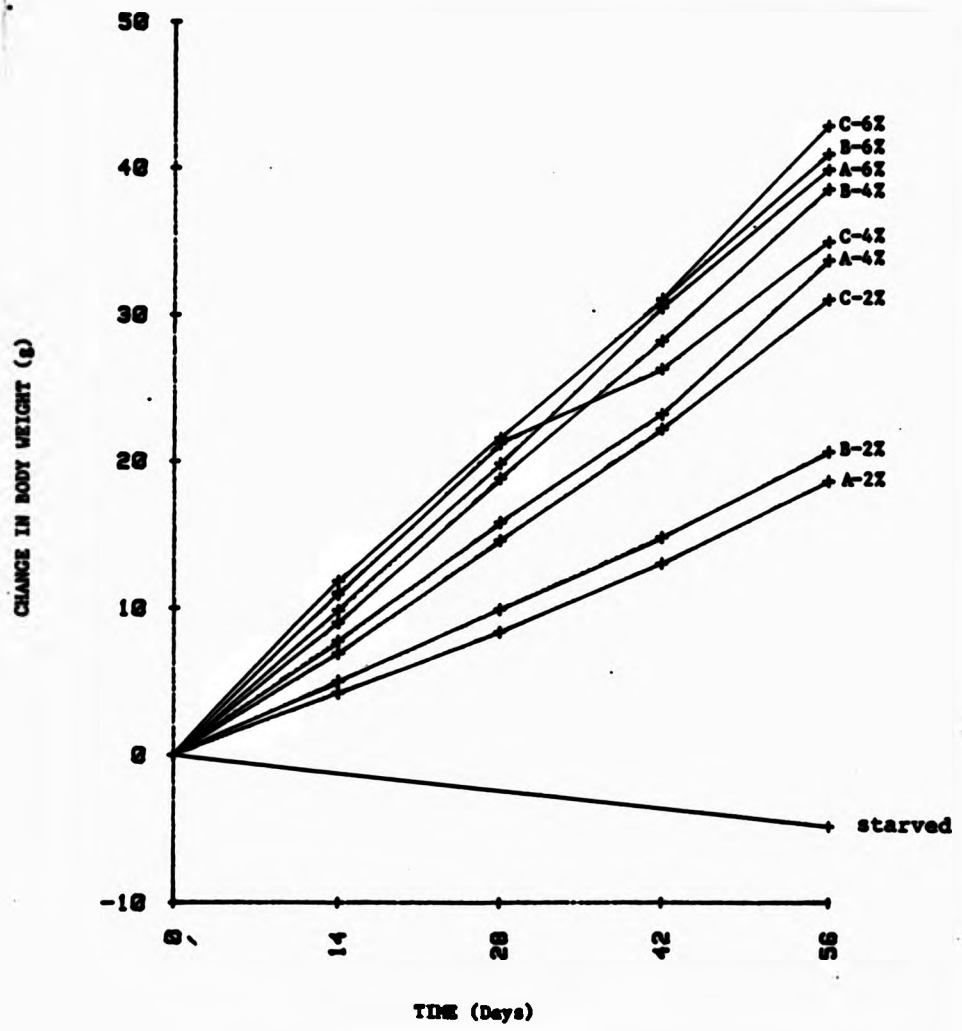


Figure 3.1 Growth rate of small O.spilurus fed on 24.94% (A), 34.44% (B) and 43.35% (C) protein diets at 3 feeding rates 2, 4 and 6% BW/D.

Table 31 Experimental feeding regimes applied for the two sizes of O. spilurus in seawater.

Fish Size	Feeding Rate	Protein %	Protein Intake (g/kg/d)	Gross Energy Intake (Kcal/kg/d)
Small	0.00	0.00	—	—
	2	24.94	4.99	97.0
		34.44	6.89	97.0
		43.35	8.67	96.0
	4	24.94	9.98	194.0
		34.44	13.78	194.8
		43.35	17.34	192.8
	6	24.94	14.96	291.0
		34.44	20.66	292.2
		43.35	26.01	289.2
Large	0.00	0.00	—	—
	0.5	20.00	1.00	22.00
		30.00	1.50	22.15
		41.25	2.06	22.40
	1.75	20.00	3.50	77.00
		30.00	5.25	77.53
		41.25	7.22	78.40
	3.00	20.00	6.00	132.00
		30.00	9.00	132.90
		41.25	12.38	134.40

Table 3.2 Summary Table for the Experimental Results

Treatment No.	Fish Size	Feeding Rate % BW/day	Protein % in diet	Total Fish No.		Survival %	Mean Body Wt.(g)		Fish Growth Rate (g/fish/day)	SGR
				Initial	Final		Initial	Final		
1	Small	2	24.94	60	60	100.00	27.83	46.46	0.33	0.91
2			34.44	60	60	100.00	27.61	48.24	0.37	0.99
3			43.35	60	60	100.00	28.13	59.15	0.55	1.33
4		4	24.94	60	59	98.35	27.75	61.60	0.60	1.42
5			34.44	60	60	100.00	28.87	67.45	0.69	1.52
6			43.35	60	60	100.00	28.62	63.59	0.62	1.42
7		6	24.94	60	60	100.00	27.46	67.38	0.71	1.59
8			34.44	60	55	91.65	29.81	71.21	0.74	1.56
9			43.35	60	60	100.00	28.98	71.88	0.77	1.62
10		0	---	60	59	98.35	36.44	31.64	(-0.09)	-0.25
1	Large	0.5	20.00	20	12	60.00	255.82	270.69	0.18	0.07
2			30.00	20	11	55.00	268.42	386.85	1.41	0.41
3			41.25	20	14	70.00	265.18	262.54	-0.03	-0.01
4		1.75	20.00	20	19	95.00	282.38	275.94	-0.08	-0.03
5			30.00	20	19	95.00	273.71	296.04	0.27	0.09
6			41.25	20	8	40.00	255.45	308.26	0.63	0.22
7		3.00	20.00	20	18	90.00	278.09	303.22	0.30	0.11
8			30.00	20	19	95.00	269.33	283.21	0.17	0.05
9			41.25	20	19	95.00	277.54	273.13	-0.05	-0.00
10		0	---	20	13	65.00	257.30	211.50	-0.55	-0.25

whereas only starved fish lost weight. Generally the growth rate for the fed fish followed the same trend during the whole experiment, where higher growth rate is observed with higher feeding rate and higher protein percentage in the diet at any particular feeding rate. However, the 43.35% protein diet fed at 4% bw/d resulted in a slower growth rate only during the second month.

Contrary to these results the pattern of growth of large O. apilurus over time was not clear. As expected the starved fish showed a negative growth rate. While some fed fish showed a positive growth, some other fed fish did not grow or showed negative growth. This is shown in Fig. 3.2. These observed differences in growth rate did not appear to correlate with specific experimental feeding regimes. The high growth rates observed with 30% protein diet fed at 0.5% bw/d and 41.25% protein diet fed at 1.75% bw/d probably reflected the high mortality (40% and 60%, respectively) among the smaller fish which occurred during these treatments, where subsequently only larger fish survived.

(3) Growth v/s Food Intake:

Food intake had a highly significant effect ($p>0.01$) on growth rate in experiment 1. Starved fish showed a negative growth rate during the two month experimental period, with average weight loss of

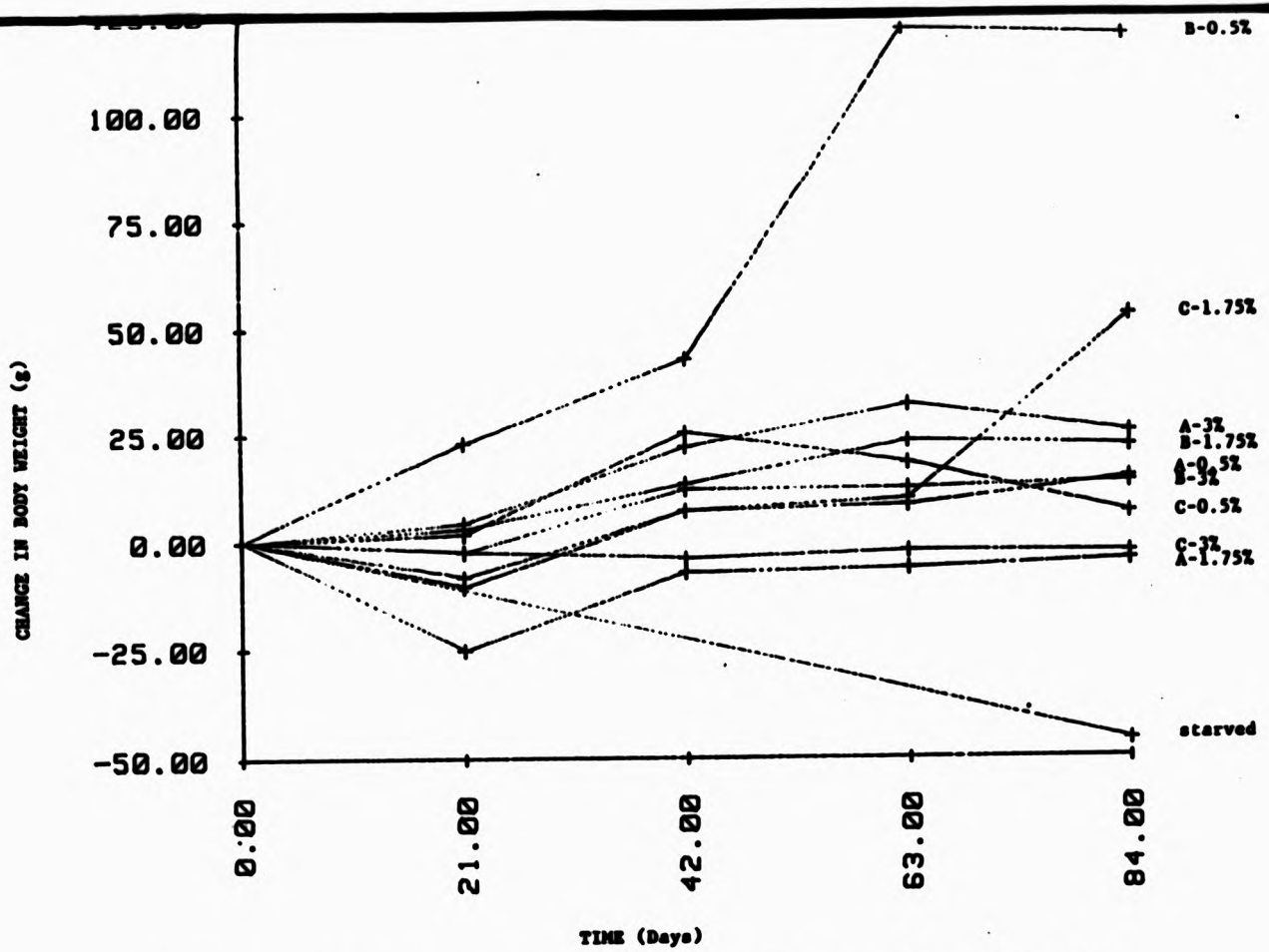


Figure 3.2 Growth rate of large *O.spilurus* fed on 20% (A), 30% (B), and 41.25% (C) protein diets at 3 feeding rates 0.5, 1.75 and 3% BW/D.

0.09g/fish/day. However, in experiment 2 (larger stocks) food intake did not have a significant ($p<0.05$) effect on growth rate. The average weight loss of large O. spilurus during the 3 months experimental period was 0.55g/fish/day.

For experiment 1, the results of 2-way ANOVA indicated that feeding rate had a highly significant effect ($p>0.01$) on SGR, whereas protein percentage does not show a significant effect ($p<0.05$) on SGR. The combined affect of the two factors showed a significant affect ($p>0.05$) on growth rate. These relationships suggested that protein percentage in the diet becomes an important factor for growth only at a certain critical level. To determine this level the Student-Newmans-Keuls (SNK) test was carried out to differentiate between the feed combinations. The SNK test shows that 43.35% protein diet fed at 2% bw/d produced a significantly higher growth rate ($p>0.05$) than 24.94% and 34.44% protein diets fed at the same feeding level, while it did not produce significantly different results ($p<0.05$) from any other feed combinations tested in this experiment. This protein intake seems to be the critical level.

To check further the significance of protein percentage in the diet in determining growth rate its

effect was tested against SGR by one-way ANOVA for each feeding rate separately. The results indicate the protein percentage in the diet gave a highly significant effect ($p>0.01$) on SGR only at 2% bw/d feeding rate. At higher feeding rates the protein percentage in the diet has no significant effect ($p<0.05$) on SGR.

Fig. 3.3 shows the relationship between feeding rate and SGR for the 3 experimental diets. The relationship is quadratic and the components of these regressions are shown in Table 3.4. The negative values of b_0 indicated weight loss of starved fish. Whereas the negative values of b_2 indicated that the effect of feeding rate on SGR decreases at higher feeding rates. With such a quadratic polynomial regressions it is possible to calculate the maximum SGR (Zar, 1984). The maximum values of SGR with the corresponding values of feeding rates are shown in Table 3.4. It is clear that there is a negative relationship between maximum SGR and protein percent of the diet. The maximum values of feeding rates obtained from the regressions indicated also that the fish were overfed at 6% bw/d for all diets.

Although the 9 feeding regimes used in this experiment represent a combination between protein percent of the diet and feeding rate, it is probably

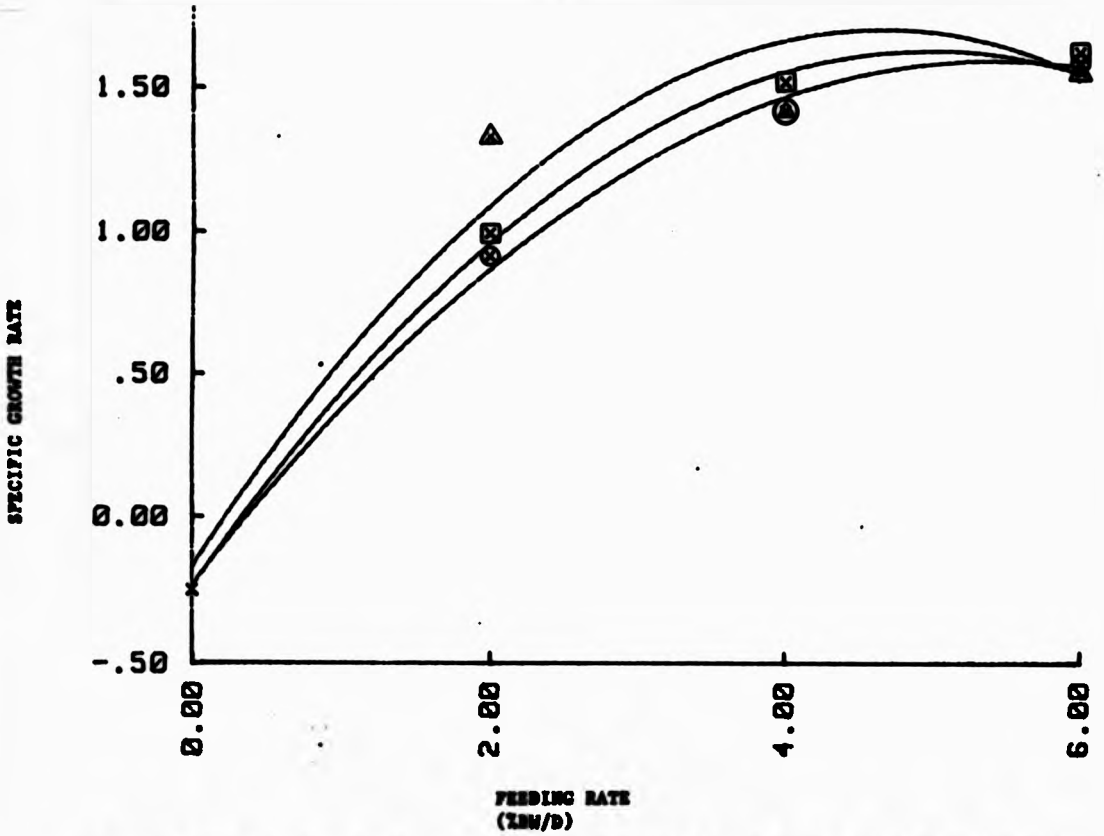


Figure 3.3 Relationship between feeding rate (%BW/D) and growth rate (SGR) for small *O.spilurus* fed on 3 diets: 24.94% (○), 34.44% (□), and 43.35% (△) protein.

Table 3 . Diets evaluation.

Fish Size	Feeding Rate % BW/D	Protein %	SGR	FCR	PER	PPV	Apparent digestibility
Small	2	24.94	0.91^{a}	1.60^{b}	2.38^{d}	35.95^{f}	62.91%
	2	34.44	0.99^{a}	1.49^{b}	1.82^{c}	30.67^{e}	78.57%
	2	43.35	1.33^{b}	1.09^{a}	2.01^{c}	36.14^{f}	81.32%
	4	24.94	1.42^{b}	2.08^{d}	1.84^{c}	28.69^{d}	63.84%
	4	34.44	1.32^{b}	1.94^{c}	1.44^{b}	23.01^{c}	59.29%
	4	43.35	1.42^{b}	2.15^{d}	1.02^{a}	17.97^{b}	73.10%
	6	24.94	1.59^{b}	2.82^{e}	1.36^{b}	25.52^{c}	39.98%
	6	34.44	1.56^{b}	3.23^{f}	0.86^{a}	14.96^{a}	53.65%
	6	43.35	1.62^{b}	2.71^{c}	0.81^{a}	12.87^{a}	85.43%
Large	0.5	20	0.07	1.78			
	0.5	30	0.41	0.90			
	0.5	41.25	-0.01	-1.29			
	1.75	20	-0.03	-24.89			
	1.75	30	0.09	-4.32			
	1.75	41.25	0.22	-2.79			
	3	20	0.11	95.32			
	3	30	0.05	0.98			
	3	41.25	-0.00	1364.86			

Same letters are not significantly different at 0.05 level.

Table 3.4 (1) Relationship between growth rate, SGR (Y) and feeding rate (% BW/D):

(2) Relationship between growth rate, SGR (Y) and protein intake (% BW/D), for small O.spilurus.

Item		b_0	b_1	b_2	Standard Error at Estimate	r^2	r	Maximum Feeding Rate % BW/D	Maximum SGR
[1]Protein %	24.94	-0.2363	0.6764	-0.0625	0.0072	0.997	0.936	5.41	1.59
	34.44	-0.2406	0.7504	-0.0754	0.0053	0.997	0.910	4.97	1.63
	43.35	-0.1724	0.8038	-0.0863	0.3574	0.943	0.854	4.68	1.70
[2]Protein intake: %BW/D		-0.0697	1.8423	-0.4820	0.1708	0.930	0.795	1.91	1.69

Table 3.5 Multiple linear regression components where Y = SGR, X_1 = feeding rate (%BW/D); X_2 = (feeding rate)^2 ; X_3 = FR x Protein % of the diet.

Variable	Coefficient	Standard Error of Reg. Coefficient	R^2	Standard Error of Estimate
a-Constant	-0.1532	0.1584	0.94	0.1704
X_1-FR	0.7072	0.1038		
X_2-FR^2	-0.0736	0.0187		
X_3-FR x Pr.%	0.0001	0.0003		

possible to express these combinations as one factor such as protein intake. The relationship between growth rate (SGR) and protein intake (% bw/d) could also be explained by a quadratic regression which is shown in Fig. 3.4 and Table 3.4. The maximum protein intake was found to be 19.1g/kg fish/day which supported an SGR of 1.69.

A multiple linear regression model was formulated to show the combined effect of protein level in the diet and feeding rate. Only those factors which have a significant effect on growth as shown by the orthogonal contrast polynomial ANOVA test were included in the model. The model components are shown in Table 3.5. Comparing the regression coefficients it seems that the linear feeding rate has the highest effect followed by quadratic feeding rate, and then the interaction between feeding rate and protein percent of the diet.

Neither feeding rate nor protein percent of the diet causes a significant ($p<0.05$) growth for large *O. spilurus* in experiment 2. Subsequently, no further statistical analysis was performed.

(4) Food Conversion Ratio (FCR):

FCR values are shown in Table 3.3. Protein percent of the diet did not show any significant effect

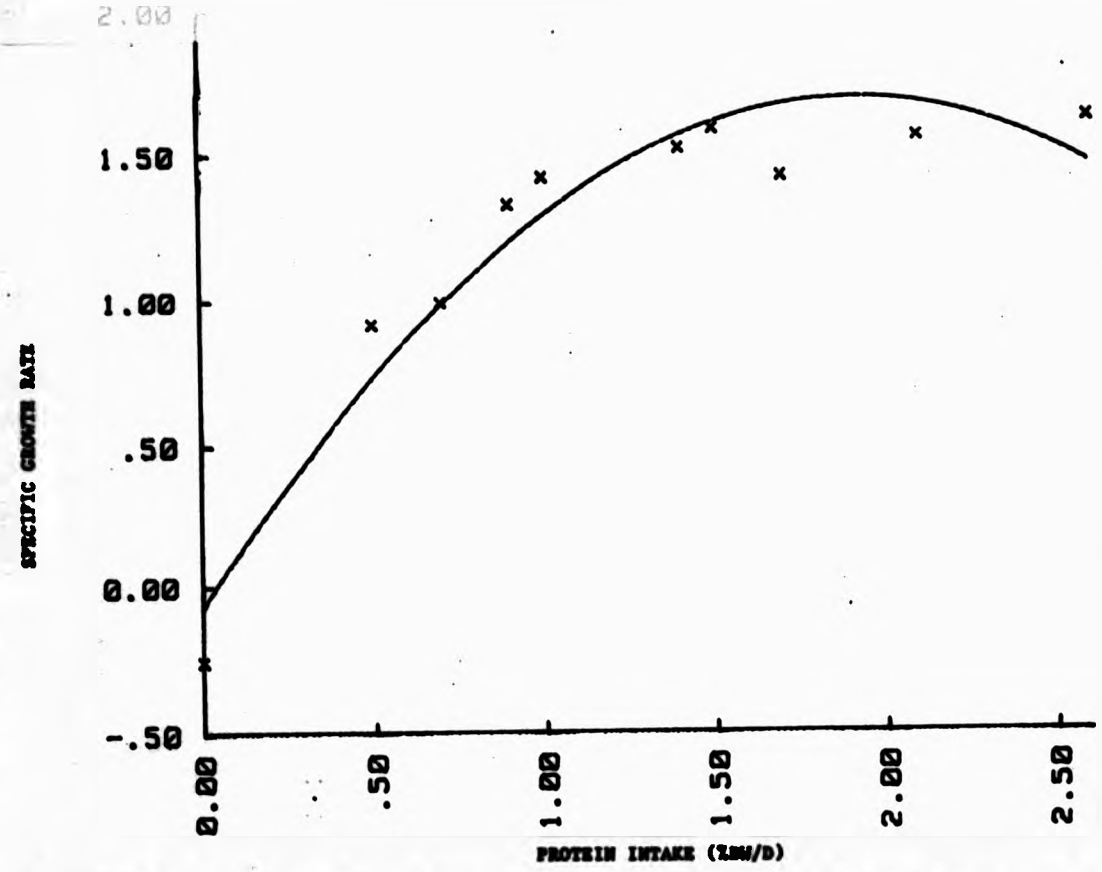


Figure 3.4 Relationship between protein intake (%BW/D) and growth rate (SGR) for small O.spilurus.

on FCR, while feeding rate had a highly significant effect. The combined effect between protein percentage in the diet and feeding rate showed a significant effect on FCR. This suggests also that protein percentage in the diet becomes important at a certain critical point.

Fish fed 6% bw/d appeared to show the highest FCR (approx 3) which is significantly higher from all other feeding regimes. Fish fed 4% bw/d show a medium FCR (approx 2) which is significantly different from all other feeding regimes. Fish fed 2% bw/d were divided into two groups. First, diets containing 24.94% and 34.44% protein show low FCR (approx 1.4) which is significantly different from all other feeding regimes. Second, diets containing 43.35% protein show the lowest FCR (approx 1) which is significantly different from all other feeding regimes. This feeding regime seems to be the critical because higher feeding rates (one direction) or lower protein percentages in the diet (another direction) result in a higher FCR.

The relationship between feeding rate and FCR for all experimental diets is shown in Fig 3.5 and summarised in Table 3.6. There is a positive linear relationship between feeding rate and FCR. Protein percent of the diet seems to have a significant effect on the slope.

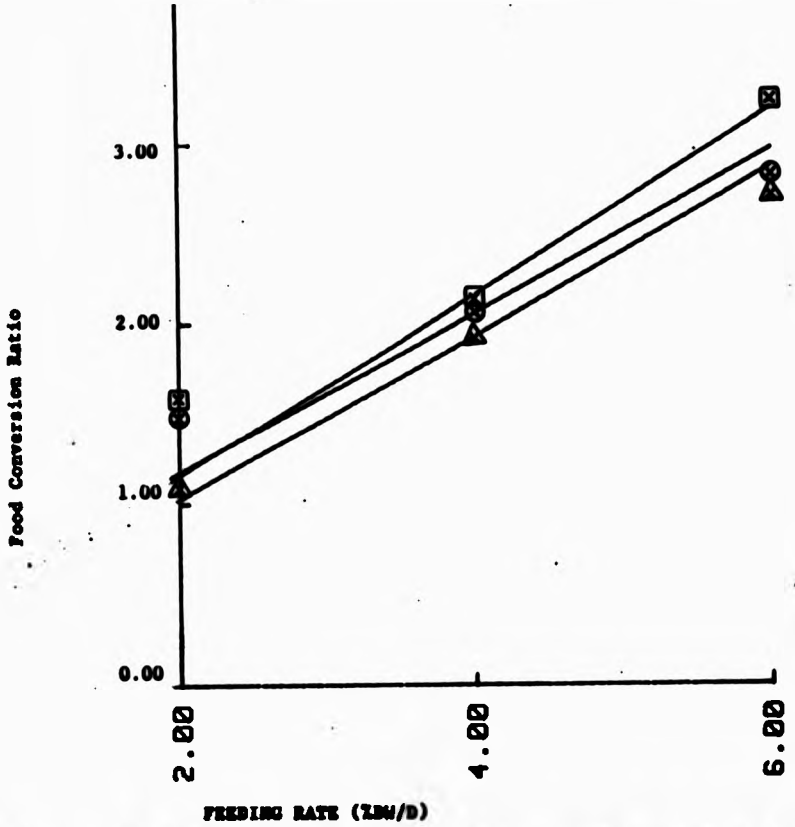


Figure 3.5 Relationship between feeding rate and food conversion ratio for small O.spilurus fed on 3 diets: 24.94% (O), 34.44% (□) and 43.35% (△) protein.

Table 3.6

Item	b_0	b_1	Standard Error of Estimate	r^2
Protein %: 24.94	0.2840	0.4470	0.3742	0.93
34.44	0.1440	0.5070	0.3055	0.96
43.35	0.1090	0.4590	0.2016	0.99

Relationship between PCR (Y), and feeding rate (X) for the 3 experimental diets for small O. spilurus in seawater.

Table 3.7

Item	b_0	b_1	Standard Error of Estimate	r^2
Protein %: 24.94	2.88	-0.255	0.002	0.99
34.44	2.40	-0.253	0.006	0.99
43.35	2.48	-0.300	0.318	0.88

Relationship between protein efficiency ratio, PER (Y) and feeding rate for small O.spilurus.

Table 3.8

Item	b_0	b_1	Standard Error of Estimate	r^2
Protein %: 24.94	40.48	-2.61	1.67	0.95
34.44	38.59	-3.93	0.159	0.99
43.35	45.59	-5.82	5.34	0.90

Relationship between protein productive value, PPV (Y) and feeding rate for small O.spilurus.

The results of FCR of large O. spilurus obtained from experiment 2 did not show any significant ($p<0.05$) difference between the experimental treatments. Therefore, no more statistical analysis was performed.

(5) Protein Evaluation:

To evaluate protein utilisation efficiency both protein efficiency ratio, PER (body protein v.s. protein intake) and protein productive value, PPV (increase in body protein v.s. protein intake) were calculated for small fish only. These values are shown in Table 3.3. The same trend was found for the two estimates. The highest protein utilisation efficiency was found for 24.94% protein diet fed at 2% bw/d followed by the 43.35% protein diet fed at the same feeding rate. The lowest value was recorded for 43.35% protein diet fed at 6% bw/d. PER and PPV were affected by protein percent of the diet ($p>0.05$) and feeding rate ($p>0.01$).

A negative linear relationship was found between feeding rate and PER for the 3 diets (Table 3.7, Fig. 3.6). There was no significant difference ($p<0.05$) between the 3 regressions suggesting that the effect of feeding rate on PER is the same regardless of the protein content of the diet. However, the 24.94% protein diet gave significantly higher ($p>0.05$)

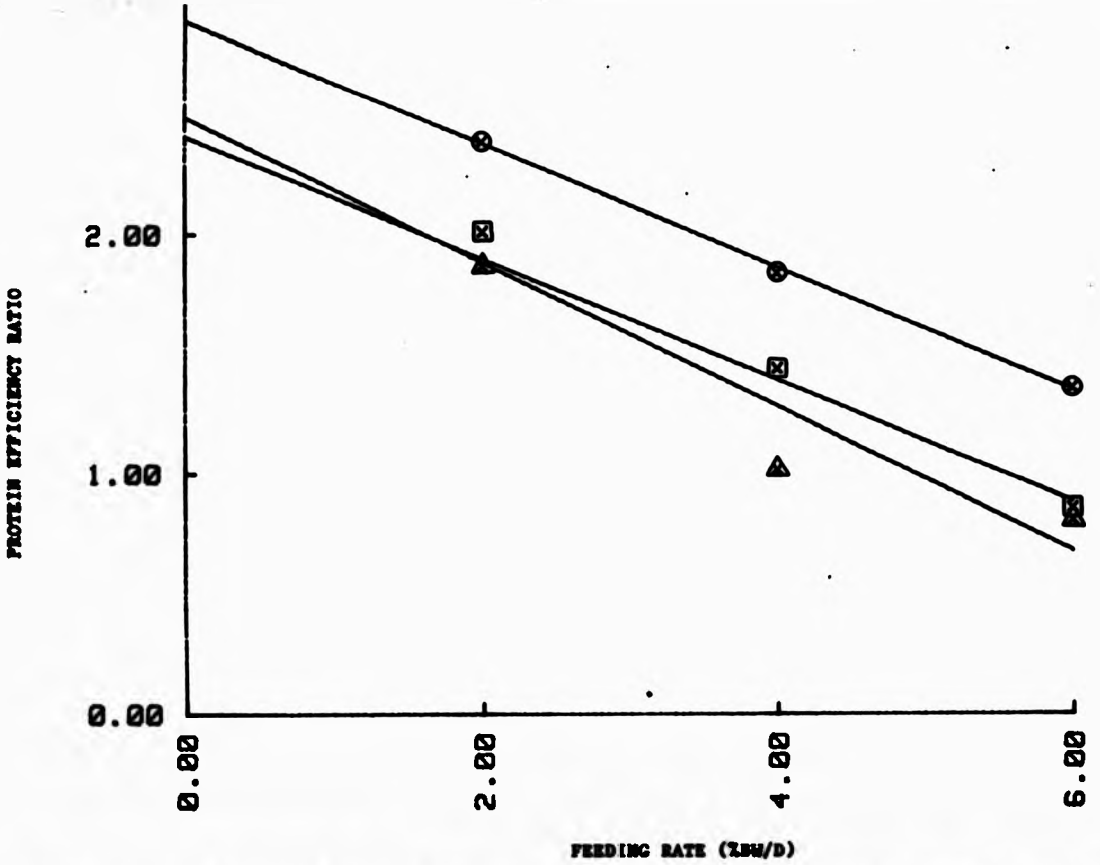


Figure 3.6 Relationship between feeding rate and protein efficiency ratio for small O.spilurus fed on 3 diets: 24.94% (O), 34.44% (□) and 43.35% (△) protein.

elevation than the other two diets suggesting that PER is higher with low protein diet.

Similarly a negative linear relationship was found between feeding rate and PPV for the 3 diets (Table 3.8, Fig. 3.7). Although there was no significant difference ($p<0.05$) between the slopes and the elevations of those 3 regressions there is a trend of smaller slopes with increasing protein content of the diet. This suggests that PPV is better with low protein diets and it is decreasing with increasing the protein content of the diet.

When the 9 feeding regimes were expressed as a protein intake, a negative linear relationship was found with both PER and PPV suggesting that the lower the protein intake the better protein utilisation.

(6) Digestibility:

The apparent protein digestibility of small O. spilurus for the 9 feeding regimes are shown in Table 3.3. These digestibilities were statistically not different at the $p=0.05$ level as indicated by the two-way factorial ANOVA without replication. The apparent protein digestibility for large O. spilurus was 29.60, 9.56 and 47.79 for 20, 30 and 41.25% protein diets respectively. No statistical analysis was carried out

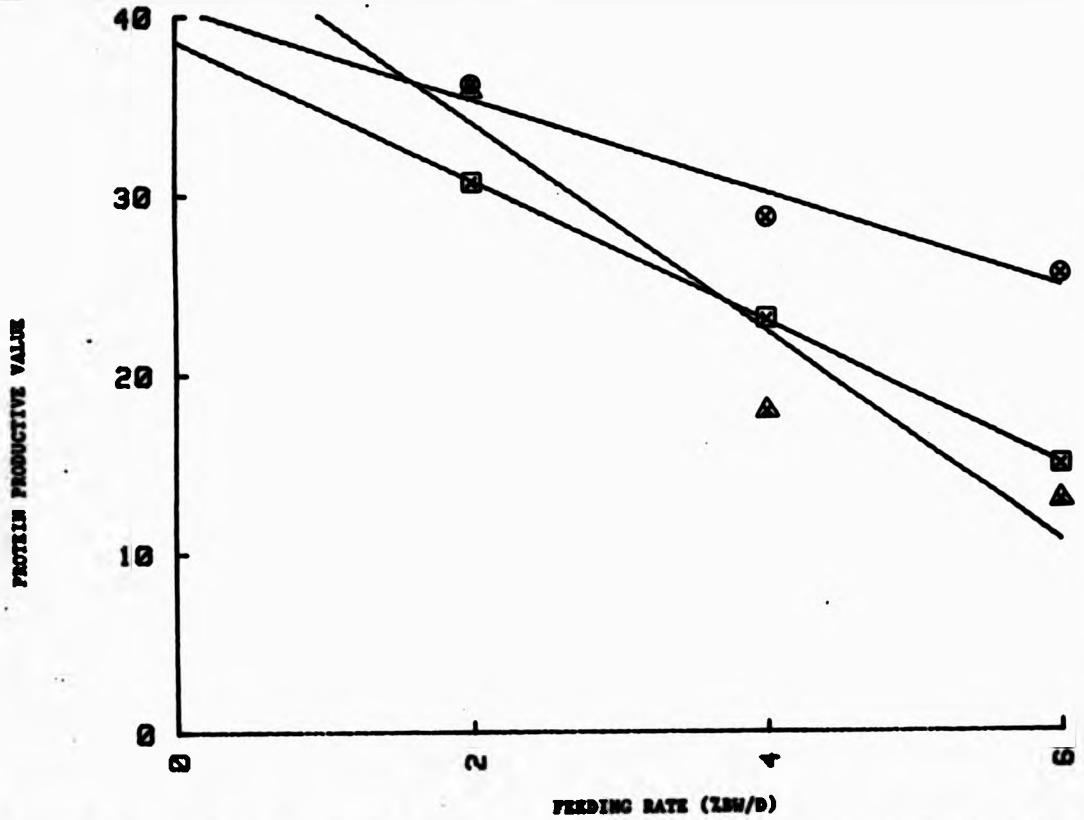


Figure 3.7 Relationship between feeding rate and protein productive value for small O.spilurus fed on 3 diets: 24.94% (⊗), 34.44% (⊠) and 43.35% (△) protein.

because these values represents only one replicate, as the small amount of faeces available did not allow replication.

(7) Body Composition:

The body composition on a percentage basis of small and large O. spilurus is shown in Table 3.9. Statistical analysis was carried out on the total change in body composition over the experimental period. Generally, there was little change in body composition in reference to food intake in either small or large O. spilurus. For small fish, protein and ash body contents show no significant ($p<0.05$) changes as a result of food intake. Lipid and moisture contents, however, appeared to be inversely related to the feeding rate. A positive linear relationship was found between body moisture content and feeding rate. Protein percent of the diet had no significant effect ($p<0.05$) either on lipid or on body moisture contents. The interaction between feeding rate and protein percent in the diet also had no significant effect on body lipid and moisture content.

For large O. spilurus neither protein level of the diet nor feeding rate induced significant ($p<0.05$) changes on body protein, or moisture content. However, feeding rates affected body lipid content sigaficantly ($p>0.05$), while body lipid content was not affected by

Table 3.9 Major constituents of fish tissue at the beginning and the end o the experiment.

Fish Size	Time of Analysis	Feeding Level % BW/D	Protein % in Diet	Moisture %	Major Components (% Dry)		
					Protein	Lipid	Ash
Small	Initial	---	---	71.87	15.65	6.05	5.20
	Final	2	24.94	71.37	15.42	6.29	4.75
			34.44	71.84	15.96	5.65	4.80
			43.35	72.03	16.86	6.17	5.01
		4	24.94	70.63	15.62	6.81	4.21
			34.44	71.03	15.85	6.67	4.23
			43.35	69.92	16.68	7.85	4.43
		6	24.94	67.85	17.45	8.10	4.70
			34.44	69.70	16.62	7.57	4.48
			43.35	69.61	15.80	8.44	3.83
		0	—	76.20	13.67	2.94	6.56
Large	Initial	-	—	73.18	15.09	8.74	2.95
	Final	0.5	20	69.59	15.25	8.29	4.42
			30	77.43	14.00	4.73	1.58
			41.25	74.23	14.28	7.14	2.03
		1.75	20	72.73	13.93	9.68	1.75
			30	74.13	15.28	7.32	1.57
			41.25	74.94	16.07	4.95	1.62
		3.0	20	71.85	13.29	9.32	2.15
			30	70.55	16.03	10.05	2.05
			41.25	70.83	15.20	12.56	1.63
		0	—	78.88	11.83	3.59	2.26

protein percent in the diet. Body ash content was significantly ($p>0.05$) affected by both protein level of the diet and feeding rate.

Total body moisture, protein, and lipid content of starved small O. spilurus decreased 7.94, 24.04 and 57.73%, respectively, while the body ash content increased 0.04%. Approximately the same trend was noticed in starved large O. spilurus. The total body moisture, protein and lipid content decreased 11.40, 13.22, and 66.25%, respectively. In contrast to the small fish the ash body content of large O. spilurus decreased 37.02%.

Discussion:

(1) Optimum Dietary Protein Requirement for Growth:

The criteria used for selecting the optimum protein requirement for growth were based on the presence of statistical differences among treatments. Other criteria for defining the optimum protein requirement such as FCR or PER will be discussed in a later section. The lowest protein intake that produces the statistically highest growth rate (SGR) was considered the optimum for growth. By the end of experiment 1 the 9 feeding regimes were divided into 2 groups. Diets containing 24.94 and 34.44% protein fed at 2% bw/d yielded statistically lower SGR than all

other feeding regimes. Among the other 7 feeding regimes higher growth rates were obtained with higher protein intake. The maximum SGR (1.61) was obtained with the maximum tested protein intake 26.01g/kg/d. However, these high growth rates were not significantly different by SNK test at 0.05 level. Therefore the lowest protein intake (8.67g/kg/d) among those 7 feeding regimes was considered the optimum level. This protein intake results from the 43.35% protein diet fed at 2% bw/d and produces an SGR of 1.33.

Using the method of Brett (1979) in which a growth/ration curve is drawn starting from zero ration, with a tangent is drawn from the origin (zero growth) passing through the point where the ratio of growth to ration is maximal, optimum ration was found approximately at 2% bw/d for all diets. The maximum growth obtained with this low ration was with 43.35% protein diet. This is the same optimum feeding regime selected by statistical analysis.

Tacon and Cowey (1985) reviewing the protein requirements in a fixed feeding rate regime, for different fish species and sizes (0.01-125g bw) recorded optimum protein requirement ranges from 52.5 to 9.21g/kg/d. Although the value obtained in this experiment (8.67g/kg/d) is lower than this range it fits

the linear relationship developed by Tacon and Cowey (1985) between daily protein requirement and the specific growth rate. However, Viola and Arieli (1982) studying the combined effect of protein percent of the diet and feeding rate on the growth rate of tilapia hybrid (O. niloticus and O. aureus, 120g bw) found that the maximum growth rate (2.25g/fish/d) was obtained with 29.9% protein diet fed at 3% bw/d which is equivalent to 8.97g protein/kg/d. Wange et. al. (1985a) found the optimum dietary protein level for O. niloticus (6g bw) for the maximum growth was 25% when the feeding rate is 3.5% bw/d (8.75g protein/kg/d). Those two values, although slightly higher, are very close to the results obtained in this experiment.

Feeding rate has a major role in defining the optimum protein requirement for growth in this study, while P:E ratio of the diet appeared to show only a secondary effect, mainly at a low feeding rate. Contrary to this conclusion Viola and Arieli (1982) found that both protein level and feeding rate have a highly significant effect on the growth rate of tilapia hybrids. The reason for this is probably the difference in feeding rates applied in the two experiments. The maximum feeding rate used by Viola and Arieli (1982) was 3% bw/d, at which low feeding rate the same conclusion could have been drawn from this experiment. Thus, at 2%

bw/d in this experiment protein level had a highly significant effect on growth rate, while at higher feeding rates (4 and 6% bw/d) protein content becomes unimportant. It must be noted that the maximum rations calculated from the growth-ration quadratic regressions were 5.41, 4.97 and 4.66% bw/d for 24.94, 34.44 and 43.35% protein diets, respectively. This probably indicates also that the protein percent of the diet becomes unimportant only at near maximum ration level. Furthermore, although at high feeding rates (>4% bw/d) protein content becomes less important it still appears that overall protein intake is the limiting factor for growth. The relationship between protein intake (% bw/d) and growth rate was significant indicating that the growth rate was a function notonly of the food consumed but mainly of its protein content.

Increasing protein level in the diet and feeding at low rates appears to be an advantageous approach to providing the optimum protein requirement. By contrast Viola and Arieli (1982) working with carp reported that at low feeding rates the additional protein in the diet was not used for growth, but served mainly as a source of energy, and thus it was unwise to try to save feed by using high-protein pellets at low feeding rates. Although the difference between the two species cannot be ignored a possible reason for this discrepancy is

that Viola and Arieli (1982) did not incorporate high enough protein in their diets (25-30% protein) and that low feeding rates were used (3-4% bw/d). Under such circumstances, low daily protein intake (g/kg/d) is probably limiting optimum growth.

(2) Effect of P:E Ratio on Growth-Ration Relationship:

The relationship between growth and feeding rate was similar for the three experimental diets. Growth rises sharply from zero ration to 2% bw/d, after which the increase moderates. With increasing ration a plateau of maximum growth rate occurs at the point of maximum ration. Brett (1979) reviewing the growth-ration relationship described the same pattern. It appears, therefore, that the P:E ratio of the diet does not itself affect the overall shape of the growth-ration curve.

However, the P:E ratio of the diet appears to cause a quantitative difference in the growth-ration curve. The maximum SGR obtained in experiment 1 was 1.59, 1.63 and 1.70 for 24.94, 34.44 and 43.35% protein diets, respectively. Thus, there is a positive linear relationship between maximum growth rate and protein level of the diet. There was also a negative linear relationship between P:E ratio of the diet and maximum feeding rate. The maximum feeding rate obtained was

5.41, 4.97 and 4.68% bw/d for 24.94, 34.44 and 43.35% protein diets respectively. Several papers have shown such a relationship; Wange et. al. (1985a) working with O. niloticus found a decrease in food consumption with an increase in the amount of dietary protein.

This inverse relationship between dietary protein level and feeding rate may reflect the difference in the metabolizable energy contents of these diets. Although it was originally planned to formulate isocaloric diets, this was calculated on a gross energy basis, and so differences in the metabolizable energy are possible. The carbohydrate source used in this study to produce isocaloric diets was dextrin. Jackson and Capper (1982) reported that dextrin is less than 95% digestible at an inclusion level of 50% for T.Zilli. They concluded that diets containing different ratios of dextrin will be non-isocaloric with respect to metabolizable energy and thus this probably explains the improved growth rates with higher protein levels when the fish were fed fixed food rations. As with different rations the fish have the chance to eat according to their metabolizable energy demand (Jobling, 1983) it would be concluded that the three diets used in experiment 1 in this study were non-isocaloric which, would explain the difference in the maximum food rations obtained with these diets.

(3) Food and Protein Utilization Efficiency:

FCR seems to be affected mainly by feeding rate. The higher the feeding rate the higher the FCR. This could be partly due to incomplete consumption of the food supplied at higher feeding rates. However, the higher FCR might reflect also higher gut evacuation rate. Pandian and Vive Kanandan (1985) reported that the gastric evacuation rate increased in proportion to the feeding rate.

On the other hand P:E ratio seems to have a significant effect on FCR only at low feeding rate, at which the higher the P:E ratio the better the FCR. This could be due to higher digestibility of high protein diets (Tacon and Cowey, 1985). The best FCR obtained in experiment 1 was 1.09. This FCR was obtained with a high protein diet (43.35%) fed at low ration (2% bw/d). This feeding regime also produced the optimum growth rate.

One of the main aims in fish culture is to produce PER and PPV values which are as high as possible (Knights, 1985). The highest PER and PPV values recorded in experiment 1 were 2.38 and 35.95, respectively. These values were, however, obtained with low protein intake and subsequently low growth rate, while the optimum growth rate was obtained with PER and

PPV values equivalent to 2.01 and 36.14, respectively. Lower values were obtained by Jauncey (1982) with O. mossambicus fed on different P:E ratio diets at a fixed ration, while higher values were recorded by Wange et. al. (1985a, b) with O. niloticus fed on different P:E ratio diets at different rations. It seems, therefore, that the high PER and PPV values obtained in this study and in Wange's et. al. (1985a, b) studies is due to incorporating different feeding rates. Feeding rate seems to have a highly significant effect on protein utilisation efficiency; the lower the feeding rate the higher the PER and PPV.

The P:E ratio of the diet has a secondary effect on the PER and PPV values. Generally, the higher the P:E ratio the lower the protein utilisation efficiency. This could be due to the fact thatprotein energy substitutes the reduction in the non-protein energy sources. However, at low feeding rate a very good PER (2.01) and PPV (36.14) were achieved with 43.35% protein diet (P:E ratio = 89.9mg protein Kcal) which are higher than the PER (1.82) and PPV (30.67) obtained with 34.44% protein diet (P:E ratio = 70.7mg protein/Kcal). Jackson and Capper (1982) suggested that energy provided by protein catabolism leads to improved growth at high protein levels, which could explain the good PER and PPV obtained with high P:E ratio.

(4) Body Composition:

Over the period of the trial the gross body composition was not greatly affected by changing dietary protein level of the diet but was affected by feeding rate, which affected fat and moisture content but not protein or ash, in small O. spilurus carcasses. Similarly, Reinitz (1983), working with rainbow trout reported that diet affected fat and moisture but not protein or ash. Body water and lipid levels appeared to be inversely related as has been noted with O. mossambicus (Jauncey, 1982). There was no abnormal fat deposition in small O. spilurus, suggesting a good P:E ratio in the test diets. Fat deposition with unbalanced P:E ratios was frequently observed in fish nutrition studies (Millikin, 1982).

It should be noted, however, that the observed changes in fish body composition can not be simply related to feeding regime only. It has been mentioned earlier that the higher the feeding rate the higher the growth rate. This means that at the end of the experiment the fish fed on high ration will be "bigger" than those fed on small ration. Therefore, comparing the body composition of these fishes will reflect not only their different feeding history but also their different body sizes. Hogendoorn et. al. (1983) concluded that in experiments using a single size group,

the effect of feeding on body composition can not be evaluated because feeding level and body weight are confounded.

(5) Digestibility:

Although there is an increasing trend in the apparent digestibility with increased protein content of the diet, this is not statistically significant. Feeding rate also seems to have no significant effect on apparent digestibility although there was an increasing trend with decreasing feeding rate.

In this experiment stripping was used to collect the faeces. This method introduces a stress to the fish and might force the fish to void the fecal matter or intestinal contents. However, the other methods available for fecal collection that do not interfere with the normal activity of the fish such as fecal settling or trough netting might be not suitable in sea water. Ferraris et. al. (1986) noticed salt contamination of sample from sea water milkfish, which was not shown in the fresh water samples. Thus, it seems at present that there is no optimum method for fecal collection in sea water. This point requires further research.

It is realised that manual stripping may lead to the collection of incompletely digested food resulting in an underestimation of digestibility (Talbot, 1985). The apparent protein digestibility obtained in this study appeared to be lower than that reported by Jauncey (1982) for fish meal in spite of the fact that in both studies fecal matter was collected by manual stripping. The very good growth rate of O. spilurus with low protein intake suggests a good protein digestibility. Al-Ahmad et. al. (1986) found that O. spilurus in sea water are more prone to stress caused by handling. Therefore, the low apparent digestibility observed in this study could be due to stress caused by manual stripping in sea water.

(6) Growth of Large O. spilurus:

The large O. spilurus used in experiment 2 failed to grow. The condition of the fish before the experiment was poor, as evident by the poor performance of the fish during the second overwintering period. Conditions were expected to improve under the environment and feeding conditions provided by the experiment, but no such improvement was noticed within the first 1.5 months of the experiment. The experiment was continued because of the unavailability of other stock of fish. This failure in growth of large O. spilurus raises three possibilities; the requirements

of the fish were not met, the fish were stressed, or the fish were physiologically impaired by earlier conditions.

It must be noted that the same feed ingredients were used for small fish as for the large ones. The results obtained from the small O. spilurus indicated that the feeds formulated from those ingredients were utilised and efficient in promoting fish growth. However, a different batch of ingredients was used for formulating the feeds of large O. spilurus. Differences in the nutrient availability between the two batches are possible (Millikin, 1982; Michael Kew, pers. comm.).

In order to evaluate the availability of these diets a confirmatory test was carried out using a stock of fish which became available later. These fishes were grown up to 200-250g bw before use, to avoid any size effect. In this test only one diet of the three experimental diets (41.25% protein) was used, fed at 2% bw/d in duplicate (10 fish/500l tank) and compared with a control commercial diet of 40% protein. The experiment was performed for 2.5 months.

The results show that those fishes fed on the test diet were able to grow comparably with those fed on the commercial diet and there was no significant difference

in the growth rate between the two. It was thus concluded that the test diets used to feed the large O. spilurus in experiment 2 were not at fault, but that the problem lay in the fish themselves.

The failure of those fishes to grow probably indicates that they were stressed, possibly from early exposure to high salinity which might have had a comulative negative effect on the fish growth, becoming particularly apparent only in large fish. This would tend not to be supported by the results obtained on the same species in Kuwait, which generally show very good growth rate of large O. spilurus in sea water in both sea cages and tanks.

Although there was no disease detected with these fishes, the problem of pathological stress can not completely be ignored. Another factor may have been that the large O. spilurus were the same as those used in the first trial the year before. During the first experiment the fish were sampled every two weeks, during which the collected fry and eggs were thrown away. This procedure probably causes the fish to spawn more frequently than normal. By the end of the trial, the end of the summer season prevented any chances for the stock to overcome the exhaustion caused by overspawning.

The sampling procedure is similar to the weekly fry collection procedure practiced at KISR. In both procedures, the fry and the eggs are removed from the parent's tank. Similar effects have been noticed on the growth rate of O. spilurus spawners over a two year period at KISR. Al-Ahmad et. al. (1986) showed that both small and large spawners in brackish and sea water managed to spawn and grow during the first spawning year (May - October), but when used in the following year, spawning potential decreased with almost negligible growth during the 5 months spawning period.

CHAPTER 4: The Effect of Protein Intake on Ammonia Excretion of *O.spilurus* in sea water.

Introduction:

The estimation of ammonia excretion rate can provide valuable information for more than one field in aquaculture. Since ammonia originates mainly from protein metabolism (Vaarde, 1983) its measurement can be useful in explaining protein utilisation, particularly as a measure of the balance between protein and energy contents of the diet. Brafield (1985) reported that the amount of energy lost in nitrogenous excretion, including ammonia, is modest (approx 7%) but significant, and account must be taken of it when compiling energy budgets. Finally, since ammonia is toxic to fish, Meade (1985) reported that although many factors affect ammonia toxicity the effect of ammonia depends largely on exposure, which is a function of excretion or ammonia production by the fish themselves.

Ammonia excretion is influenced by many factors: as ammonia originates mainly from protein metabolism, any factor that affects protein metabolism and/or fish metabolism would affect ammonia excretion rate. Nevertheless, Paulson (1980) studying the effect of protein intake, temperature, and fish size on ammonia output in brook and rainbow trout found that nitrogen consumption was by far the most important factor influencing ammonia excretion, followed by fish size.

Table 4.1 summarises some of the available literature on the effect of protein intake on ammonia output from which two major approaches can be identified; manipulating feeding rate and changing dietary protein level. From this table it appears that most of the feeding rate studies were performed with natural diets and only limited information was available on the effects of formulated feeds. On the other hand, the effect of dietary protein level on ammonia excretion was usually studied in protein sparing studies. The combined effect of feeding rate and protein content of the diet was studied only by Beamish and Thomas (1984) with rainbow trout, while the effect of body weight on ammonia output was studied for rainbow trout, bream, and seabass.

There is little available data on the effect of protein intake or body weight on ammonia excretion rate for tilapia. The objective of this study is, therefore, to define the effect of both feeding rate and protein: energy ratio of the diet on ammonia excretion rate for two sizes of O. mpilurum held in sea water.

In addition to its effect on ammonia output feeding rate in particular has a significant contribution on other important metabolic parameters such as oxygen consumption (Jobling, 1981b).

Table 4.1 Some experimental methodologies used for studying ammonia excretion as a function of food.

SPECIES	TANK COVER	TANK SIZE	WATER FLOW	FISH SIZE	OTHER PARAMETERS MEASURED
Atlantic cod	covered	175L	1 hr closed : 3 hr open	256-482g	oxygen
Sockeye salmon	covered	73L	3 hr closed : 3 hr open	28.6g	oxygen and urea
Rainbow trout	covered	10 cm dia. 36cm length	80-120ml/min	250-550g	urea, urine, faces
Rainbow trout	open	6L	static	-	-
Rainbow trout	open	700L	1hr closed : 1 hr open	-	-
Rainbow trout	open	40 x 48 L	static	60g	Urea, faces
Rainbow trout Common Carp	open	40L	2L/min	130-350g	Urea
Rainbow trout Brook trout	open	178L	static	15-360g	-
Perch	covered	10 dm^3	2.5dm^3/hr	29-78g	Urea, amino acids, oxygen
Bream	open	10L	-	8-34g	Urea
Gilthead Seabream	open	190L	static	3-90g	Total dissolved nitrogen, total oxidised nitrogen, urea
Atlantic cod	open	336L	70L/hr	199g	-

Table 4.1 Continued

SPECIES	FEED TYPE	FEEDING LEVEL	FEEDING FREQUENCY	PRE-FASTING DAY	REFERENCE
Atlantic cod	Pellets	113KJ/Kg	once	yes	Laid and Bratten (1984)
Sockeye salmon	Oregon moist pellets	3%BW	once	yes	Brett and Zala (1975)
Rainbow trout	Pellets, 35 and 49% protein	0.5-1.0%BW	once	yes	Beamish and Thomas (1984)
Rainbow trout	Pellets	ad.libitum	once	yes	Rychly and Marina (1977)
Rainbow trout	Oregon moist pellets	satiation	once	yes	Ming (1985)
Rainbow trout	Gammarus pulex	10-100% of maximum ration	-	yes	Elliot (1976)
Rainbow trout Common carp	Pellets	0 - satiation	once & twice	no	Kaushik (1980)
Rainbow trout Brook trout	Invertebrates Insects Zooplanktons	2.5 & 5% BW	once	yes	Paulson (1980)
Perch	G.pulex	-	-	yes	Solomon and Brafield (19
Bream	Tubifex sp.	5-10% BW	once	yes	Tatrai (1981)
Gilthead Sea Bream	Pellets	5-1.4% BW	once	no	Porter et. al. (1987)
Atlantic cod	Pellets	0.5-4.1% BW	once & twice	no	Rasmarine et. al. (1987)

Furthermore, urea, the second major nitrogenous waste (Vaarde, 1983) might be affected also by protein intake. Estimation of these parameters and correlating them with food input might add a useful contribution to understanding the effect of food on fish metabolism as a whole, and in turn on the overall protein intake/metabolite output/growth relationships typical of intensive aquaculture.

Materials and Methods:

Overall methodology was similar to that described in Chapter 2. Table 4.2 summarizes some of the experimental conditions.

(1) Fish Stock:

The small and large O. spilurus used in this study were the same batch used in the growth experiment (Chapter 3). The size of the smaller fish ranged between 50.93 to 110.93g bw, while it was between 271.18 to 310.78g bw for the large ones. Three different stocking densities; 20, 15 and 7 fish/tank were used with small fish fed on 2, 4 and 6% bw/d respectively. As feeding rate was found to have a significant effect on oxygen consumption rate, this was necessary for maintaining a constant oxygen level in the tanks over the very wide range of feeding rates used. In order to maintain a minimum acceptable oxygen level (3mg/l) with

Table 4.2 Summary tables for the experimental conditions.

Fish Size	Feeding rate %BW/D	Protein %	Fish number /tank	Fish size (g)	Water flow rate L/min,/tank
Small	0.00	0.00	20	83.64	3.52 ± 0.20
	2	24.94	20	50.93	3.46 ± 0.10
		34.44	20	59.70	3.69 ± 0.07
		43.35	20	56.12	3.33 ± 0.09
	4	24.94	15	85.69	3.30 ± 0.23
		34.44	15	110.93	3.73 ± 0.23
		43.35	15	97.16	3.35 ± 0.23
	6	24.94	7	89.00	3.57 ± 0.27
		34.44	7	105.88	3.42 ± 0.13
		43.35	7	87.75	3.53 ± 0.14
Large	0.00	0.00	10	301.56	3.48 ± 0.19
	0.5	20.00	10	297.63	3.57 ± 0.17
		30.00	10	310.78	3.66 ± 0.15
		41.25	10	303.25	3.50 ± 0.13
	1.75	20.00	10	275.94	3.38 ± 0.20
		30.00	10	305.15	3.50 ± 0.16
		41.25	10	303.50	3.61 ± 0.19
	3.0	20.00	10	303.23	3.59 ± 0.22
		30.00	10	298.93	3.49 ± 0.17
		41.24	10	271.18	3.44 ± 0.17

all feeding rates it was necessary to adjust either the water flow rate or fish biomass. It has been mentioned in Chapter 1 that fish activity, which is affected by the water flow rate, makes a significant contribution to ammonia excretion rate. On the other hand, the effect of stocking density on ammonia excretion rate is not yet clear. Therefore, in the absence of a clear relationship between stocking density and ammonia excretion rate and with the presence of a clear relationship between ambient oxygen concentration and flow rate with ammonia excretion rate, it was decided to adjust the stocking density to maintain a constant oxygen level.

(2) Food and Feeding:

The same feeding regimes used with small and large fish in Chapter 3 were used in this study. The fish were allowed to acclimatize to the experimental feeding regime 10 days before the measurements were taken. They were fed 3 times a day at 8, 12 and 16 hr and on the day of water quality measurement each meal was weighed individually.

(3) Holding Tanks:

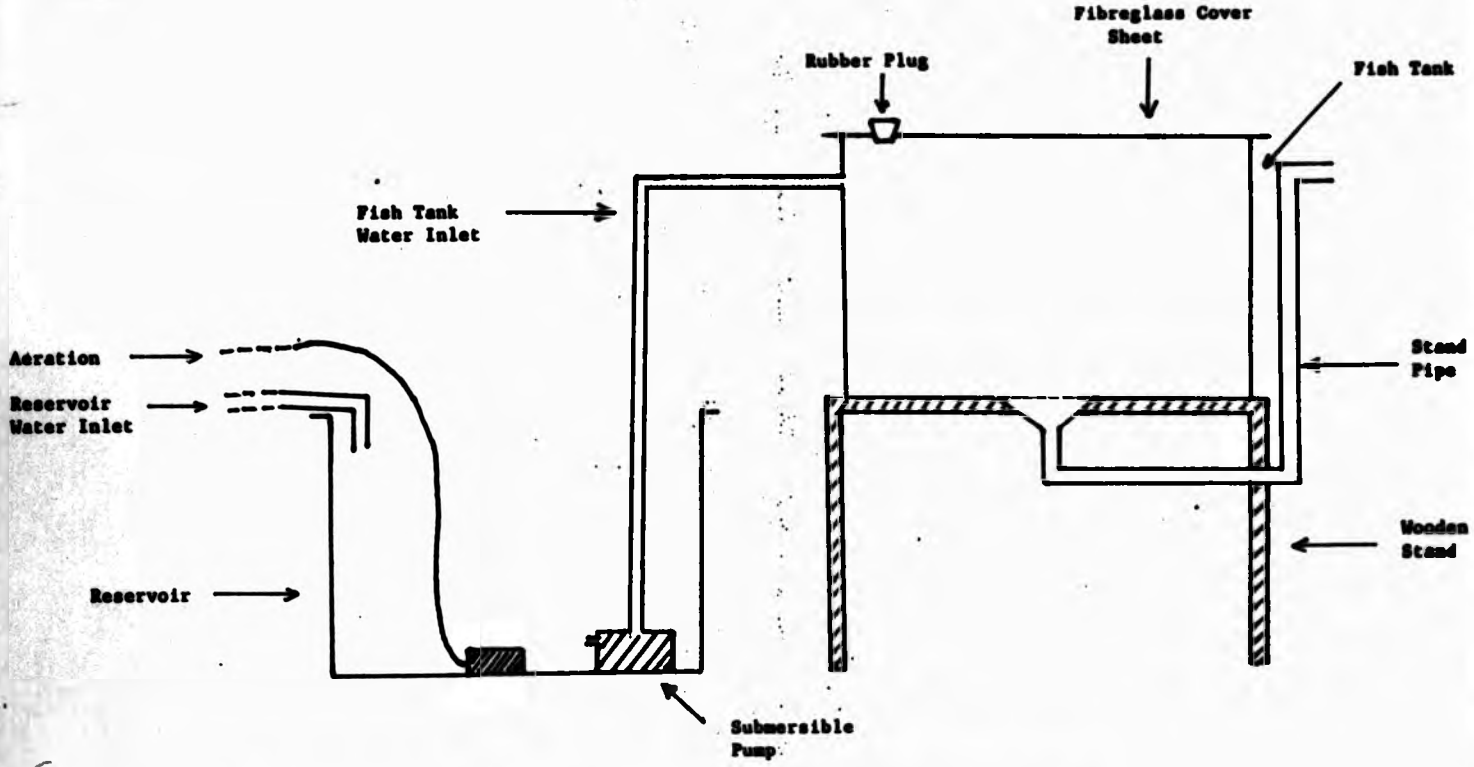
Three 500l EVOS tanks were covered with transparent 2mm thick fibreglass sheets, bolted around the tank edges. Rubber strips were fitted between the

tanks and the sheets as a water and air sealant. A small hole of 3cm diameter was made in one corner of the cover, serving for fish observation, feeding, and emergency aeration. During the sampling day this hole was closed with a rubber stopper. Another hole of approximately the same size was made on the upper end of the tank. To this hole a coupling was fitted which was used for the water inlet. Fig. 4.1 shows the basic tank design used with small O.spilurus.

For the larger fish the same system was applied but without using the tank covers. Six uncovered tanks were prepared for experiment 2.

(4) Water Flow System:

In order to have a constant and controlled water flow rate a fixed speed small aquarium pump was used for water inlet. To stabilize inlet water pressure another 500l fibreglass tank was used as a constant head reservoir. This reservoir was supplied with vigorous aeration provided by air stone. This was the only means of aeration used. The pumped water flowed through a small hose to the tank. The 2" overflow pipe was used as usual except that a 1" elbow was fixed to the pipe end. To fill the tank completely with water the stand pipe was elevated and the water was allowed to overflow from the tank edges. This was done to prevent air



Fif 4.1 Basic tank design used in ammonia excretion experiments with small O.spilurus.

bubbles being trapped between the water surface and the covers. After 2-3 minutes the screws were closed tightly, the small observation hole was covered and the water was allowed to drain from the stand pipe which was kept at a fixed position. This method of tank filling was not necessary for the uncovered tanks used in experiment 2. The water flow rate in both experiments was measured every two hours during the sampling day from the outlet stand pipe by measuring the time required to fill a 1l measuring cylinder and was found to be about 3.5l/tank/min. (Table 4.2)

(5) Experimental Protocol:

Since only 3 tanks were prepared for experiment 1 with small O. spilurus, each tank was assigned for a treatment, so that within each sampling day only 3 feeding regimes were tested. The feeding regime was selected on the basis of feeding rate, so that within each sampling day the 3 groups of fish received one feeding rate but 3 different diets. To test another feeding rate the same diet was fed to each group and only the feeding rate was adjusted (Table 4.3). For large O. spilurus in experiment 2 the same experimental protocol was followed except that each feeding regime was applied to two replicate tanks.

Table 4.3 Summary for experimental protocool used with small O.spilurus.

Tank No:	1	2	3
Feed Type (protein %)	24.94	34.44	43.35
Feeding Rate (1st sampling day):	2%	2%	2%
(2nd sampling day):	4%	4%	4%
(3rd sampling day)	6%	6%	6%

Note: Ten days separates the sampling day from the next one.

(6) Fish Measurements:

Total fish weight was measured with the initial stocking to the tank, ie, 10 days before sampling day. The total weight was measured again 3 days after the water sampling day and the total fish weight on the sampling day was calculated from these two measurements.

(7) Water Sampling:

The tanks were filled and sealed one day before water sampling. With small O. spilurus water sampling started from 6 am and continued every 2 hrs until 6 am the following day, while with large fish the water sampling started from 6 am and continued every 2 hrs also until 10 pm of the same day. The ammonia excretion and oxygen consumption rates between 10 pm and 6 am was

calculated as the mean of those two values. With small fish another sampling day was taken for ammonia analysis only from 8 am until 6 pm only to check day to day variation. In all cases 3 samples for each parameter were measured at each sampling time. To calculate the amount of ammonia, excreted urea and oxygen consumed at any time the following formula was used as suggested by Kaushik (1980):

$$E_t = V_o . C + C_t . V$$

where, V_o = volume of water in the tank

C = variation in ammonia-nitrogen ($C_1 - C_{1-t}$) concentration

C_t = mean of ammonia-nitrogen concentration between two intervals ($C_1 + C_{1-t}/2$)

V = flow water/unit of time

t = unit of increment in time (2hr)

E_t = ammonia-nitrogen excreted by fish per unit time retained

The mean value of 3 water replicates was calculated first for each 2hr period, and then the average daily mean was used for statistical analysis.

Results:

A. Ammonia Excretion:

(1) Effect of Protein Percent of the Diet on Ammonia Excretion Rate:

Protein level of the diet significantly affects ($p>0.01$) ammonia excretion rate in both small and large O. spilurus. Ammonia excretion rates for both sizes are shown in Table 4.4. The orthogonal polynomial contrast ANOVA test shows that the effect of protein percent of the diet on ammonia excretion is linear for both sizes. This means that at constant feeding rate the higher the protein content of the diet the higher the ammonia excretion rate.

This linear relationship is summarised in Table 4.5 and shown in Figs 4.2 and 4.3. The negative values of b_o indicates the endogenous ammonia excretion rate at starvation which represents a fish in a negative nitrogen state. The zero ammonia excretion rate of this linear relationship does not represent a condition of no ammonia excretion because, in practice, this is not true. However, it represents a fish in a balanced nitrogen state, where protein catabolism, and subsequently ammonia excretion, required for maintenance purposes is drawn from exogenous dietary protein. An alternative procedure is to plot ammonia output against food intake as it is measured in the fish tanks. With

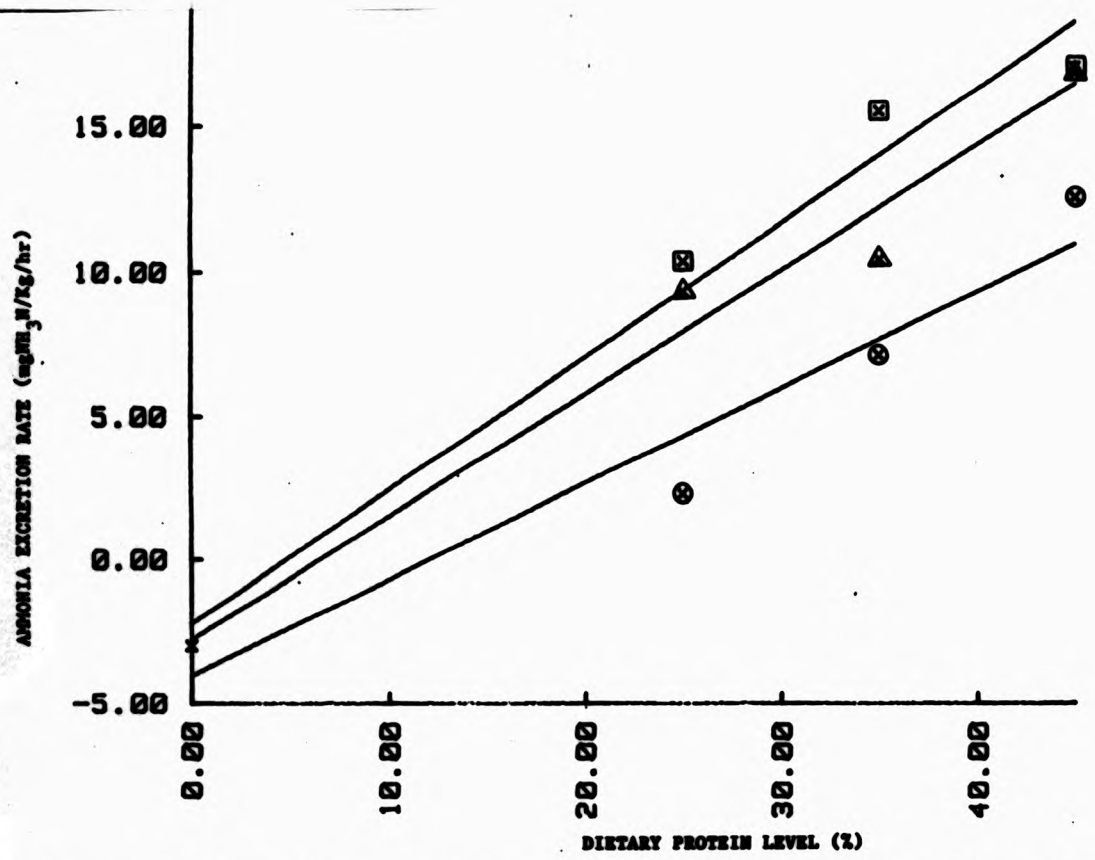


Figure 4.2 Relationship between ammonia excretion rate and dietary protein level for small *O.spilurus* fed at 3 feeding rates: 2 (○) 4 (□) and 6 (△) %BW/D.

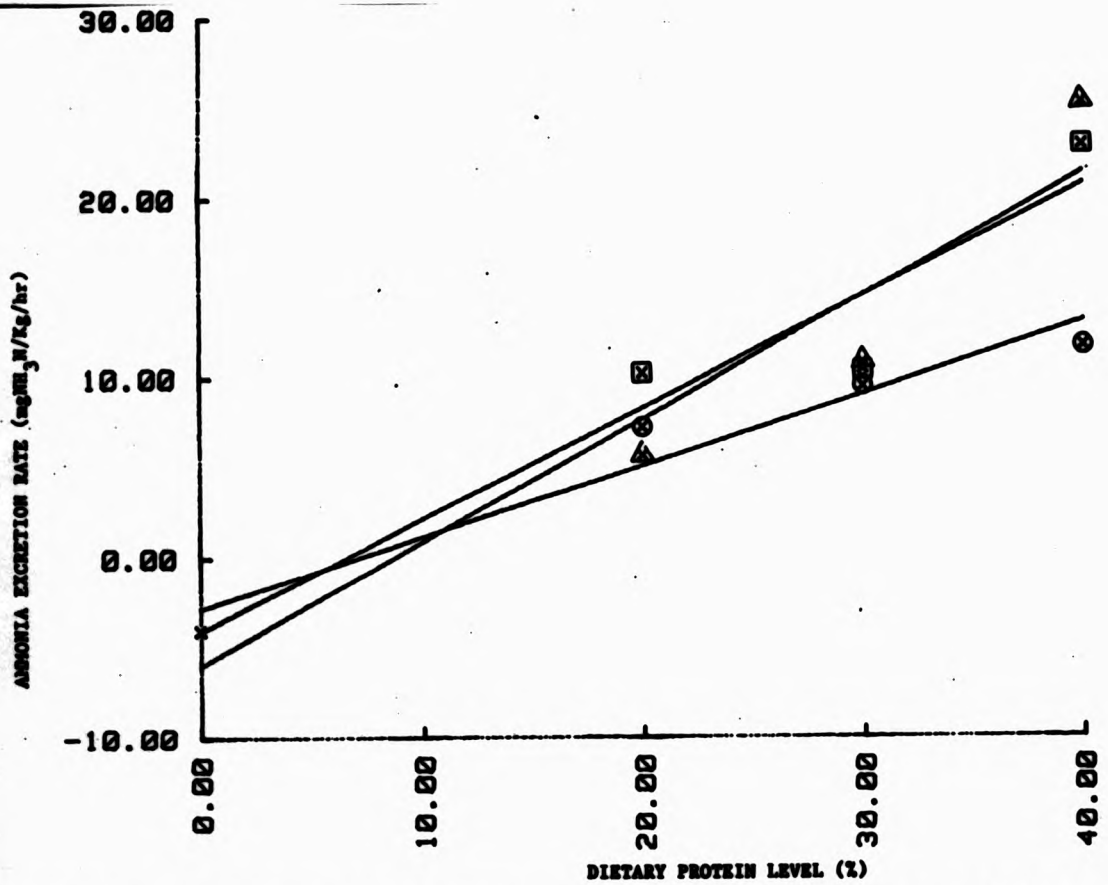


Figure 4.3 Relationship between ammonia excretion rate and dietary protein level for large *O.spilurus* fed at 3 feeding rates: 0.5 (○), 1.75 (□) and 3 (△) %BW/D.

this procedure neither negative nor zero ammonia outputs will be plotted. However, this procedure does not differentiate between the source of the excreted ammonia whether endogenous or exogenous. Therefore, the first procedure was adopted in this study.

The analysis of covariance suggests that the 3 slopes for the 3 regression equations representing the 3 feeding rates are not equal at 0.05 level for the two sizes, and therefore a common regression slope cannot be computed. The Tukey test shows that the regression slope for the lowest feeding rate (2% for small and 0.5% for large size fish) is significantly lower than the other two slopes, between which there was no significant difference. There was a high correlation ranging from 0.96 to 0.99 between ammonia excretion rate and protein percent of the diet for the two sizes.

(2) Effect of Feeding Rate on Ammonia Excretion Rate:

The effect of feeding rate on ammonia excretion rate for small O. spilurus was linear ($p>0.25$) but with some curvature ($p>0.025$). The relationship for the 3 experimental diets is explained by a quadratic (second degree polynomial) regression. The regression components are summarised in Table 4.6 and shown in Fig 4.4. The negative values of b_0 indicate the endogenous ammonia excretion at starvation. The 3 regression

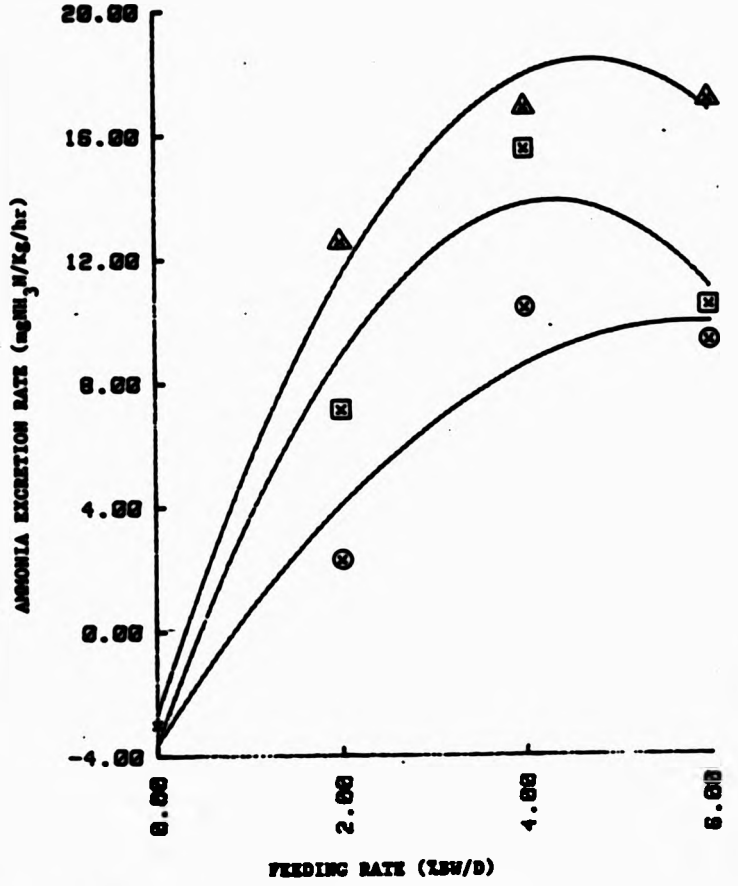


Figure 4.4 Relationship between ammonia excretion rate and feeding rate for small *O.spilurus* fed on 3 diets: 24.94% (○), 34.44% (□) and 43.35% (△) protein.

Table 4.4 Effect of feed on metabolites production in O.spilurus.

Fish Size	Feeding Rate %BW/D	Protein %	Ammonia excretion mgN/Kg/D	Oxygen consumption gO_2/Kg/D	Urea excretion mgN/Kg/D
Small	0.00	0.00	71.52	3.83	-
	2	24.94	54.84^{a}	6.11^{a}	28.80
		34.44	171.17^{b}	6.26^{a}	130.92
		43.35	301.18^{bc}	8.13^{a}	124.73
	4	24.94	249.50^{b}	8.73^{a}	99.77
		34.44	372.65^{c}	8.25^{a}	126.34
		43.35	405.24^{c}	7.99^{a}	107.33
	6	24.94	223.78^{b}	12.48^{b}	256.97
		34.44	251.30^{b}	11.94^{b}	221.98
		43.35	410.18^{c}	11.65^{b}	96.14
Large	0.00	0.00	97.20	1.92	
	0.5	20.00	174.00^{ab}	1.86^{a}	
		30.00	228.00^{bc}	2.43^{ab}	
		41.25	279.84^{c}	1.91^{a}	
	1.75	20.00	245.52^{bc}	2.53^{ab}	
		30.00	250.56^{bc}	3.37^{b}	
		41.25	547.68^{d}	3.27^{b}	
	3.10	20.00	136.56^{a}	3.24^{b}	
		30.00	258.48^{bc}	3.04^{b}	
		40.00	604.32^{d}	3.17^{b}	

Note: same letters are not significantly different at 0.05 level.

Table 4.5 Ammonia excretion rate, mg/Kg/hr(Y) as a function of protein percent of the diet (X).

Fish Size	Feeding Rate %BW/D	b_0	b_1	Standard error of Estimate	r	R^2
Small	2	-4.024	0.334	1.985	0.97	0.94
	4	-2.207	0.463	1.879	0.99	0.97
	6	-2.772	0.428	1.640	0.99	0.97
Large	0.5	-2.854	0.398	2.017	0.97	0.94
	1.75	-4.096	0.620	3.515	0.97	0.93
	3.0	-5.976	0.683	4.271	0.96	0.92

Table 4.6 Ammonia excretion rate, mg/Kg/hr(Y) as a function of feeding rate, %BW/D (X) for small O.spilurus.

Protein % of diet	b_0	b_1	b_2	Max,Y	MaxX	Standard Error of estimate	r	R^2
24.94	-3.601	4.638	-0.397	9.94	5.84	2.685	0.92	0.94
34.44	-3.586	8.136	-0.949	13.85	4.28	2.619	0.81	0.96
43.25	-2.646	8.984	-0.959	18.40	4.68	1.584	0.88	0.99

Table 4.7 Ammonia excretion rate, mg/Kg/hr(Y) as a function of protein intake g/Kg/D(X).

Fish Size	b_0	b_1	b_2	Standard error of estimate	r	R^2
Small	-2.615	1.630	-0.037	3.359	0.81	0.79
Large	3.463	1.563	-	5.813	0.75	0.56

curves show a positive linear relationship between ammonia excretion rate and feeding rate up to 4% bw/d. At higher feeding rate (6% bw/d) it seems that there is a declining trend in ammonia excretion. This is evident with the negative value of b_2 for all the 3 regression curves.

With such a quadratic regression it is possible to calculate the maximum value of Y with its corresponding value of X. These values for the 3 regressions are shown also in Table 4.6. This indicates that there is a positive relationship between protein percent of the diet and the maximum ammonia excretion rates. It also indicates that this maximum ammonia excretion rate is achieved at lower feeding rates with high protein diets.

Similarly the factorial ANOVA test shows that feeding rate has a significant effect ($p>0.025$) on ammonia excretion for large O. spilurus (Table 4.4). However, the orthogonal test fails to demonstrate whether the effect is linear or quadratic. Actually the relationship from zero ration up to 1.75% bw/d seems to be linear for all diets. However, the conflicts come at the highest food ration. With the low protein diet (20%) there was a reduction in ammonia excretion at a rate of 3% bw/d, whereas with the medium protein diet (30%) ammonia excretion rate did not increase with the

increase of feeding rate from 1.75 to 3% bw/d, suggesting that a plateau has been reached. However, with high protein diet (41.25%) the linear rise in ammonia excretion noticed from zero ration up to 1.75% bw/d was continued up to 3% bw/d.

(3) Combined Effect of Protein Percent of the Diet and Feeding Rate on Ammonia Excretion Rate:

Multiple linear regression equations were formulated for small and large O. spilurus to show the simultaneous effect of the two experimental factors on ammonia excretion rate. Only those factors which have a significant affect by the orthogonal polynomial contrast ANOVA test were included in the regressions (Table 4.8). For small O. spilurus it seems that feeding rate has the major effect on ammonia excretion rate, whereas protein level of the diet has only a small contribution. For large O. spilurus, however, feeding rate was not included in the multiple regression because it was not possible to define its effect, whether linear or quadratic. This is probably the reason for the fairly low R^2 value.

(4) Effect of Protein Intake on Ammonia Excretion Rate:

When the 9 feed combinations were expressed as a protein intake (g/kg/d) two different relationships were

found for the two sizes. For small O. spilurus a quadratic regression explains the relationship whereas for large fish it was linear. The regression components are shown in Table 4.7 and Figs 4.5 and 4.6. The correlation between protein intake and ammonia excretion was not very high although it was higher for small fish (0.81) than for large (0.75). This probably reflects the difference in the mode of action between protein level of the diet and feeding rate on ammonia excretion rate.

(5) Diurnal Pattern of Ammonia Excretion:

The diurnal pattern of ammonia excretion for small O. spilurus is shown in Figs 4.7-4.16. Generally the effect of feeding rate on the diurnal ammonia excretion pattern for small O. spilurus was more pronounced than the effect of protein level of the diet. However, the effect of protein percent of the diet on the diurnal ammonia excretion pattern was evident at the lowest feeding rate (2% bw/d). Table 4.9 summarised some of the most important characteristics of the daily ammonia excretion pattern such as peak ammonia excretion rate, time of peak appearance, and duration of the peak for the two sizes.

For small O. spilurus and at 2% bw/d there was a small peak of 15mg NH_2N/kg/hr starting after the morning

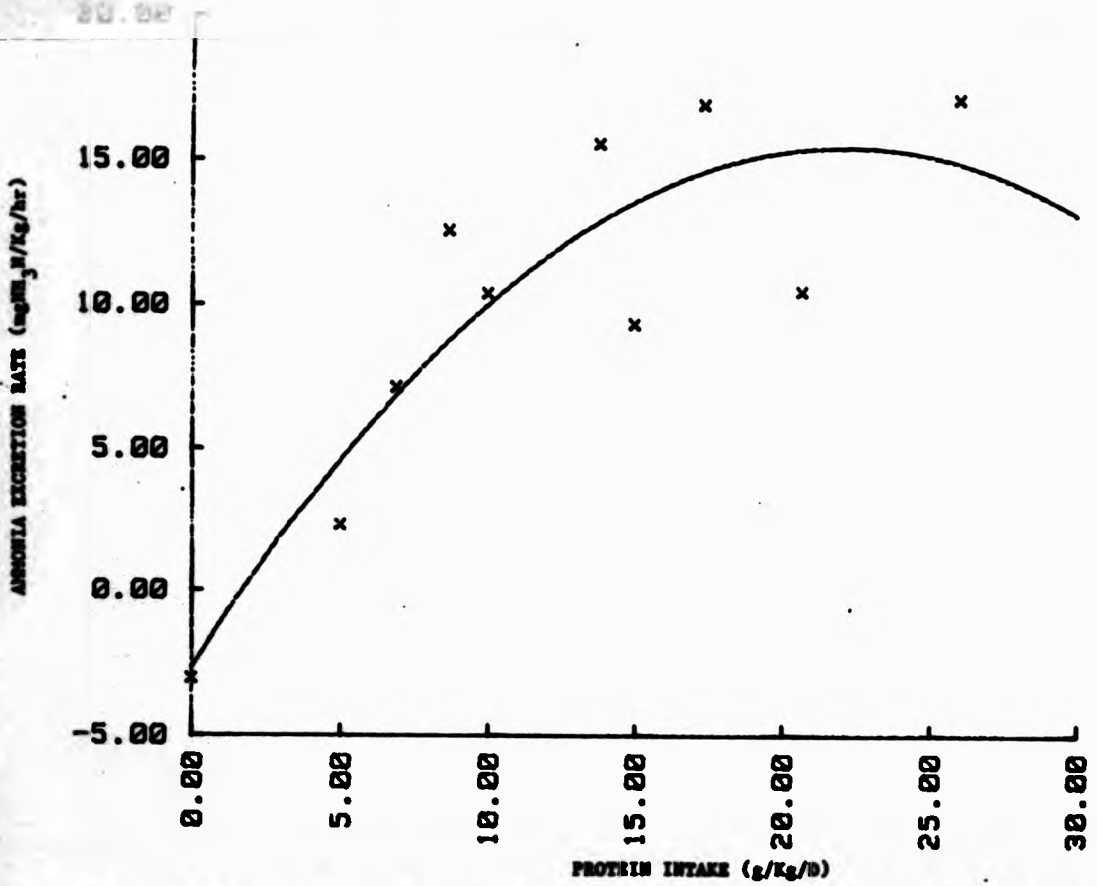


Figure 4.5 Relationship between ammonia excretion rate and protein intake for small O.spilurus.

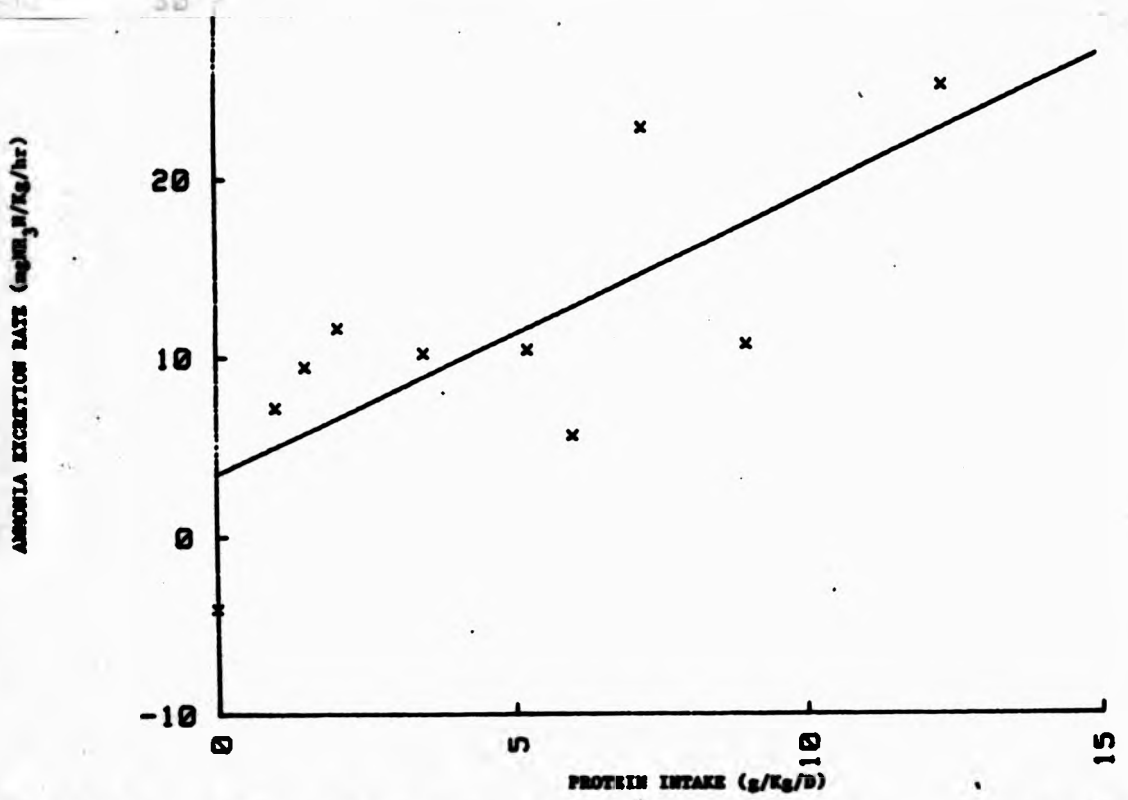


Figure 4.6 Relationship between ammonia excretion rate and protein intake for large O.spilurus.

Table 4.8 Multiple linear regression components for estimation of Ammonia excretion rate, mg/Kg/hr (Y) from feeding rate (FR, %BW/D) and protein percent of the diet (P,%)

Fish Size	Variable Item	Coefficient	St. Error of Reg. Coeff.	St. Error of Estimate	R^2
Small	Constant	-23.729	4.426	1.506	0.94
	FR	10.165	2.152		
	$(FR)^2$	- 1.115	0.266		
	P%	0.443	0.067		
Large	Constant	- 4.415	2.669	3.457	0.86
	P%	0.429	0.109		
	P% x FR	0.075	0.036		

Table 4.9 Some important parameters of the diurnal ammonia excretion rate.

Fish Size	Protein %	Feeding rate %BW/D	Peak Value mg/Kg/hr	Time of Peak appearance	Duration of the peak (hr)
Small	24.94	2	5.45	7pm	2
	34.44	2	14.99	11am	6
	43.35	2	28.81	11am	2
	24.94	4	15.17	7pm	2
	34.44	4	22.61	7pm	2
	43.35	4	26.90	7pm	2
	25.94	6	23.35	9pm	4
	34.44	6	26.17	9pm	4
	43.35	6	39.39	11pm	6
Large	20.00	0.5	10.22	5pm	2
	30.00	0.5	15.64	1pm	2
	41.25	0.5	16.76	11am	2
	20.00	1.75	15.72 & 15.12	7pm & 11am	2
	30.00	1.75	17.45 & 12.34	1pm & 5pm	2
	41.25	1.75	41.13 & 23.05	11am & 7pm	2
	20.00	3.0	8.79	7pm	2
	30.00	3.0	26.81	3pm	2
	41.25	3.0	34.25	7pm	2

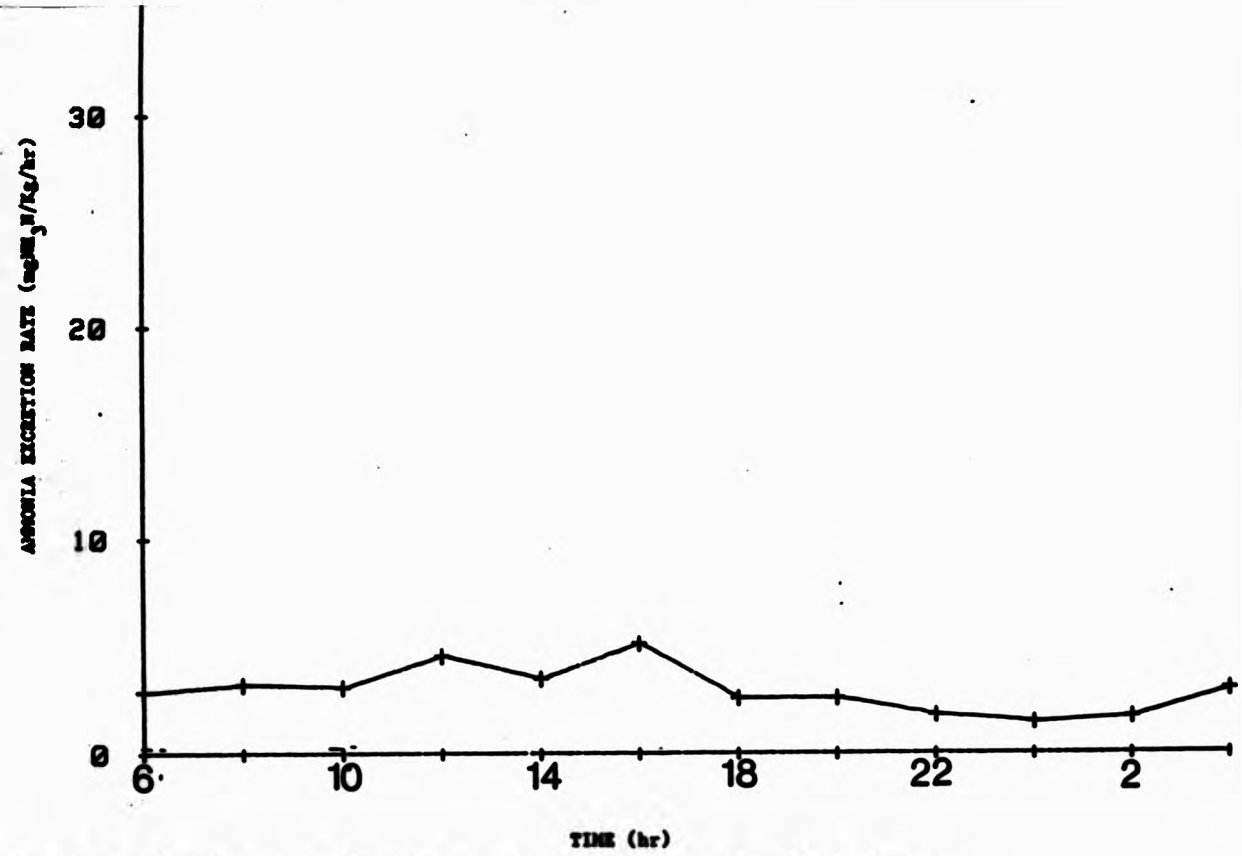


Figure 4.7 Diurnal pattern of ammonia excretion rate for starved small O.spilurus.

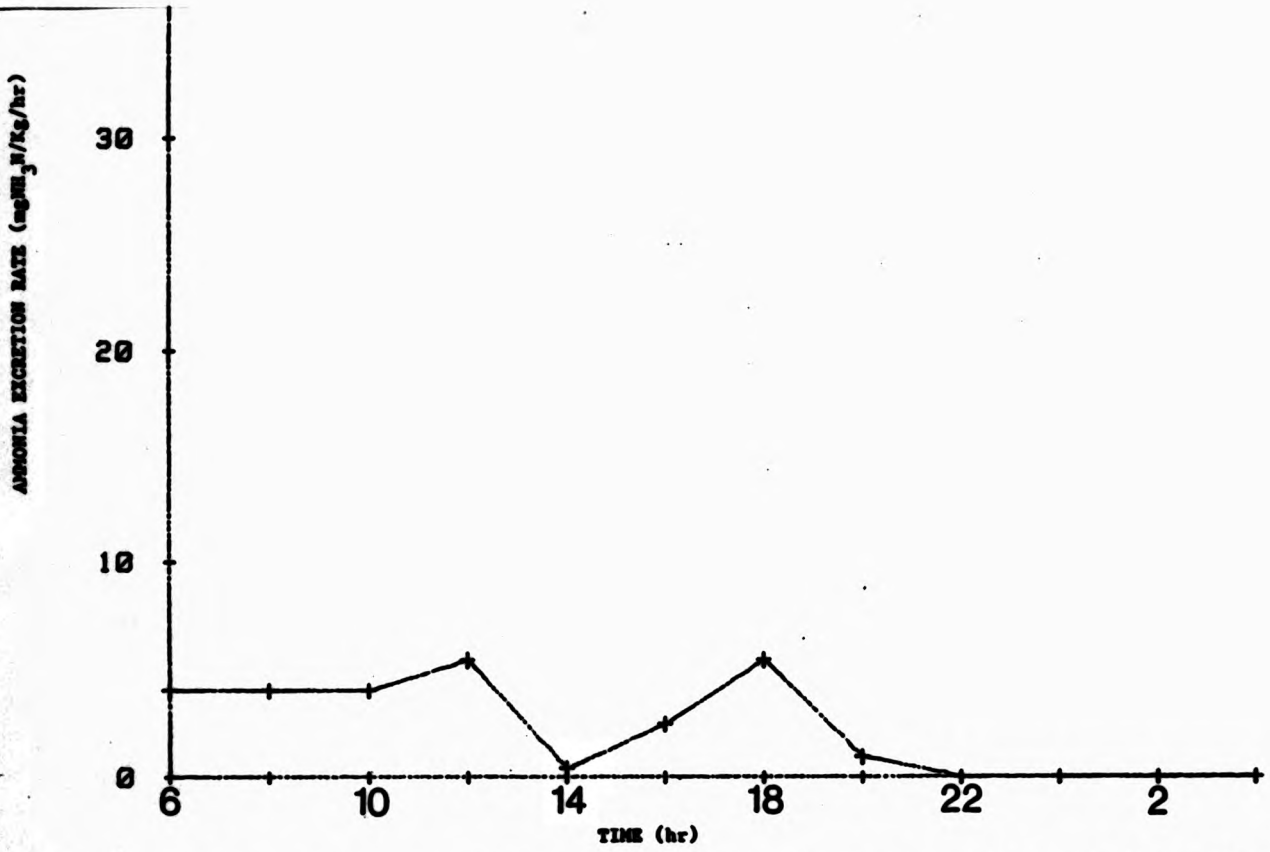


Figure 4.8 Diurnal pattern of ammonia excretion rate for small O.spilurus fed on 24.94% protein diet at 2%BW/D.

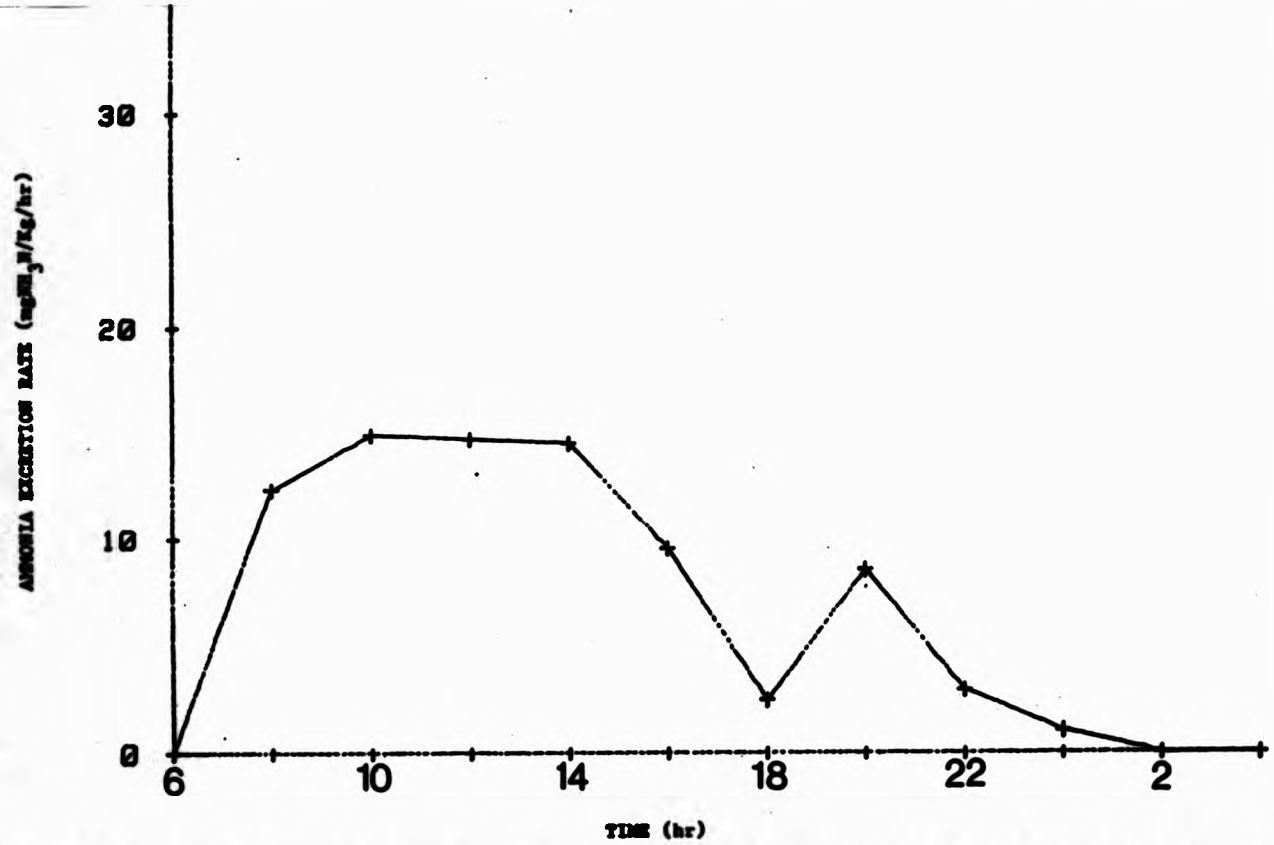


Figure 4.9 Diurnal pattern of ammonia excretion rate for small O.spilurus fed on 34.44% protein diet at 2%BW/D.

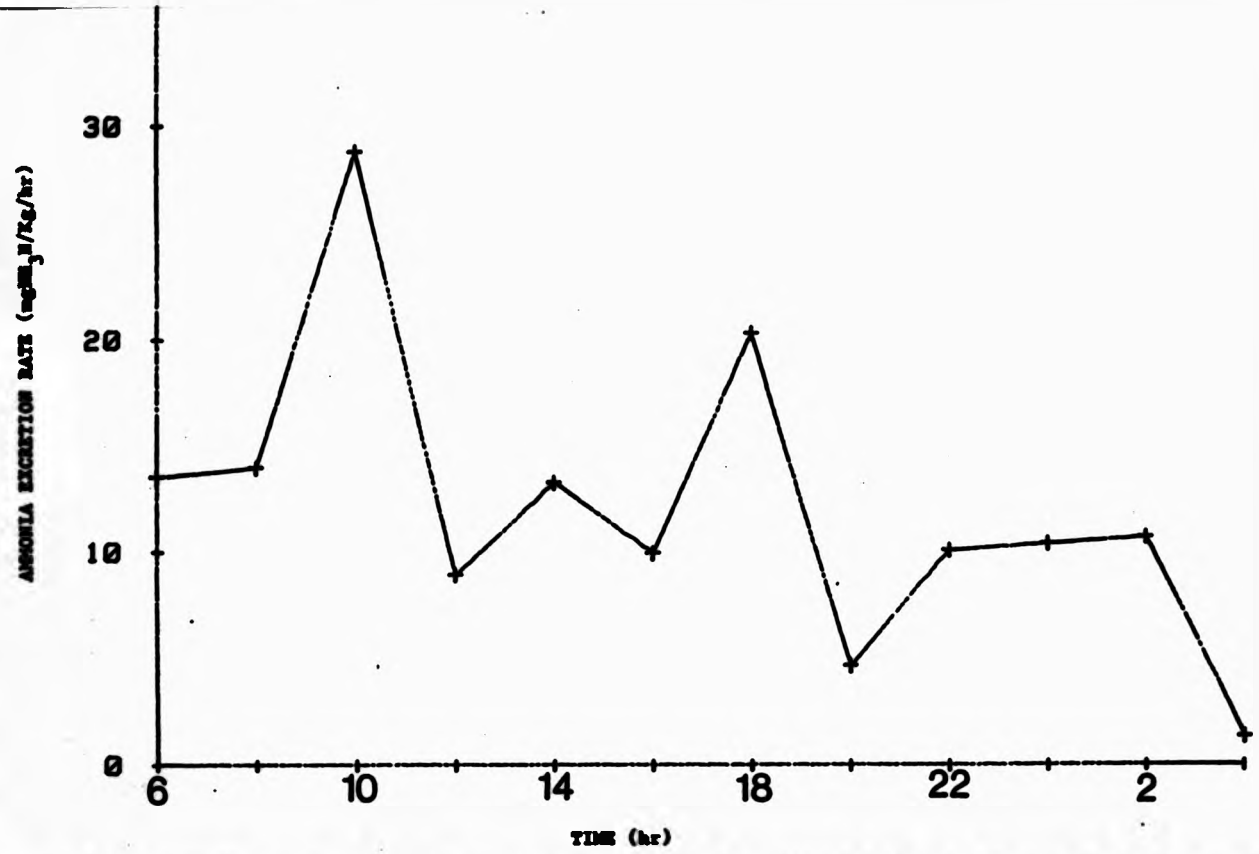


Figure 4.10 Diurnal pattern of ammonia excretion rate for small *O.spilurus* fed on 43.35% protein diet at 2%BW/D.

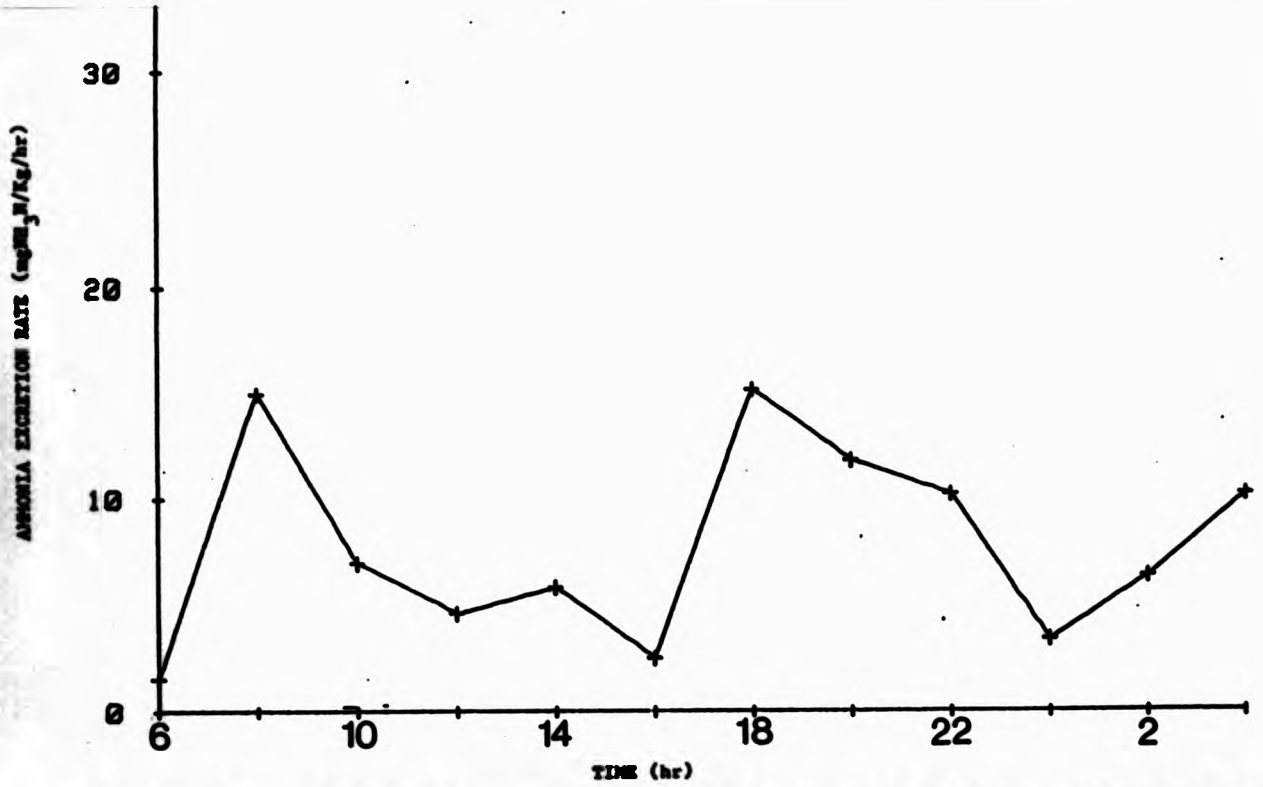


Figure 4.11 Diurnal pattern of ammonia excretion rate for small O.spilurus fed on 24.94% protein diet at 4% BW/D.

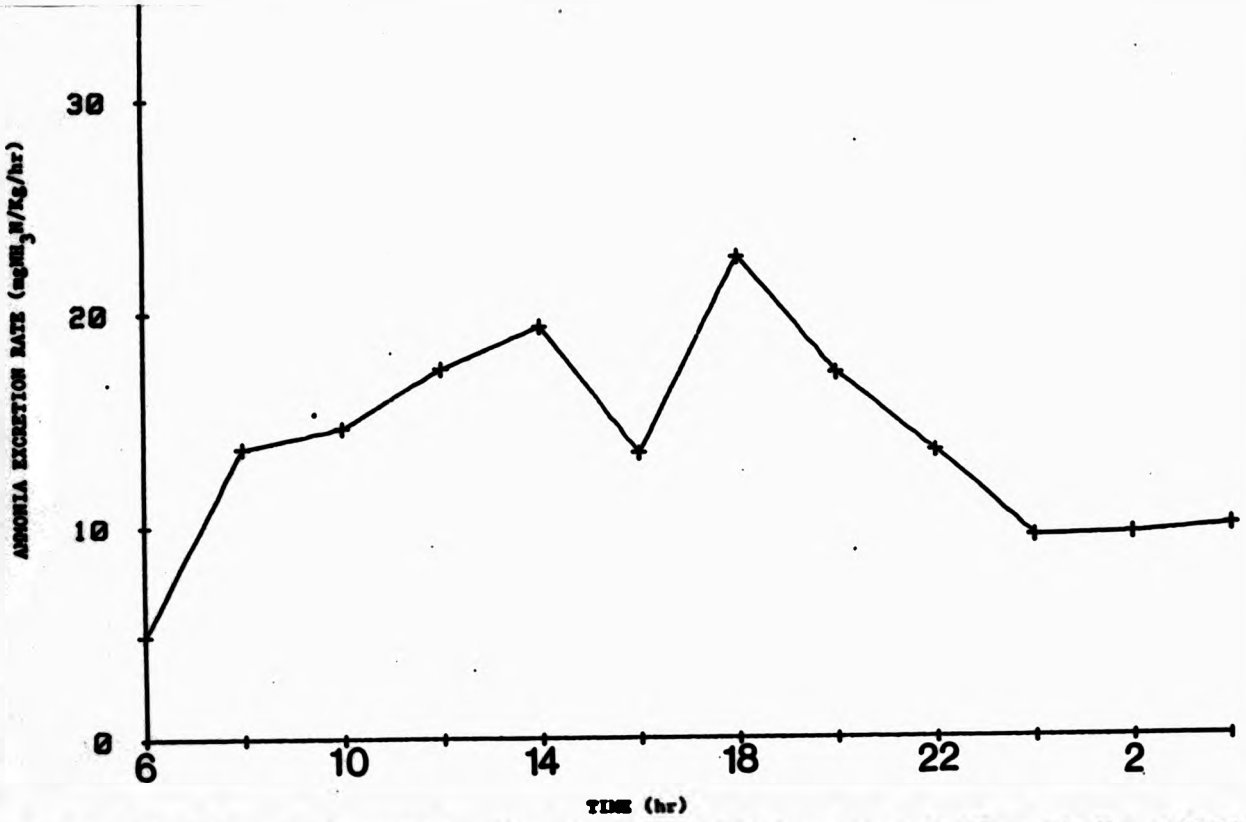


Figure 4.12 Diurnal pattern of ammonia excretion rate for small O.spilurus fed on 34.44% protein diet at 4% BW/D.

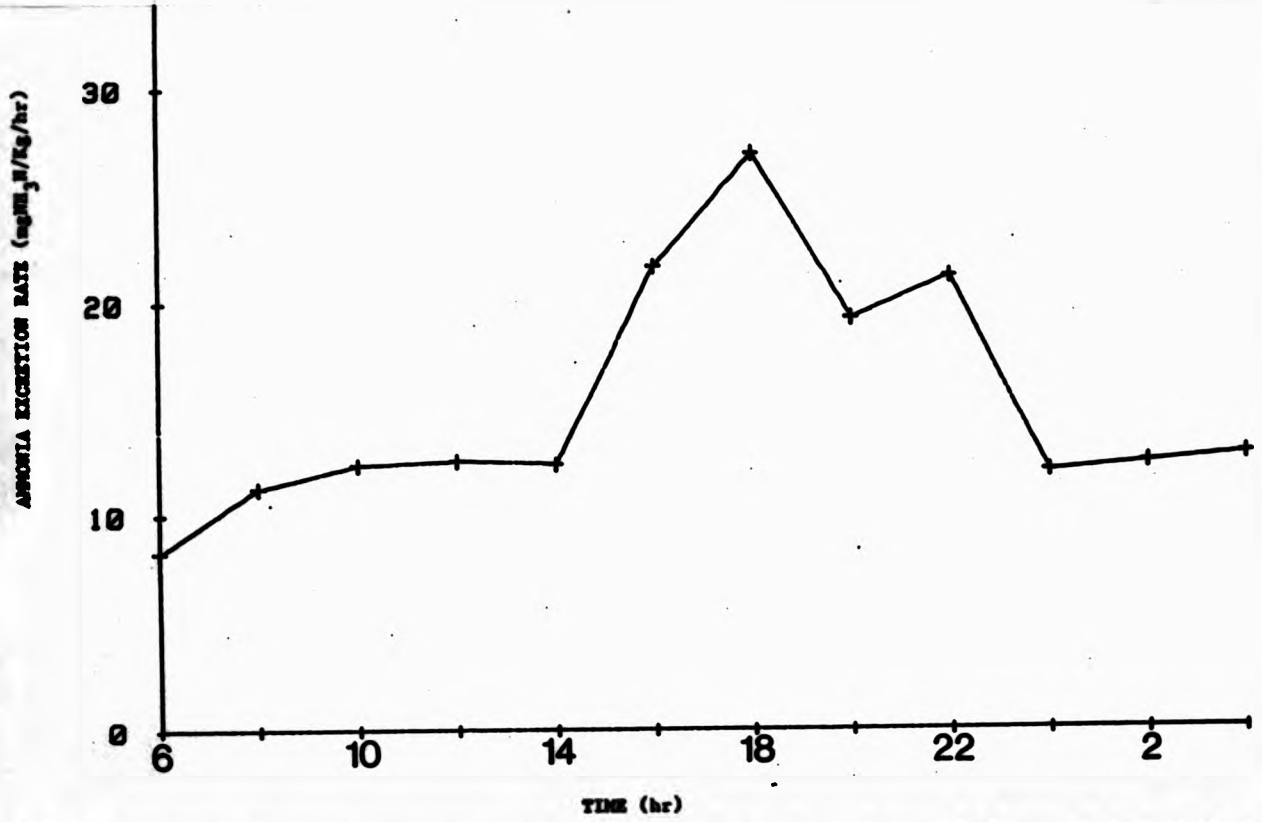


Figure 4.13 Diurnal pattern of ammonia excretion rate for small O.spilurus fed on 43.35% protein diet at 4% BW/D.

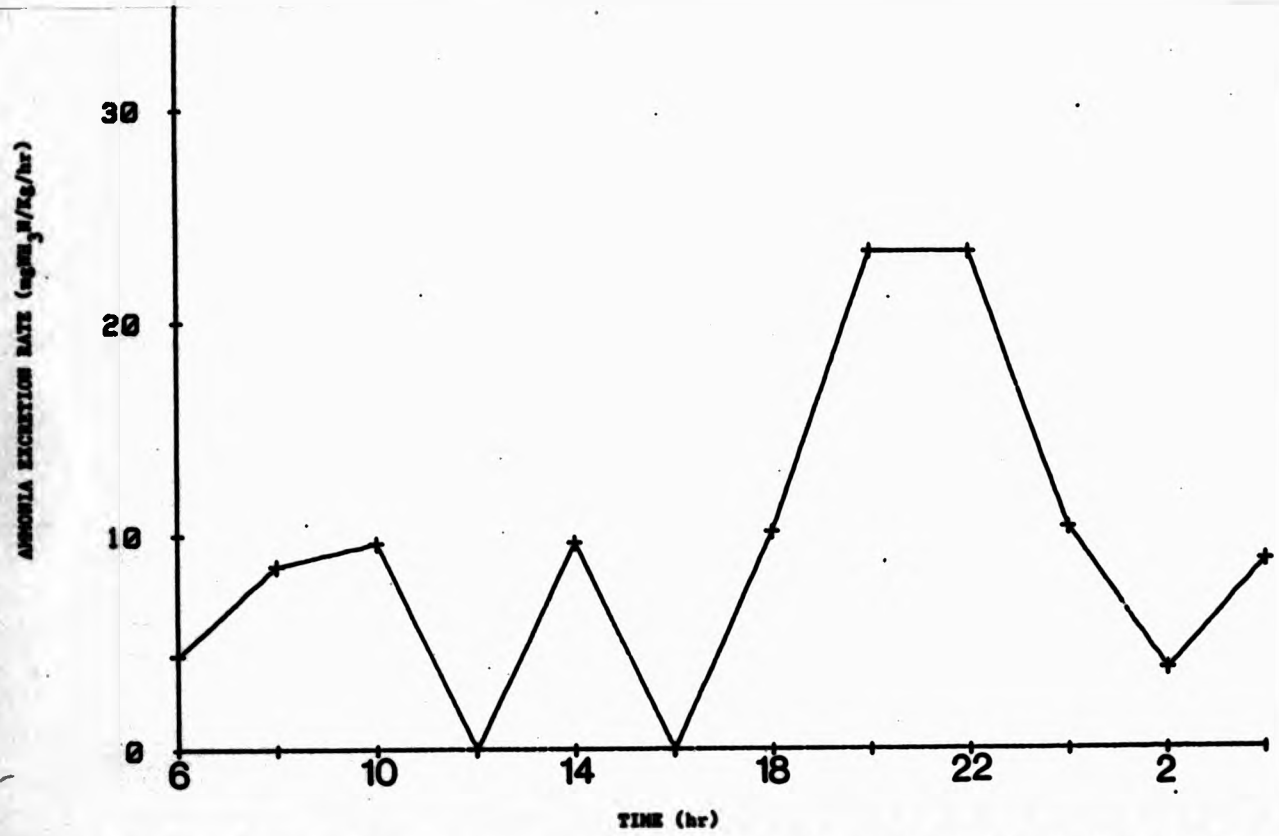


Figure 4.14 Diurnal pattern of ammonia excretion rate for small *O.spilurus* fed on 24.94% protein diet at 6%BW/D.

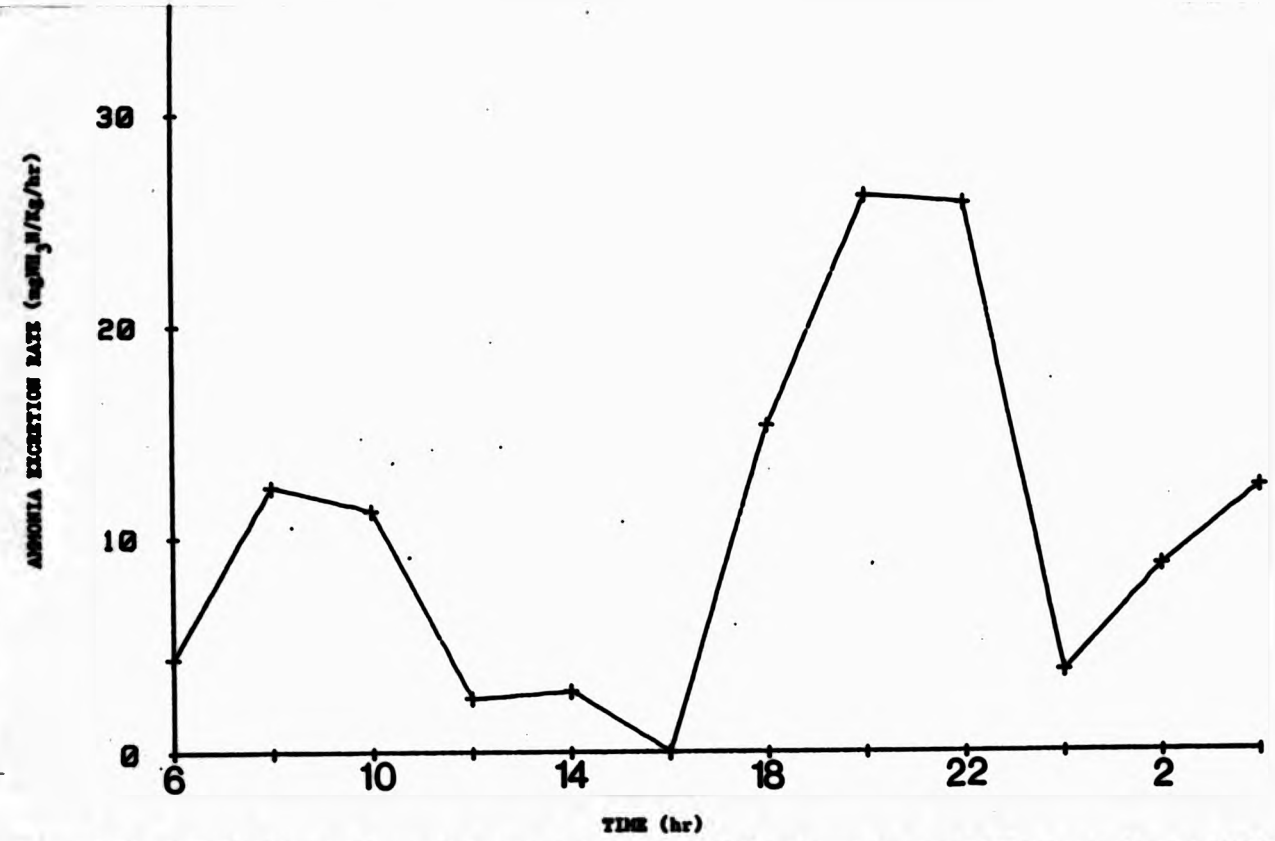


Figure 4.15 Diurnal pattern of ammonia excretion rate for small *O.spilurus* fed on 34.44% protein diet at 6%BW/D.

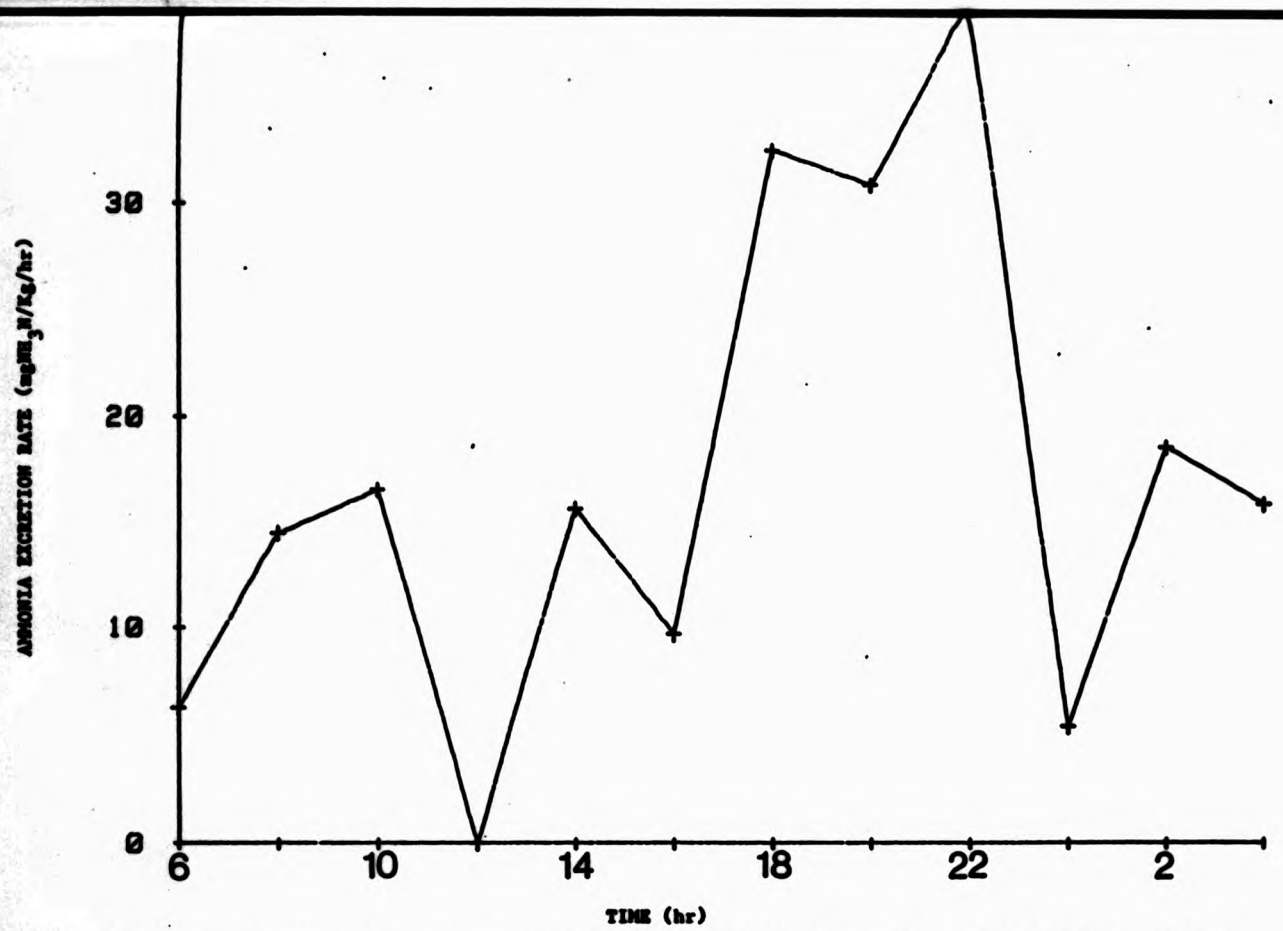


Figure 4.16 Diurnal pattern of ammonia excretion rate for small O.spilurus fed on 43.35% protein at 6%BW/D.

meal lasting for long periods (6-7 hrs). With the 43.35% protein diet two high peaks of short duration were noticed. The first peak, 28mg NH_3N/kg/hr, was noticed at 11 am while the second peak, 19mg NH_3N/kg/hr at 7 pm.

At 4% bw/d the same pattern was noticed for the 3 diets. The highest peak was recorded at about 7 pm for all diets, at 14, 21 and 26mg NH_3N/kg/hr for 24.94, 34.44 and 43.35% protein, respectively.

At 6% bw/d the same pattern was also evident for the 3 diets. After the morning meal ammonia rose until about 11 am and then declined to reach a level of almost no ammonia excretion at 1 pm. Thereafter ammonia excretion rose again to reach a peak of 23, 25 and 35mg NH_3N/kg/hr between 7 and 11 pm for 24.94, 34.44 and 43.35% protein diets, respectively.

The duration of these peaks were about 2 hrs for diets fed at 2 and 4% bw/d. However, 34.44% protein diets fed at 2% bw/d show a longer duration (6 hrs). At 6% bw/d the peak lasted 4 hrs for 24.94 and 34.44% protein diets, whereas it lasted 6hrs for 43.35% protein diet.

In experiment 2 ammonia measurements were started at 6 am, ending at 10 pm, and therefore the ammonia excretion rates between 10 pm and 6 am next morning are estimated values. At 0.5% bw/d there were no true peaks. However, ammonia excretion rate was higher with the higher protein content of the diet. At 1.75% bw/d there were two peaks for all diets. The time of the appearance of the peak was not clear as shown for small O. spilurus.

There was no great differences in the rate of ammonia excretion rates between small and large O. spilurus. For small O. spilurus the peak ammonia excretion ranges from 5.45 to 39.39 mg NH_3N/kg/hr, whereas for large O. spilurus it ranges from 8.79 to 34.25mg NH_3N/kg/hr. However, the time of ammonia peak appearance was more clear for small fish than large individuals.

(6) Urea Excretion:

The urea excretion rate is summarised in Table 4.3. It has been found that urea excretion is not affected either by dietary protein level or by feeding rate. Urea excretion rates ranged from 28.80 to 256.97mg urea-N/kg/d for small O. spilurus. No urea excretion measurements were collected for large O.

apilurus. There was no daily variation in urea excretion rate corresponding to feeding.

(7) Nitrogen Balance:

The proportion of daily ammonia-nitrogen excretion to total nitrogen intake for small O. apilurus ranges from 6.86 to 21.67% (Table 4.10). It must be noted that nitrogen intake represents the actual food supplied, not necessarily that consumed. The highest variation in the proportion of ammonia-nitrogen excretion was noticed at 2% bw/d, increasing with increasing protein content of the diet. However, at the other two feeding rates the proportion of ammonia-nitrogen excretion did not vary appreciably with the protein content of the diet.

The proportion of daily ammonia-nitrogen excretion to total nitrogen intake for large O. apilurus ranges from 14.23 to 95.00% (Table 4.10). A negative nitrogen balance (-8.75%) was found with a low protein diet fed at low ration. By contrast with small fish the proportion of ammonia-nitrogen at the lowest feeding rate was very high (87.45-95.00%). At 1.75% bw/d there was no clear trend in the proportion of ammonia-nitrogen excretion with dietary protein level whereas at 3% bw/d it increases with increasing protein level.

Table 4.10 Proportion of ammonia - nitrogen excretion from total nitrogen intake for small and large O.anilurus.

Fish Size	Feeding Rate %BW/D	Protein Level %	Nitrogen Intake mg/Kg/D	Ammonia Excretion rate ($mgNH_3N/Kg/D$)	NH_3N as % of Nitrogen intake
Small	2	24.94	798.4	54.84	6.87
		34.44	1102.4	171.17	15.53
		43.35	1387.2	301.18	21.71
	4	24.94	1596.8	249.30	15.63
		34.44	2204.8	372.65	16.90
		43.35	2774.4	405.24	14.61
	6	24.94	2993.6	223.78	9.35
		34.44	3305.6	251.30	7.60
		43.35	4161.6	410.18	9.86
Large	0.5	20.00	160	174.00	-8.75
		30.00	240	228.00	95.00
		41.25	322	279.84	87.45
	1.75	20.00	560	245.52	43.84
		30.00	840	250.56	29.83
		41.25	1127	547.68	48.47
	3.00	20.00	960	136.56	14.23
		30.00	1440	358.48	17.95
		41.25	1932	604.32	31.31

B. Oxygen Consumption:

(1) Effect of Protein Percent of the Diet:

The oxygen consumption rates for the different feeding regimes for the two fish sizes are shown in Table 4.4. The orthogonal contrast ANOVA test shows that protein percent of the diet has no significant effect ($p<0.05$) on oxygen consumption rate for the two fish sizes.

(2) Effect of Feeding Rate:

Feeding rate has a highly significant effect ($p>0.01$) on oxygen consumption rate for small O.spilurus. The relationship between feeding rate and oxygen consumption was linear for all diets. The regression components are summarised in Table 4.11 and the relationship is shown in Fig 4.17. Comparing the slopes of these linear regression equations by the Tuckey test shows that they are significantly different ($p>0.05$) from each other, and therefore a common slope cannot be computed. This suggests that protein percent of the diet has a significant effect on the slope of the feeding rate - oxygen consumption regression curve.

Feeding rate was found also to have a significant effect ($p>0.05$) on oxygen consumption for large O. spilurus. However, the orthogonal contrast test fails to demonstrate the mode of this effect whether it is

Table 4.11 Relationship between oxygen consumption rate, mg/Kg/D, (Y), and feeding rate, %BW/D, for small O.spilurus.

Item		b_o	b'	St. Error of Est.	R^2
Protein %:	24.94	146.24	59.44	22.31	0.99
	34.44	151.26	54.76	23.27	0.98
	43.35	183.71	48.52	54.96	0.89

Table 4.12 Multiple linear regression components where Y=oxygen consumption rate (mg/Kg/D); X_1=feeding rate (%BW/D); X_2=FR x Protein percent of the diet.

Item	b_o	St. Error of Reg. Coeff.	St. Error of Est.	R^2
b_o	160.75	24.33	36.79	0.92
X_1-FR	59.23	13.54		
X_2-FR X P%	-0.145	0.348		

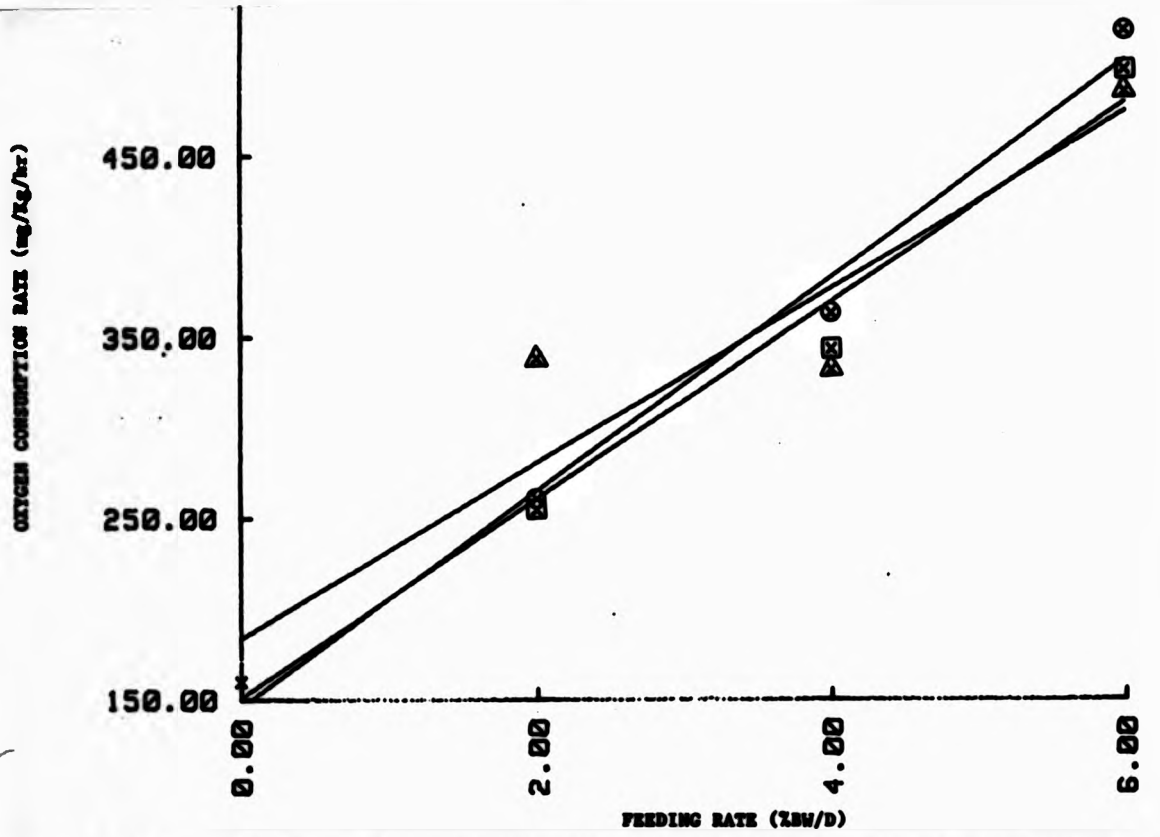


Figure 4.17 Relationship between feeding rate and oxygen consumption rate for small O.spilurus fed on 3 diets: 24.94% (O), 34.44% (□) and 43.35% (△) protein.

linear or quadratic. The relationship between feeding rate and oxygen consumption rate seems to be linear for the low protein diet (20%) whereas it is quadratic for the other two diets.

(3) Combined Effect of Percent Protein and Feeding Rate:

A multiple linear regression equation was formulated for small O. spilurus to show the effect of both P:E ratio and feeding rate on oxygen consumption rate (Table 4.12). Only those factors which have a significant effect by the orthogonal polynomial contrast ANOVA test were included in the regression. Thus feeding rate has the major effect on oxygen consumption rate, whereas P:E ratio of the diet was not significant. However, the interaction between those two factors was significant. This result suggests that P:E ratio becomes important only at a certain critical level. SNK test shows that all fish fed at 6% bw/d showed a significantly higher oxygen consumption rates from those fed at 2 and 4% bw/d.

For the oxygen consumption of large O. spilurus, however, it was not possible to formulate a multiple linear regression equation with high correlation. This was mainly because it was not possible to define the mode of effect of the feeding rate.

(4) Diurnal Pattern of Oxygen Consumption:

For small O. spilurus the diurnal pattern of oxygen consumption show approximately the same pattern for the 9 feeding regimes. Fig 4.18-4.21 show the pattern of oxygen consumption rates over the 24hr period for starved and fed fish on 43.35% protein diet at 3 feeding rates. Table 4.13 summarises some of the important characteristics of oxygen consumption rates such as peak values and time of peak appearance.

Feeding rate has the greatest effect on peak values, though at the lowest feeding rate protein level of the diet shows a significant effect on the peak value of oxygen consumption. At 2% bw/d there is a positive linear relationship between peak value and protein level of the diet. However, at 4 and 6% bw/d there is a trend, although not significant, of lowering peak value with higher dietary protein levels.

Generally, the peak oxygen consumption appeared at about 5 pm for all diets. Two exceptions were noticed: 24.94% protein diet fed at 2% bw/d and 34.44% protein diet fed at 4% bw/d where the peak was noticed at 7 pm and 3 pm, respectively.

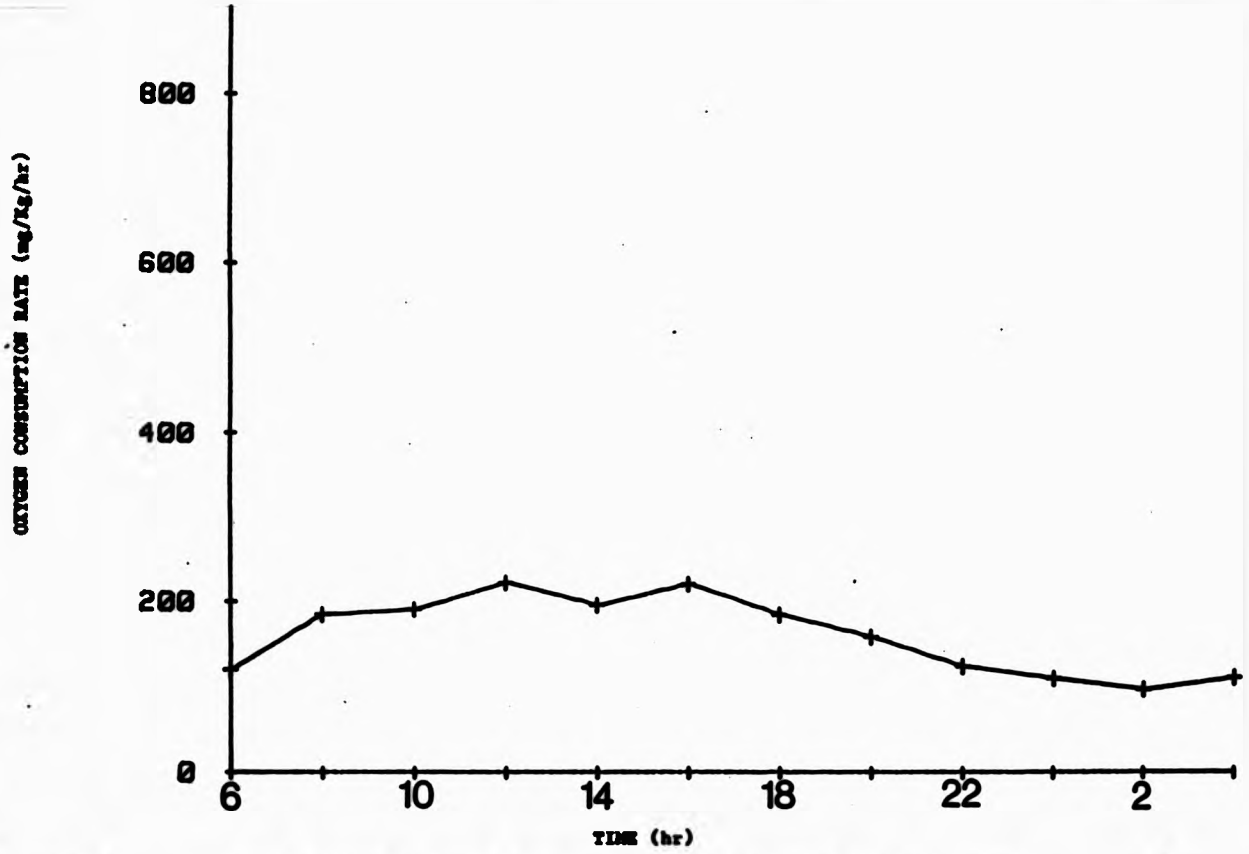


Figure 4.18 Diurnal pattern of oxygen consumption rate for starved, small O.spilurus.

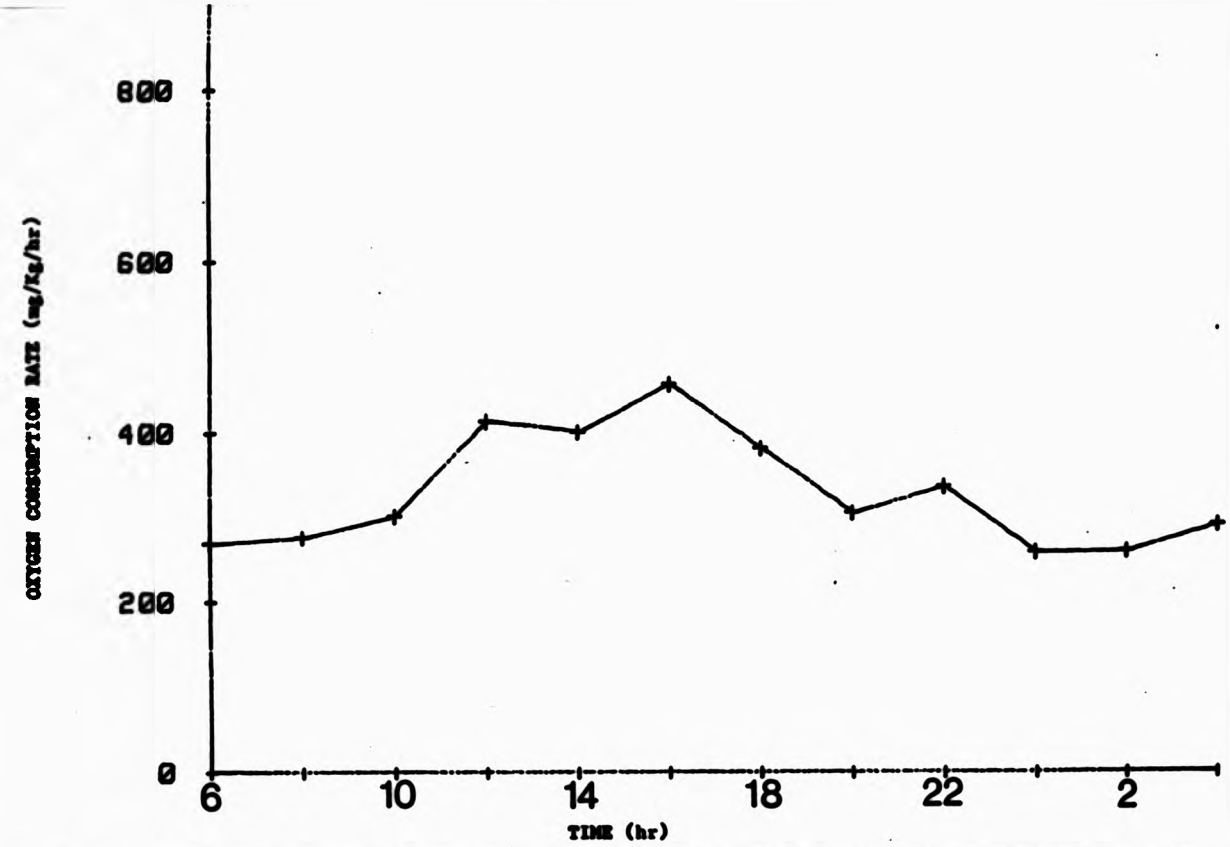


Figure 4.19 Diurnal pattern of oxygen consumption rate for small O.spilurus fed on 43.35% protein diet at 2%BW/D.

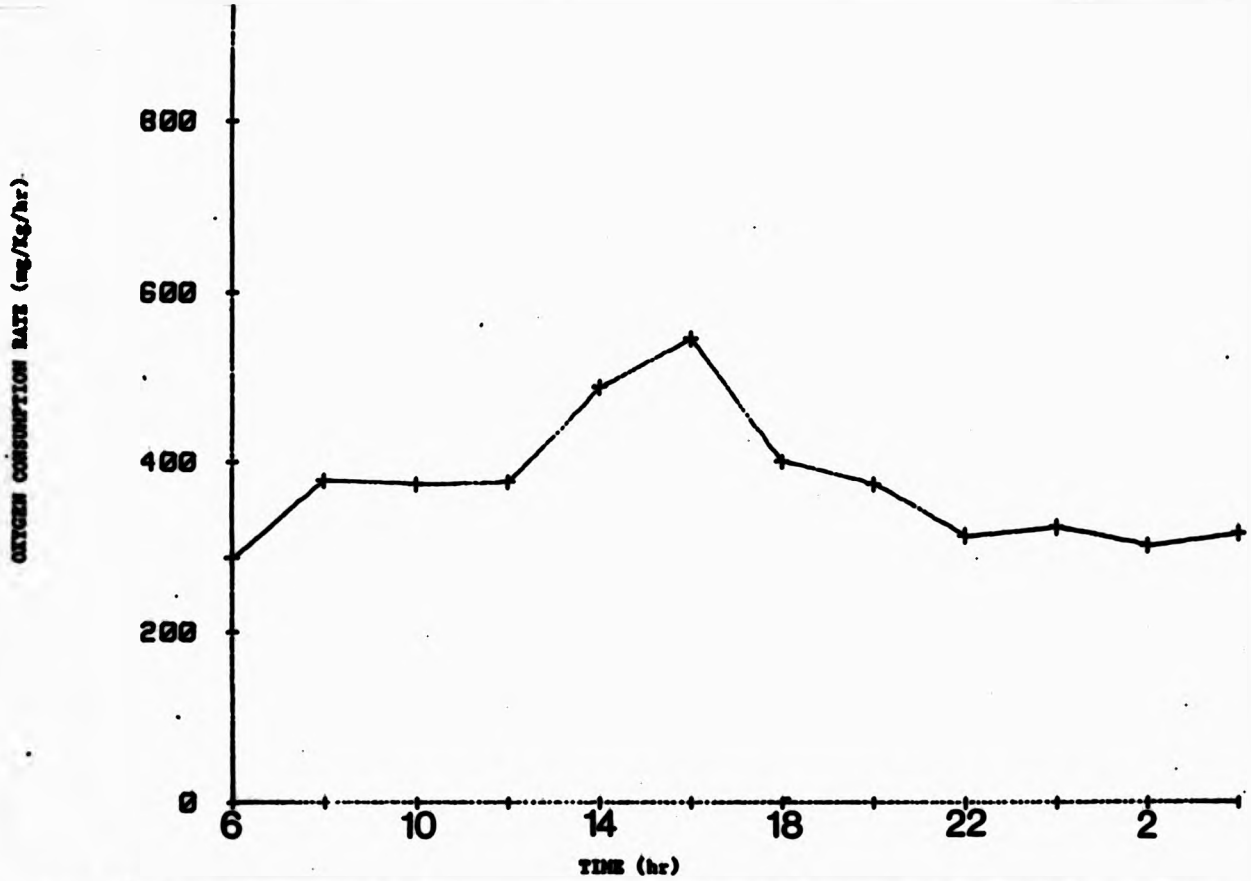


Figure 4.20 Diurnal pattern of oxygen consumption rate for small *O.spilurus* fed on 43.35% protein diet at 4%BW/D.

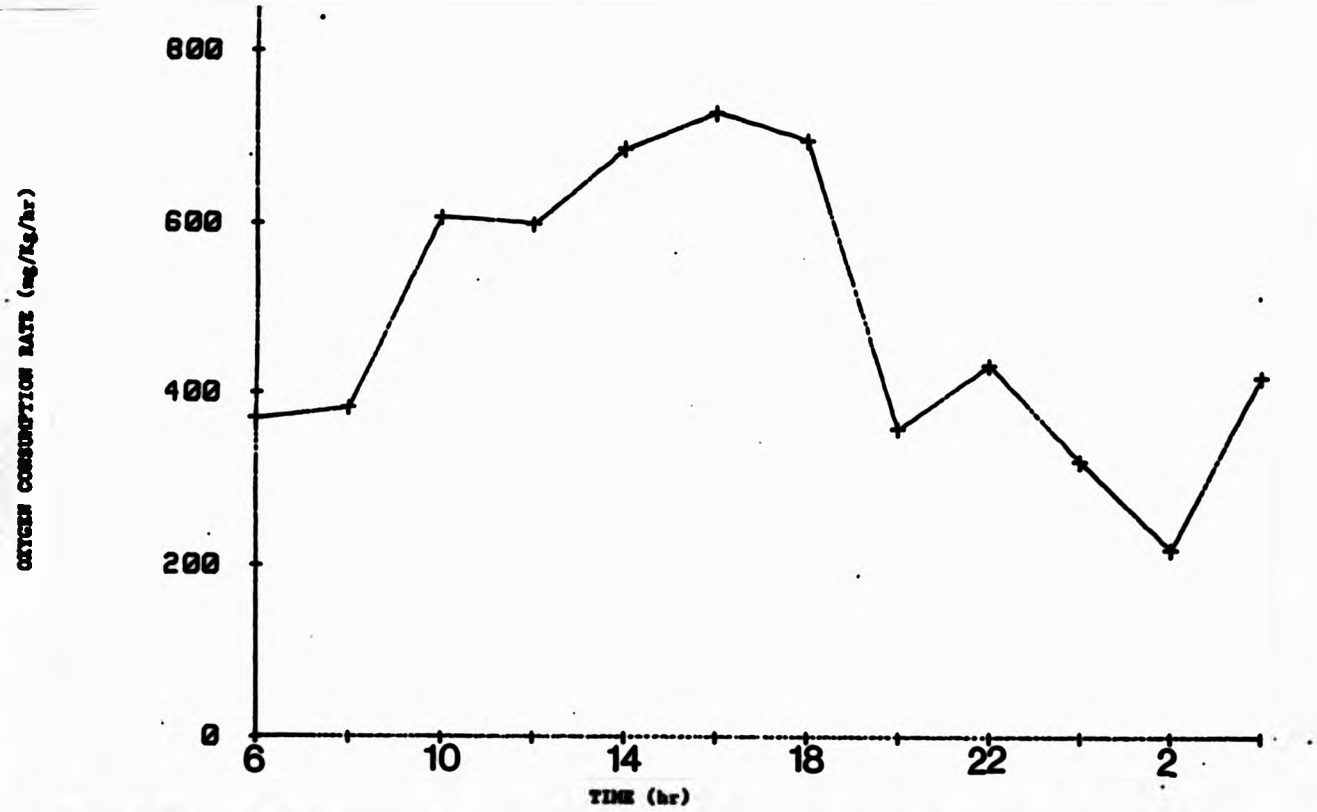


Figure 4.21 Diurnal pattern of oxygen consumption rate for small O.spilurus fed on 43.35% protein diet at 6% BW/D.

It was difficult to estimate the actual duration of those peaks because oxygen consumption increased and decreased gradually.

Table 4.13 Some important parameters of the diurnal oxygen consumption rate.

Fish Size	Protein %	Feeding Rate %BW/D	Peak Value mg/Kg/hr	Time of peak appearance (hr)
Small	24.94	2	417.79	7 pm
	34.44	2	349.24	5 pm
	43.35	2	456.51	5 pm
	24.94	4	509.07	5 pm
	34.44	4	548.12	3 pm
	43.35	4	545.53	5 pm
	24.94	6	824.21	5 pm
	34.44	6	878.63	5 pm
	43.35	6	727.91	5 pm
Large	20.00	0.5	138.82	5 pm
	30.00	0.5	125.07	11 am
	41.25	0.5	93.94	1 pm
	20.00	1.75	142.76	5 pm
	30.00	1.75	179.77	1 pm
	41.25	1.75	156.54	5 pm
	20.00	3.0	171.05	5 pm
	30.00	3.0	175.31	7 pm
	41.25	3.0	164.71	5 pm

Discussion:

(1) Evaluation of Experimental Methodology:

Before discussing the results obtained in this study it is appropriate to consider the methodology used, in view of the wide variation in the methodologies applied for studying this relationship (Table 1). These variations include: pre-fasting v.s. non-fasting; feeding frequency, tank coverage, water flow, stocking density ... etc.

The importance of the methodology arises from the fact that ammonia excretion is affected by many factors other than nitrogen intake (Chapter 1) such as: temperature (Paulson, 1960); oxygen (Kutty, 1972); fish activity (Kutty, 1972) and adaptation to a particular feeding regime (Kaushik, 1980). The reason for these differences is probably the different objectives for which those studies were performed, as a result of which there is an absence of a standard methodology.

The basic intention in this study was to maintain the fish in comparable conditions to those of the growth experiment (Chapter 3), so that data collected would be comparable. This necessitated the use of a constant water flow. It is well realised that measuring fish metabolites in a constant water flow is difficult (Cho

and Kaushik, 1985). The system used in this study appears to yield very reproducible flow rates. The average flow rate was about 3.5l/min/tank which results in a theoretical residence time of 2.38 hrs.

No attempt was made in this study to measure the effect of flow rate on the water mixing in the tank. However, Rammarine et. al. (1987), in a similar study, claimed that more than 95% of the water, as measured by a dye, was removed from the system (rectangular tanks: 1.2 x 0.7 x 0.4m depth; water volume = 130l; flow rate = 1.17l/min/tank; theoretical residence time = 1.85 hrs) within 3-4 hrs of introduction. Although the water residence time is variable and system specific, this comparison suggests that there was some mixing in this study between earlier and later output.

Therefore, the gradual decline in ammonia excretion rate may be partly due to the flushing characteristics of the open flow system used. An open flow system, however, does have advantages, avoiding the build-up of water ammonia level and possible suppression of ammonia excretion (Rammarine et. al., 1987).

Temperature control was fortunately not necessary because of the pumped well sea water supply maintained relatively constant temperature (25.15 ± 1.35°C). Tank

coverage was needed mainly to permit a parallel measurement of ammonia excretion with oxygen consumption rates, with minimal interference from atmospheric oxygen transfer across the tank surface. However, the long acclimation period for the experimental feeding regime (10 days) together with the very wide feeding rates results in an accumulation of uneaten food and faeces in the tank. Tank cleaning was difficult and this was the main reason for using uncovered tanks with large fish. As there appeared to be little ammonia loss to the atmosphere, it is probably not necessary to use covered tanks in ammonia excretion studies, though where oxygen consumption data are required, it may be useful.

It is mentioned earlier that adjusting stocking density with different feeding rates was necessary to maintain an acceptable ambient oxygen level in the tanks. This was carried out to avoid changing the water flow rate which has an obvious effect on the fish activity level, which in turn might affect fish metabolism and ammonia excretion rate. However, the effect of these different densities was not possible to be measured statistically. In conclusion, further research is required to optimise the experimental methodology for studying ammonia excretion rate.

(2) Ammonia Excretion:

(i) Diurnal Fluctuation of Ammonia:

Generally, the effect of feeding rate on the diurnal pattern of ammonia excretion seems to be more pronounced than the effect of protein content of the diet. However, at a low feeding rate (2% bw/d) the effect of protein content of the diet was evident. The maximum post-prandial ammonia excretion was reached at 4-5 hrs at 2% bw/d. 11 hrs at 4% bw/d and 13 hrs at 6% bw/d from the first morning meal. Most of the available literatures show that the maximum post-prandial ammonia excretion is attained within 4-6 hrs from the first meal (Brett and Zala, 1975; Leid and Bratten, 1984; Rychly and Marina, 1977; Kaushik and Teles, 1985; Elliot, 1976; Kaushik, 1980).

These results are comparable with those obtained at 2% bw/d in this study. Leid and Bratten (1984) reported that the rapid apperance of the post-prandial peak in ammonia excretion suggests a fast and efficient digestion and absorption of amino-acids in fish, leading to a flow of amino-acids into the amino-acids pool. The latter appearance of the peak of ammonia excretion observed at higher feeding rates probably reflects the effect of food ration. Kaushik (1980) reached the same conclusion and reported that the volume of feed distribution of the amount of nitrogen consumed

apparantly has an influence on the interval needed for the rate of maximal post-prandial ammonia excretion to appear, whereas the number of meals did not affect these rates.

There are some indications of the appearance of more than one peak of ammonia excretion per day at 2% bw/d. Kaushik (1980) found also that with two meals/day two peaks of ammonia excretion rate were only established 7 days after the number of meals increased. Interestingly Rychly and Marina (1977) reported that there appear to be two (or three) peaks with rainbow trout fed one meal/day. They explained this by assuming that the periodicity of nitrogen excretion is closely related to activity and oxygen consumption in fish. In this study two peaks were observed only with 43.35% protein diet fed at 2% bw/d. The two peaks were associated with morning and afternoon meals. No peak was observed with the mid-day meal. Although the existence of an internal rhythm cannot be ignored it seems that the second peak observed in this study represents a post-prandial ammonia excretion resulted from the afternoon meal.

The most interesting findings in the daily pattern of ammonia excretion were observed at 6% bw/d for all diets. Ammonia excretion rate starts to increase slowly

after morning meal. However, after about 2 hrs, it starts declining to reach a level of almost no ammonia excretion at about 5 hrs after the morning meal. Thereafter, ammonia excretion increases to reach a maximum value at about 10 pm, from which it starts declining again. The main reason for this reduction in ammonia excretion observed at 1 pm is not clear. Fish consumed the food supplied and they were metabolising the food as indicated from the oxygen consumption data (to be explained in more detail later). Very little information is available on such an observation probably because in most studies the fish were fed only once a day. Gunther et. al. (1961) reported a slightly similar diurnal ammonia production pattern for large trout fed on 42% protein diet at 1.6% bw/d with 4 feeds per day in a recirculating system. They did not observe a point of no ammonia excretion but they found a great variation in ammonia output during the 24hr period with the lowest production rate observed of about 8-9 am and the maximum level at about 10 pm.

Although no attempt in this study was taken to measure the day to day variation, the ammonia excretion rate at 6% bw/d was measured again on another day at day time to check this reduction observed at 1 pm. This measurement shows the same trend in ammonia excretion rate. This observation is supported by the results

obtained by Kaushik (1980) who found that after 7 days of feeding a fish a particular feeding regime the nitrogen excretion rate (ammonia + urea) stabilises and there is little change between day to day measurements.

(ii) Ammonia Excretion v.s. Protein Intake:

Ammonia excretion can not be simply related to total protein intake. The results of this study show that the two concepts of protein intake, protein level of the diet and feeding rate, both affect ammonia excretion rate but they have a different mode of action. The effect of protein percent of the diet on ammonia excretion is linear for the two fish sizes and at all the levels tested. The slopes of these linear regressions range from 0.334 to 0.463 for small O. spilurus and from 0.398 to 0.683 for large O. spilurus. A similar linear relationship was reported for rainbow trout (Beamish and Thomas, 1984; Rychly, 1980). Direct comparisons between the regression coefficients of different studies is complicated by differences in units of measurements, diets, temperatures, fish size ... etc.

On the other hand, expressing protein intake as a feeding rate results in a curvilinear relationship for the three experimental diets tested in experiment 1 with small O. spilurus. The relationship appears to start linearly up to 4% bw/d after which it starts declining

with higher feeding rate. This decline is probably due to the reduction in ammonia excretion rate observed with 6% bw/d after 5 hrs from first meal. Similar curvilinear regressions were found between nitrogen intake and total nitrogen excretion rates by other authors. Savitz (1971) found that the exponential equation described the relationship slightly better than a linear equation. Infante (1974) came to the same conclusion.

There appears to be some doubt as to the best mathematical description on the relationship. Several studies, including those of Savitz (1971); Savitz et. al. (1977); Infante (1974); Iwata (1970); Jobling (1981a); and Rammarine et. al. (1987), have derived linear, asymptotic or exponential relationships. Jobling (1981a) found that his data could be adequately described by all of these relationships, but he concluded that they seemed best fitted to an asymptotic relationship. The data of this experiment shows that when all feeding regimes are considered the relationship is curvilinear. However, when only the sub-satiation levels (2 and 4% bw/d) are considered the data can be adequately described by a quadratic and linear relationship. Savitz et. al. (1977) concluded that it is difficult to ascertain the proper mathemtical description of this relation without a better

understanding of the physiological mechanisms involved in SDA and nitrogen retention. Rammarine, et. al. (1987) reported that it is likely the physiological mechanisms governing the processes of protein assimiliation and nitrogen excretion may have a maximum capability and that, therefore, the relationship should be curvilinear.

It is worth noting that the lowest ammonia excretion rate in fish fed on 6% bw/d was observed at about 5 hrs after the morning meal. This time is believed to be sufficient to increase the amino acid concentrations in the blood to their maximum level. Yamada et. al. (1982) found that all essential amino acids in the plasma of O. niloticus attained their maximal levels at 4 hrs after feeding. Similarly, Plakas et. al. (1980) found maximal levels of essential amino acids in the plasma of carp to appear 4 hrs after feeding casein. The reason for not detecting any ammonia excretion after 5 hrs from morning meal with such a rapid flow of amino acids into the blood suggests that no amino acid catabolism was taking place. This might have resulted from a high intake of non-protein energy consumed with such a high feeding rate which could substitute protein completely. However, this seems to be unlikely particularly for the high protein diet (43.35%) which has a total non-protein energy

intake of 142.45 Kcal/kg/d which is very close to the non-protein energy intake (137.65 Kcal/kg/d) with 24.94% protein diet fed at 4% bw/d. But no such an observation was noted at 4% bw/d.

Alternatively, this non-protein catabolism state might have resulted from a high protein intake which led to flooding of the blood by the amino acids at which the fish started releasing these amino acids in order to bring their level to the threshold level. This hypothesis also seems unlikely in view that the protein intake with 43.35% protein diet fed at 4% bw/d is higher (17.34g/kg/d) than the protein intake with 24.94% protein diet fed at 6% bw/d (14.96g/kg/d), but no such an observation was noted at 4% bw/d. This leads to the conclusion that the non-ammonia excretion state probably resulted from both high protein and energy ingested with high feeding rate. This demonstrates the significance of feeding rate in supplying the dietary protein and energy requirements. Whether this state represents protein savings or protein loss is not clear yet and this requires further research. However, it should be noted that the peak ammonia excretion at 6% bw/d is higher than that at 2 and 4% bw/d.

There is actually a linear relationship between food ration and peak ammonia excretion rate for all

diets. Ming (1985) concluded that peak ammonia excretion rate, not total ammonia excretion rate, may be used for rainbow trout as the basis for comparing the relative efficiency of dietary protein utilisation under specific conditions. This draws the attention to the question of which parameter should be considered; total or peak ammonia excretion rate, and which in turn have the greatest predictive strength, and the greatest relevance to controlling environmental conditions in intensive aquaculture.

The relationship between feeding rate and ammonia excretion in large O. spilurus was not as clear as that obtained with small fish. Although feeding rate significantly affects ammonia excretion rate it was difficult to find the proper mathematical formula to describe this relationship. As suggested in Chapter 3 this probably reflects their abnormal physiological state and, therefore, no further discussion on the results obtained with large O. spilurus will be presented.

(iii) Strategy to Control Ammonia Production:

In earlier study Liao et. al. (1972) showed that ammonia output can be predicted from feeding rate only. The results obtained from this experiment showed that although feeding rate has the major contribution on

ammonia excretion rate, P:E ratio of the diet still has a significant part in controlling the ammonia output at any given feeding rate. For example, by using the multiple linear regression equation developed in this study for small O. spilurus, the ammonia excretion rate with 30 and 40% protein diets fed at 4% bw/d will be 12.19 and 16.59 ± 1.51mg/kg/hr, respectively. This means that increasing protein level in the diet by 25% would lead to a 26.52% increase in ammonia output, which is substantial.

Liao's approach calculates ammonia output as a fixed proportion of feeding rate and, therefore, it assumes that the relationship between feeding rate and ammonia excretion is linear. The results of this study shows that the relationship between feeding rate and ammonia excretion rate is curvilinear; thus care must be taken in applying this approach particularly at near satiation feeding at which there seems to be a decline in total ammonia output.

Because of these differences between dietary protein level and feeding rate on ammonia outputs, ammonia production rates cannot be estimated accurately from protein fed only. This is shown from the relatively low correlation (0.81 and 0.75 for small and large O.spilurus) between ammonia excretion rate and

total protein intake (g/Kg/D). Thus for an accurate estimate of ammonia production a differentiation between the two parameters must be taken. The multiple regression model developed for small O.spilurus can be used as a guideline.

(iv) Nitrogen Budget:

While a complete nitrogen budget can not be given, the proportion of ammonia-nitrogen excretion to total nitrogen intake obtained from small O. spilurus was found to range from 6.87 to 21.71%. In comparison with other published data it seems that these proportions are smaller than those published for other fish. For example, Brett and Zala (1975) found that ammonia excretion amounted to 27% of the nitrogenous intake for sockeye salmon fed on Oregon moist pellets at 3% bw/d. Basmiah and Thomas (1984) found that ammonia excretion in rainbow trout represents 20.6 - 29.3% of nitrogen intake. Kaushik and Teles (1985) reported that ammonia ranges from 28.6 to 43.7% of nitrogen intake in rainbow trout fed on 43.4 - 40.9% protein diet. Similarly Porter et. al. (1987) found that ammonia excretion ranges from 28 to 37% of nitrogen intake for gilthead seabream (3 - 90g bw) fed on rations from 1.4 to 5% bw/d.

The low proportion of ammonia-nitrogen excreted by O. spilurus in this study might reflect a difference in the physiology of digestion between tilapia and other species. There was no information available on the effect of nitrogen intake on ammonia output for other tilapia species, and therefore a direct comparison could not be made.

The results could also be a reflection of low endogenous ammonia excretion rates in sea water. There are some indications in the literature showing that endogenous ammonia excretion rate for marine fish is in general less than that for fresh water (Porter et. al., 1987).

However, it is interesting to note that in all the above mentioned studies the fish have been fed once per day whereas in this experiment the fish were fed 3 times per day. It would appear that distributing the food over more than one meal per day gave the fish a better chance for more efficient food utilisation. Very little work is available on the effect of feeding frequency on ammonia excretion. Kaushik (1980) found that rainbow trout fed twice a day lost almost the same proportion as ammonia and urea alone (39%), while this loss was still higher (44%) in trout fed once a day. Nevertheless, the results obtained in this experiment indicates the very

good protein utilisation for growth by the small O. spilurus even in the sea water environment.

Urea Excretion:

Generally the values of urea excreted are very high (28.80 - 221.98 mg urea-N/Kg/D) in comparison with other data in literature. For example Kaushik (1980) found that urea excretion in fed rainbow trout ranges from 65 to 72 mg urea-N/Kg/D. Similarly Porter et.al. (1987) found no detectable urea with fed gilthead sea bream. No clear explanation for the very high urea detected in this study can be given. However, it is possible that other excreted nitrogenous substances may have interferred with the analytical procedure and this requires further research.

Oxygen Consumption:

(1) Diurnal Oxygen Consumption:

The 9 feeding regimes result in approximately the same pattern in oxygen consumption, where it starts rising after the first morning meal to reach a peak value at about 5 pm and then starts declining again. The peak value was affected mainly by the feeding rate. This is in agreement with other published work where feeding rate affected the peak of oxygen consumption in rainbow trout (Beamish, 1974), plaice (Jobling and Davis, 1980), O. spilurus (Spencer, 1984).

The duration of the peak oxygen consumption was not affected greatly by either feeding rate or protein level in the diet. However, the 43.35% protein diet fed at 6% bw/d shows a slightly longer duration. Beamish (1974) working with rainbow trout and Jobling and Davis (1980) working with plaice found that feeding rate affects the duration of oxygen consumption peak. However, Spencer (1984) found that feeding rate (2 - 6% bw/d) has no effect on the duration of the oxygen consumption in O. spilurus in fresh water, whereas dietary protein level (20 - 60%) affects the duration of post-prandial oxygen consumption. The maximum protein level included in this study was 43.35% whereas it was 60% in Spencer's study. Therefore, it is probable that the maximum dietary protein level used in this experiment was not high enough to induce such changes in the duration of the peak oxygen consumption.

(ii) Oxygen Consumption v.s. Protein Intake:

Feeding rate has a significant effect on the oxygen consumption of small O. spilurus in experiment 1. The effect of feeding rate on oxygen consumption has been studied by several authors for several fish species; the subject was reviewed by Jobling (1981b). In order to allow a direct comparison between the data collected in this study and other published data it was necessary to recalculate the food ration and oxygen

consumption values into their energy units (equivalents). The oxygen consumption values of specific dynamic action (SDA) can be converted to energy equivalent by multiplying by the oxycalorific coefficient. Although even small differences in this coefficient might significantly affect energy estimations (Brafield and Solomon, 1972), inexact values have been commonly assumed (Cowey, 1980). The value of 3.38 cal/mgO_2, used by Tandler and Beamish (1981) and in the present study was adopted on the basis of the studies of Elliot and Davidson (1975) on the energy equivalent of oxygen consumption in animal energetics.

The gross energy content of the diet was derived by multiplying the constituents of each diet, as determined by proximate analysis by their calorific equivalents. These were 5.65, 9.45 and 4.10 Kcal for protein, lipid and carbohydrates, respectively. The result was then multiplied by the average amount of diet supplied at 2, 4 and 6% bw/d to determine the gross energy supplied to the fish.

A linear relationship was found between energy supply as food ration and oxygen consumption. A similar linear relationship was found by many authors, as reviewed by Jobling (1981b). The slope of this linear relationship represents the proportion of the energy

intake which is expended as specific dynamic action (SDA) and termed the SDA coefficient (Jobling and Davis, 1980). The SDA coefficients obtained in this study were 0.12, 0.11 and 0.10 for 24.94, 34.44 and 43.35% protein diets, respectively. Published values for the SDA coefficient ranges from 0.09 to 0.20 (Jobling, 1981b). The values obtained in this study fall within the range of published values.

The protein percent of the diet did not have a significant effect on oxygen consumption rate as indicated by the contrast polynomial ANOVA test. Similarly, Schalles and Wieming (1976) found tht SDA was unaffected by dietary composition in blue gill sunfish fed formulated diets with protein content ranging from 24 - 45%. Furthermore Hogendoorn (1983) found that the combined effect of body weight and feeding level was responsible for 99.2% of the variance in the oxygen consumption data in African catfish (Clarias Lazara) whereas diet composition had no significant effect on oxygen consumption.

It seems, however, that there is a small negative relationship between dietary protein and SDA coefficients; in that the higher the dietary protein the lower the SDA coefficient. This contradicts available published data on the effect of protein

content on SDA. Thus, a positive relationship between protein and SDA coefficient was reported by Cho et. al. (1975) and Jobling and Davis (1980) working with rainbow trout and plaice, respectively. In this study only at low feeding rate (2% bw/d) a positive relationship was found between protein content of the diet and oxygen consumption. However, at 4 and 6% bw/d there seems to be a negative relationship between dietary protein content and oxygen consumption.

This probably results from the inverse relationship between dietary protein content and actual food consumption (Chapter 3). There are some indications derived from the food ration - SGR relationship for the 3 experimental diets suggesting that there is a negative relationship between actual food consumption and protein content of the diet. The low oxygen consumption rates at medium and high feeding rates probably reflect the difference in the actual food consumption rates between the 3 experimental diets.

On the other hand the oxygen consumption of large O. mpilurus was not clear. P:E ratio did not have a significant effect on oxygen consumption as it was with small fish. Feeding rate actually has a significant effect, but the problem was to define the mode of action. The relationship was linear for low protein

diet (20%) whereas it was quadratic for the other two diets. It is not clear whether this conflict was due to the fact that those fishes were non-growing fishes as indicated in Chapter 3 or because of using uncovered tanks. However, Knights (1985), in suggesting a procedure for measuring oxygen consumption in open tanks, suggested that this method is reliable and reproducible and adequate for comparative energetic studies.

Furthermore, El-Zahr (pers comm) working on estimation of oxygen consumption rate of juvenile sea bream of KISR using the same open tank technique suggested by Knights (1985) concluded that as long as the oxygen level in sea water is above 3mg/lthe interference of the atmospheric oxygen to the water is not significant for the estimation of oxygen consumption rate. The oxygen level in this experiment was never below 3mg/l. Based on these observations it seems that the conflict in defining the proper mode of action in oxygen consumption was due to the abnormal feeding physiology of the fish themselves.

CHAPTER 5: Ammonia Toxicity to *O.spilurus* in sea water.

Introduction:

The subject of ammonia toxicity to fish has received considerable attention in aquaculture. This is mainly because ammonia is excreted by the fish themselves (Waarde, 1983) and it is toxic (Meade, 1985), and this has implications for water management in fish farms.

According to Haywood (1983) the most common method for evaluating ammonia toxicity is the 96hr-LC50 (Lethal concentration that kills 50% of the population within 96 hours), which obviously uses fish mortality as index. In aquaculture, however, the interest is not only to know the concentration of ammonia that kills the fish, but also to define any sublethal adverse effect of ammonia on fish production (Colt and Tchobanoglous, 1978). Thus, Muir (1982) reported that environmental requirements based on threshold values in short-term toxicity tests must be interpreted with care for application in the longer-term sublethal exposures.

Long-term ammonia toxicity tests necessitate fish feeding. The relationship between food consumption and ammonia toxicity has been neglected in most studies, and in fact most standard toxicity tests prohibit feeding. This subject has been reviewed recently by Bengtson et.

al. (1986) who concluded that nutritional status affects the results of toxicity tests.

Ammonia is present in water in two forms; unionised (NH_3) and ionised (NH_4^+) ammonia. The concentration of these two forms in water depends on pH, temperature and salinity, with the pH exerting the greatest effect. Research on ammonia toxicity is normally performed at a single pH assuming that NH_3 is the only toxic species and that its toxicity is constant over the pH range. However, recent research by Thurston et. al. (1981) indicated either that NH_4^+ is also toxic, or that increased H^+ concentration increases NH_3 toxicity. They recommended, therefore, that water quality criteria include consideration of the pH dependence of ammonia toxicity.

Although it has been customarily reported that tilapia are very tolerant of high ammonia levels (Balarin and Haller, 1982), there is in fact very little research on its ammonia toxicity: the paper of Reader and Stickney (1979) on O. aureus seems to be the only published source. Other studies are available but concern the culture of tilapia in recirculating systems (Otte and Rosenthal, 1979) and were not designed to evaluate the long term effect of different ammonia levels. The objective of this study was to evaluate the

effect of different ammonia levels on the growth of two sizes of O. spilurus in sea water at two pH levels.

Materials and Methods:

Fish Stock:

The small fish used were the F_1 generation of the original imported stock from Kenya, while the large fish were the original imported stock. Fish were acclimated to sea water as described in Chapter 2. The sizes of the fish used in this study are shown in Table 5.1.

Experimental Work Plan:

Three experiments were conducted over the 5-month summer season period (April to August, 1985). A summary of these experiments is shown in Table 1. The experimental ammonia levels were selected so that the median treatment will be 0.5mg NH_3N/l. This value was reported by Muir (1982) as a typical ammonia level of effluent in a recycled system. Furthermore, Colt and Tochobanoglous, 1978) reported that the concentration that reduces the growth of channel catfish by 50% is 0.5 mg NH_3N/l.

Holding System:

The same 500l EVOS tanks were used in the 3 experiments. For experiments 1 and 2 with small O. spilurus 3 small cage nets (in which the fish were

Table 5.1 Summary of Experimental Work Plan.

Experiment No.	Fish Size (g)	Ammonia Levels NH_4 Mmg/L	Ammonia Levels NH_3 Mmg/L	pH	Duration (Days)
1	19	1	0.0	7.9	30
		15	0.5		
		30	1.0		
		60	2.0		
		90	3.0		
		120	4.0		
		150	5.0		
2	12	0	0.0	7.3	40
		15	0.13		
		30	0.25		
		60	0.5		
3	140	0	0.0	7.9	30
		15	0.5		
		30	1.0		
		60	2.0		
		90	3.0		
		120	4.0		
		150	5.0		

stocked) were placed in each tank. These cages (45 x 45 x 70cm depth) are made from a rigid PVC netting with a plastic bottom. Each cage occupies one-quarter of the tank. In experiment 3 on large O. spilurus the fish were stocked directly into the tank, so that no partitioning was performed.

Water Flow System:

Water flow in these tanks was not controlled from the water inlet, as normally done, but from the water outlet. The water is actually siphoned out by a small hose inserted in the 2" stand pipe of the tank. The outflowing water is controlled by the siphon hose diameter, siphon hose height, and water depth in the tank. In this study a hose with a 4mm diameter was used. The height of the siphon is controlled by hooking the hose on a chain hung from the edge of the tank. Water depth is controlled by installing a plastic domestic water float on the ½" tank inlet. This float maintains a constant water depth regardless of the inlet water pressure. Fig 5.1 shows the basic tank design with the water flow system used in this experiment. The water flow rate was adjusted to 250ml/min in all tanks.

Ammonium Chloride Solution Dosing System:

The stock solution of NH_4Cl was prepared by dissolving NH_4Cl salt in sea water and then adjusting pH

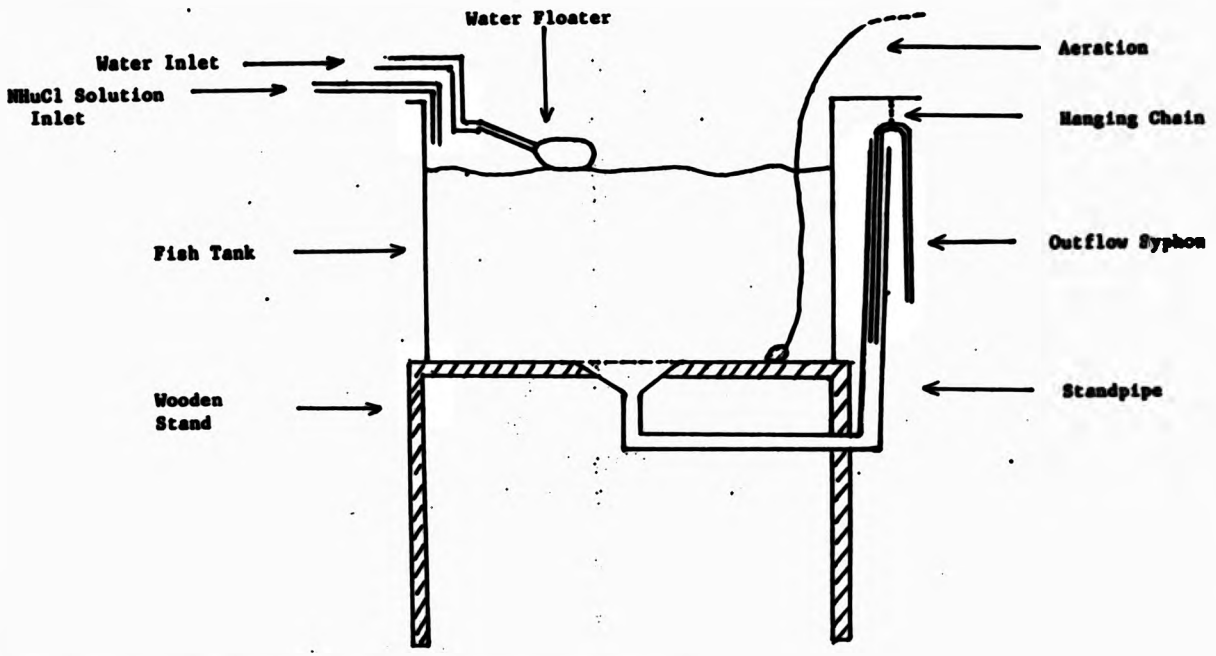


Fig 5.1 Basic tank design used in ammonia toxicity trials.

with 10N-NaOH. The NH_4Cl solution was pumped with a 4-channel peristaltic pump (Sage Instruments - 375A). One channel was assigned to each tank. Five litre plastic dark containers were used for the NH_4Cl stock solution; one container was assigned for each tank. The peristaltic pump was adjusted to a rate of 60ml/hr for all tanks. Thus, each tank received the same water flow rate and the same NH_4Cl solution flow rate, but of different concentration.

Food and Feeding:

A commercial, Taiwanese, tilapia diet (40% protein) was used in all experiments. Small fish were fed on 2mm pellets 3 times daily, while the large fish were fed twice daily on 4mm pellets. The fish were fed up to satiation as judged by cessation of feeding from the tank bottom.

Water Analysis:

Ammonia and pH levels were measured twice a week. For ammonia determination the procedure of Strickland and Parsons (1972) was followed. Un-ionozed ammonia level was caluclated from standard tables (Bower and Bidwell, 1978). Different serial dilutions with filtered, aged sea water was applied to lower the high treatment ammonia levels to detecting limit. Other parameters were measured as described in Chapter 2.

Statistical Analysis:

Survival rate was analysed as described by Sprague (1969). For weight gain and food consumption the analysis of variance was not performed because the data collected in this study represents only one replicate. Therefore, a regression analysis was developed first, and then the significance of this regression was tested statistically as described by Zar (1984).

Results:

(1) Survival Rate:

The survival rate for all the experiments is shown in Table 5.2. No threshold mortality (LC_{50}) was acheived with either the 24hr or 96hr trial with the ammonia concentrations tested. Thus, the incipient LC_{50} was calculated as suggested by Sprague (1969) by plotting the percent of killed fish over a longer time period than 4 days (2 weeks was selected in this study) on a probit-log paper. The mortality curves are fitted by eye as the best fit, and are shown for small and large fish in Fig 5.2 and 5.3, respectively. According to this procedure the incipient LC_{50} of small and large O. spilurus is 2.70 and 2.50mg NH_3N/l, respectively. No clear relationship was found between the survival rate and the total ammonia concentration in experiment 2 with low pH.

Table 5.2 Summary of the Experimental Results

Experiment No.	Fish Size	Ammonia Level NH3-N mg/L	C.V	Survival Rate Initial No.	Final No.	Survival %	Growth Rate Initial Total weight (g)	Final Total weight (g)	Weight gain (g)	Weight gain as % of control	Feeding Rate %BW/D	% of control
1	Small	0.04	48.65	15	14.3	95.56	279.50	430.63	151.13	100.00	1.67	100.00
		0.47	10.64	15	15	100.00	234.27	384.47	150.20	99.38	1.45	85.88
		1.06	51.89	15	14.7	97.78	281.83	352.10	70.27	46.50	1.01	66.47
		1.78	46.07	15	11	73.33	264.97	209.30	- 55.67	- 36.84	0.73	42.35
		2.30	46.52	15	4.3	28.89	295.00	80.23	-214.77	-142.11	-	-
		4.13	-	15	0	0	285.70	-	-	-	-	-
		4.89	-	15	0	0	300.77	-	-	-	-	-
3	Large	0.03	73.03	15	15	100.00	1951.5	2591.4	639.9	100.00	1.13	100.00
		0.63	25.32	15	15	100.00	2205.2	2459.5	254.4	39.76	0.62	80.81
		1.20	18.41	15	15	100.00	2117.6	2269.9	152.3	23.80	0.52	63.64
		1.98	16.69	15	11	73.33	2029.5	1566.3	- 463.2	- 72.39	0.40	39.39
		2.74	25.84	15	7	46.67	1976.5	924.8	-1051.5	-164.32	0.20	15.15
		3.12		15	0	0	2262.4	0	0	0	-	-
		5.29		15	0	0	2166.5	0	0	0	-	-

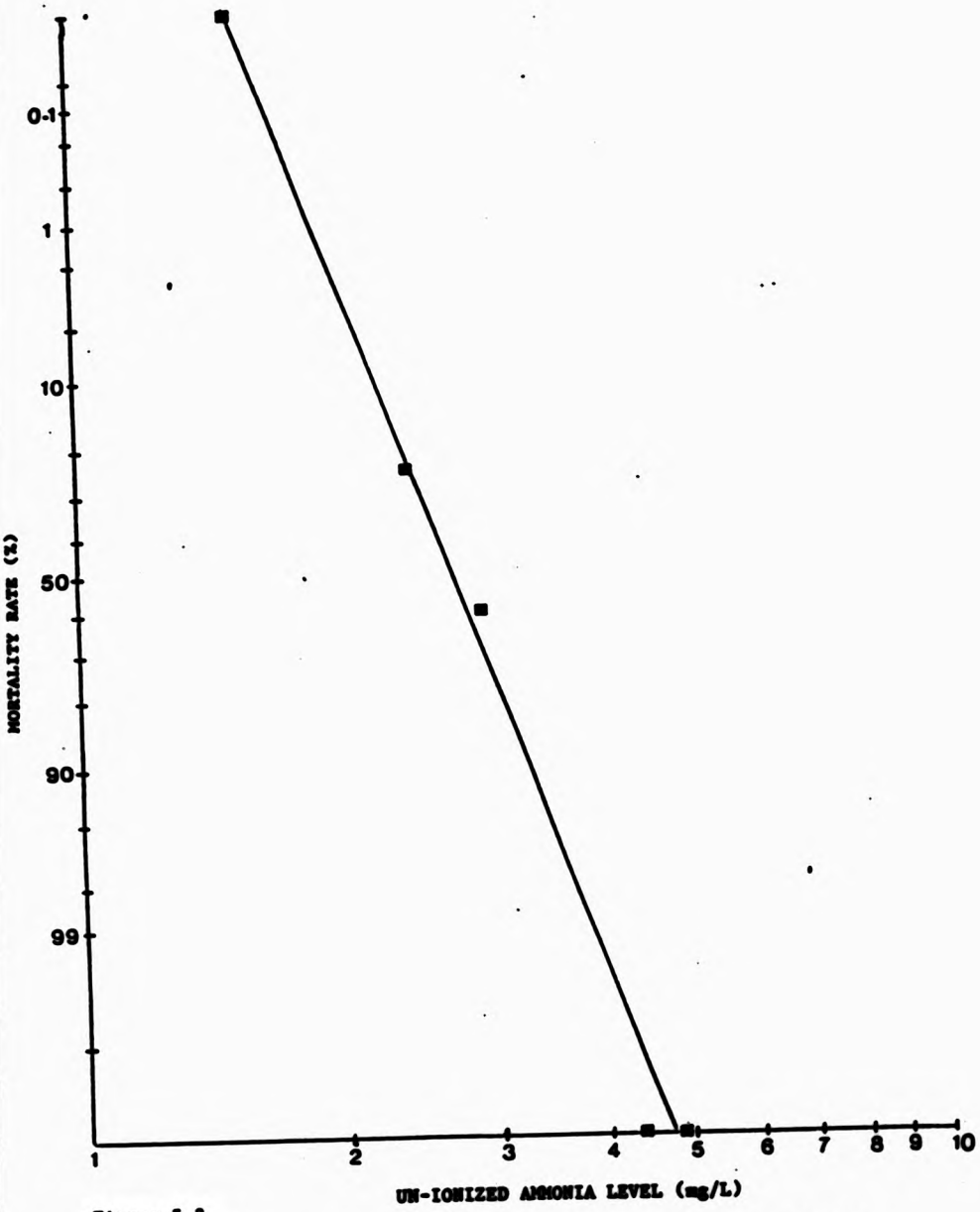


Figure 5.2
Relationship between un-ionized ammonia level and mortality rate for small O.spilurus.

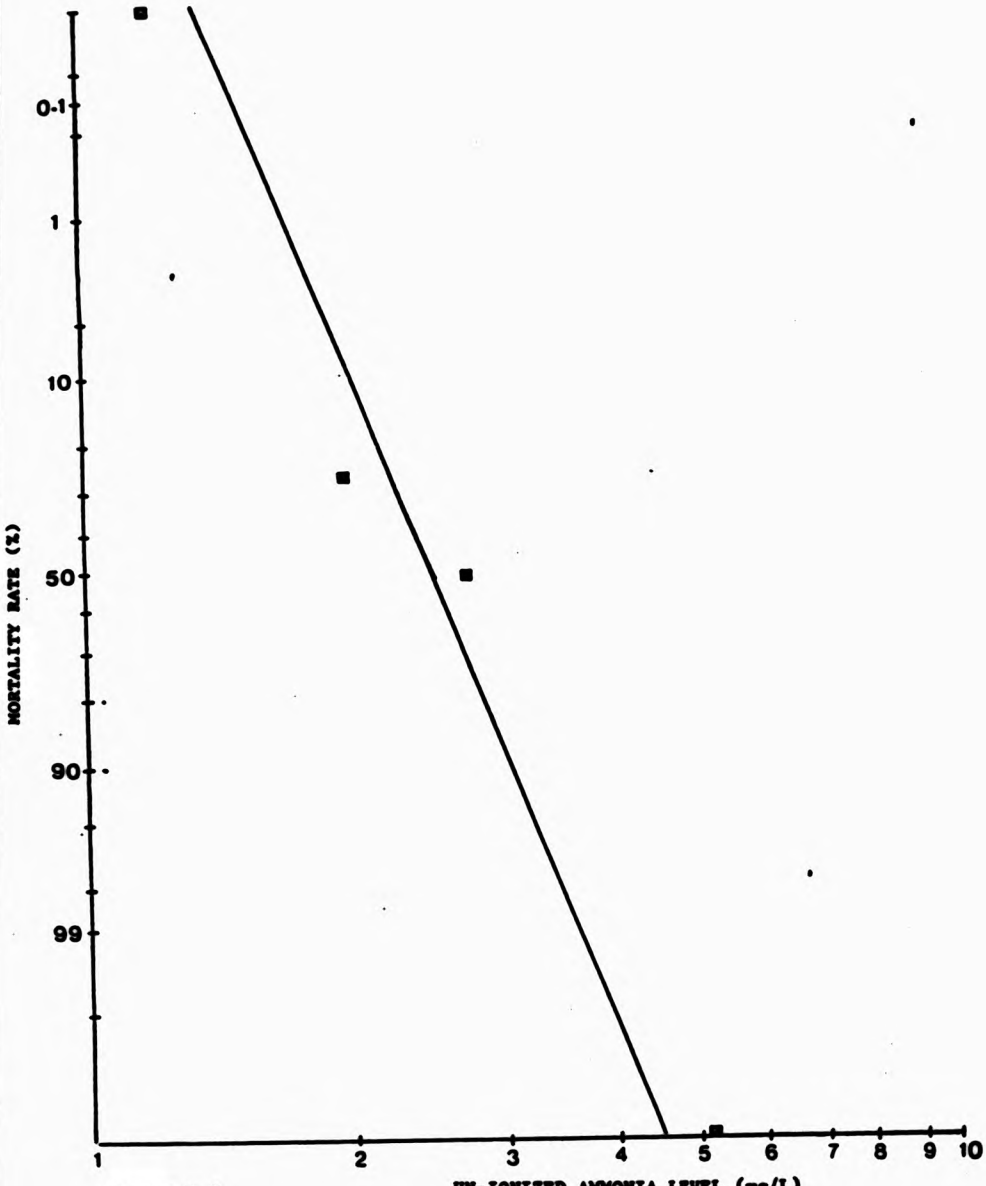


Figure 5.3

Relationship between un-ionized ammonia level and mortality rate for large O.spilurus.

(2) Growth Rate:

The initial and final weights and weight gain for experiment 1 and 3 are presented in Table 5.2. Reported weight gains represent the pooled weight of all the fish in each tank. Fish stocked in the different cage nets within each tank in experiment 1 and 2 were pooled together. Pooled weights were not corrected for either mortality or no-growth fish. therefore, weight gain (or loss) can be due to a combination of the weight response and mortality. The highest two ammonia concentrations in both experiments 1 and 3 were excluded from the regression analysis because of the complete mortality occurring at these concentrations.

Generally, there is a negative relationship between ammonia and growth rate. The relationship is linear for both NH_3-N and NH_4^+-N at the normal pH level (Figs 5.4 and 5.5). At low pH, however, the linear regression equation failed to describe the relationship between growth rate and total ammonia as evident from the low R^2 value (0.50). Table 5.3 summarises the linear regression components of the ammonia-growth relationship for the two fish sizes calculated as a percentage of the control. Size apparently seems to have no effect on ammonia toxicity. The effective ammonia concentration that reduces the growth by 50% (EC-50) was 0.65 and 0.55mg NH_3-N/l for small and large

Table 5.3 Linear Regression of Weight Gain (loss) as a percent of the Control (Y), versus NH_3mg/L(X).

Fish Size	b_0	b_1	r	R^2
Small	134.99	-107.62	-0.96	0.93
Large	111.27	- 95.67	-0.99	0.97

Table 5.4 Linear Regression of food eaten as a precent of the control (Y) versus NH_3mg/L(X).

Fish Size	b_0	b_1	r	R^2
Small	1.72	-0.56	-0.99	0.98
Large	1.00	-0.31	-0.93	0.92

O. spilurus, respectively. Whereas the "no growth" levels were 1.20 and 1.15mg NH_3N/l for small and large fish, respectively.

In experiment 2 with low pH the data collected were limited to total ammonia level of 60mg NH_4^+N/l, as there was a mechanical failure in the pump supplying NH_4Cl stock solution at higher concentrations. Nevertheless, within the range of the data collected there was a negative relationship between NH_4^+N level and growth rate. However, this relationship was with a fairly low correlation (-0.71). Furthermore, the linear regression failed to demonstrate this relationship as indicated from the R^2 value which was 0.50.

(3) Food Consumption:

Food consumption rate shows also a negative linear relationship with un-ionised ammonia. This relationship is summarised in Table 5.4 and shown in Fig 5.6 and 5.7. Feed efficiency ratio was calculated from the two regression curves derived for growth and feeding rates. Feed conversion efficiency shows also a negative linear relationship with un-ionised ammonia as shown in Fig 5.5 and 5.7. The slope of the feed efficiency ratio is lower than that of feeding rate suggesting that un-ionised ammonia affects both. It should be noted, however, that feed efficiency ratio over the whole

feeding range is curilinear (Brett 1979). The linear relationship found in this study probably reflects the limited feeding range (0-1.67% BW/D) over which the feed efficiency ratio was calculated.

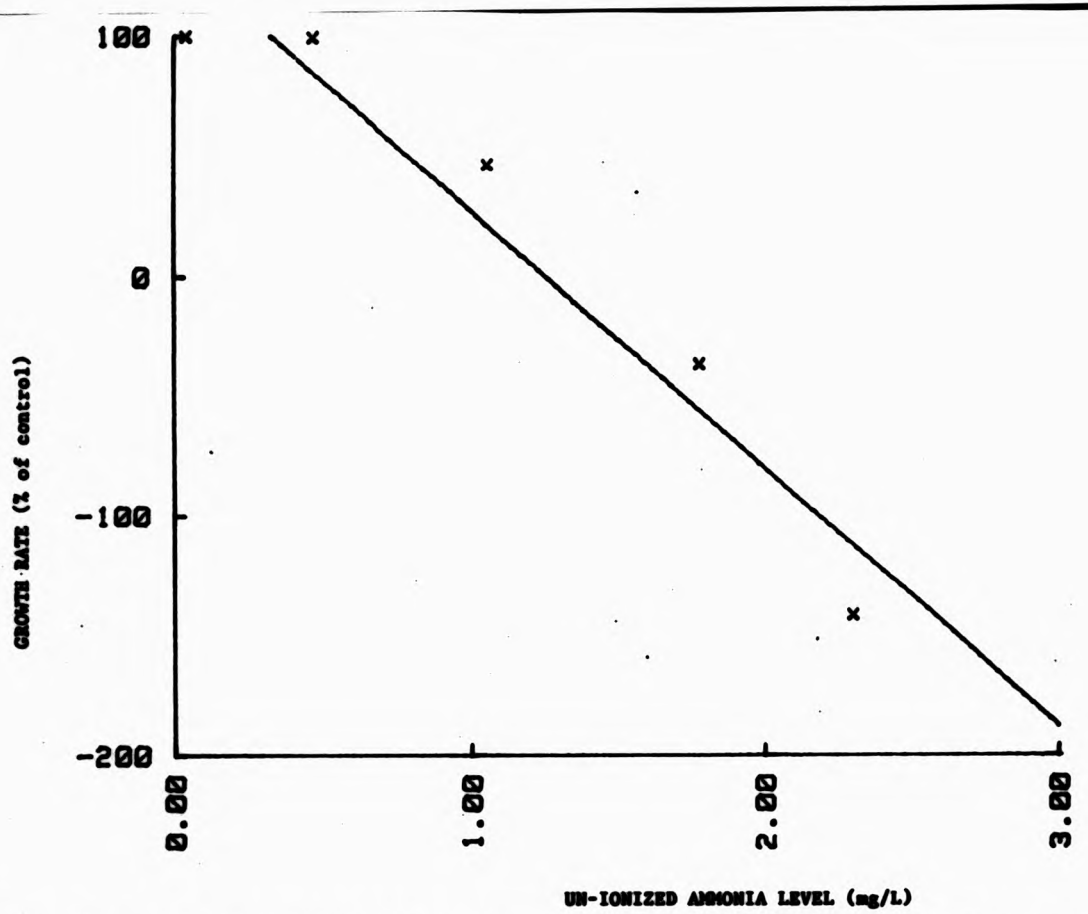


Figure 5.4 Relationship between un-ionized ammonia level and growth rate for small *O.spilurus*.

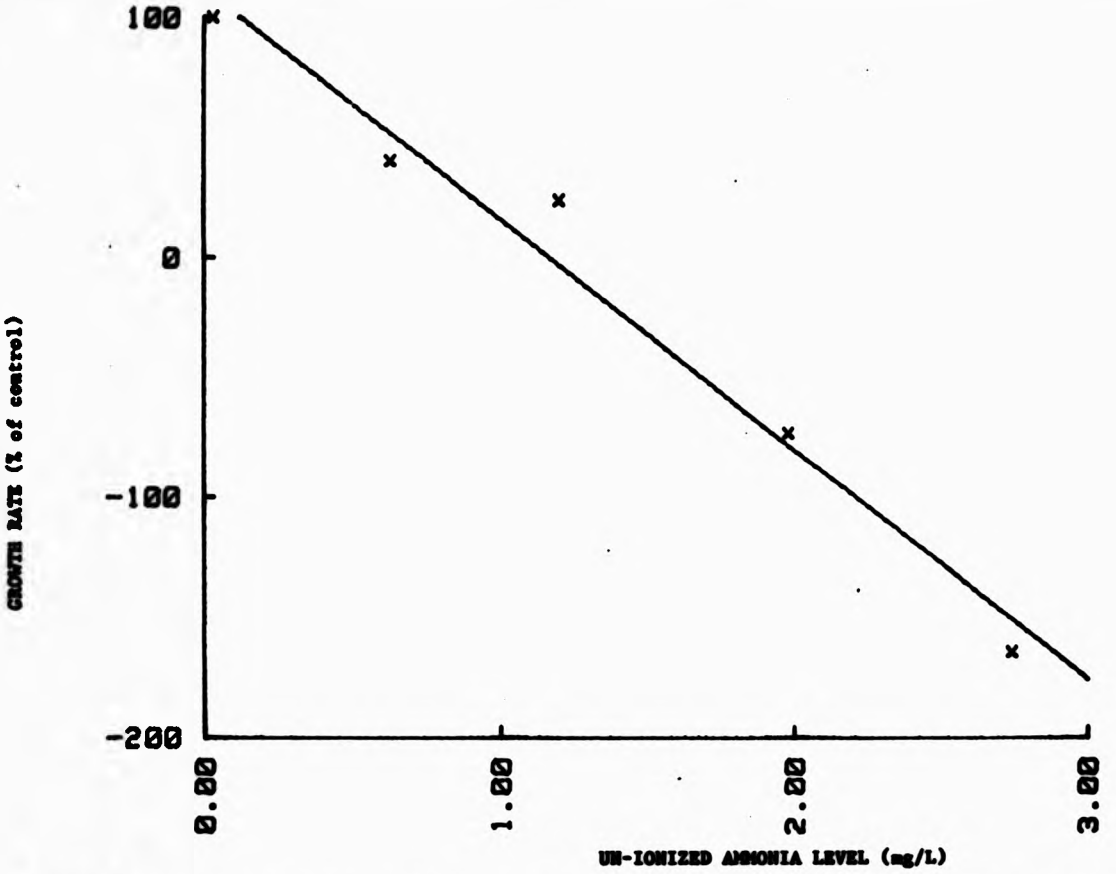


Figure 5.5 Relationship between un-ionized ammonia level and growth rate for large O.spilurus.

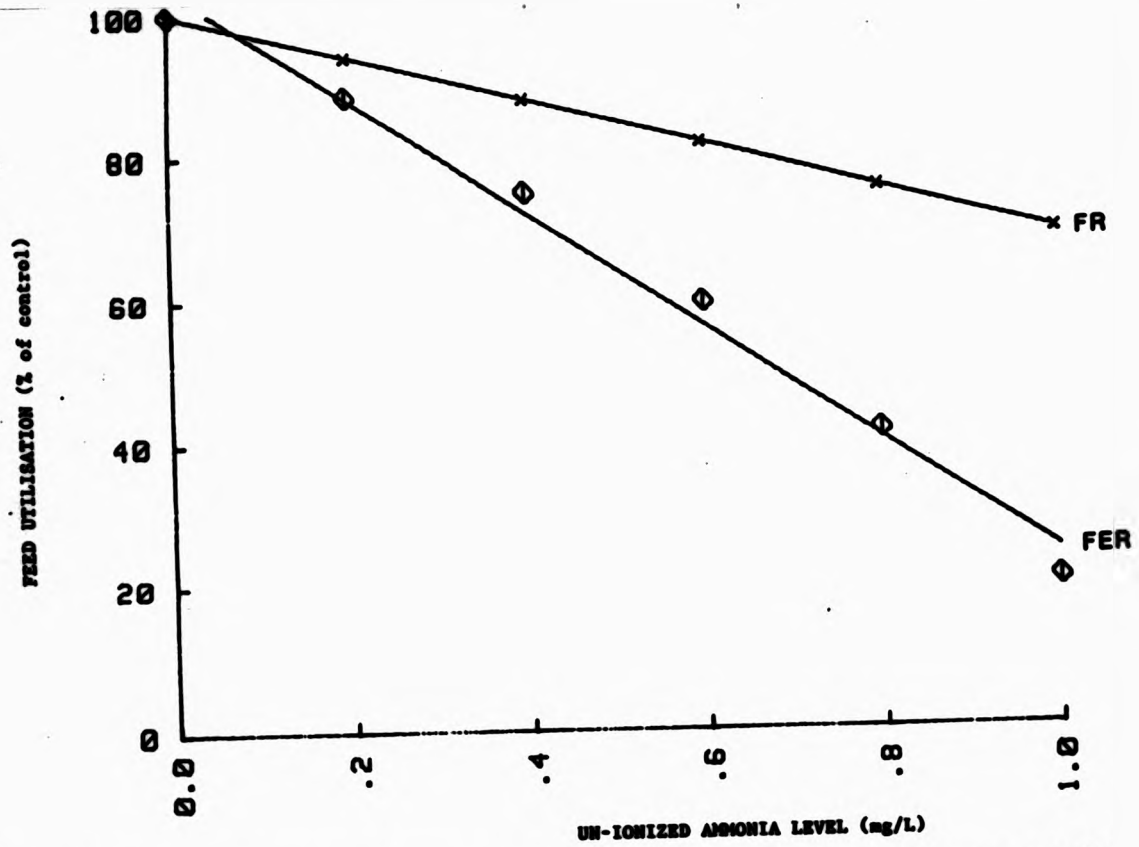


Figure 5.6 Effect of un-ionized ammonia level on feeding rate (X) and feed efficiency ratio (O) for small O.spilurus.

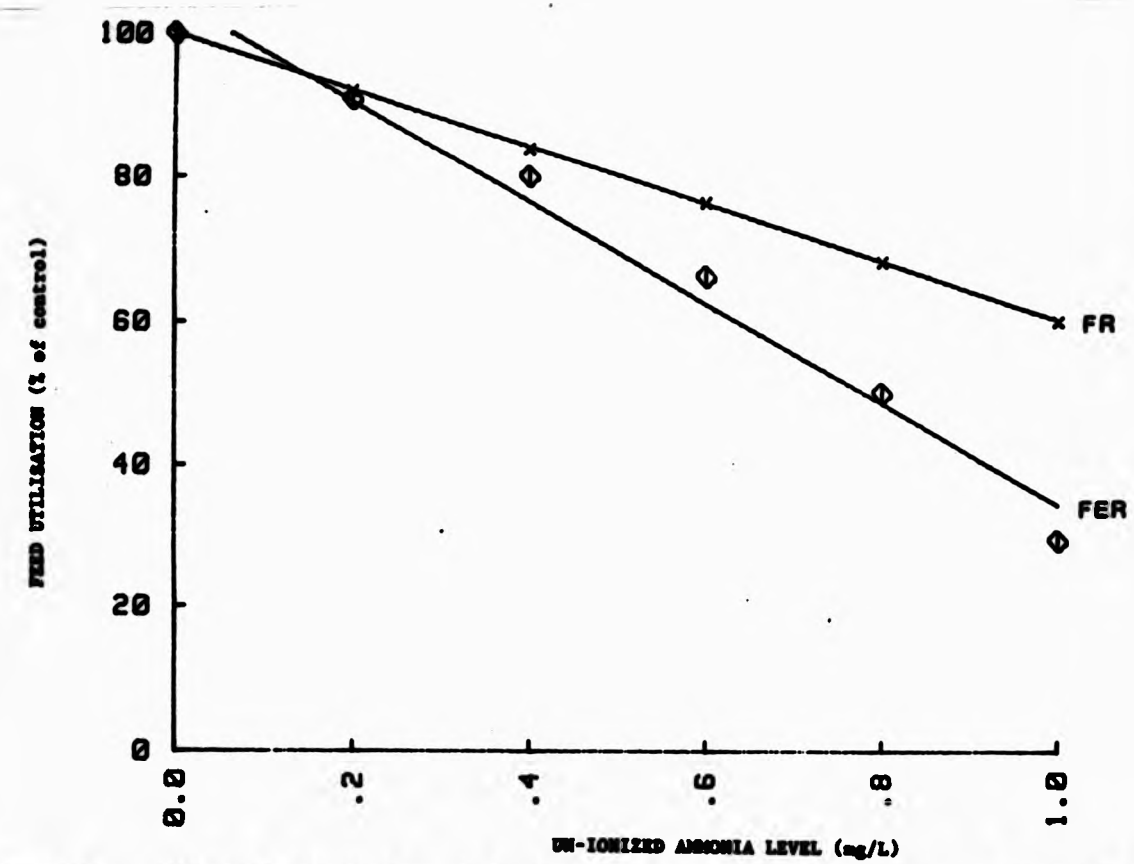


Figure 5.7 Effect of un-ionised ammonia level on feeding rate (X) and feed efficiency ratio (O) for large O.spilurus.

Discussion:

It was realised at the beginning of this study that experimental replication in such a water flow system is difficult. The ammonia levels in the tanks were found to fluctuate during the course of experiments; the average value was considered as the experimental treatment. The coefficient of variation (CV) for the ammonia levels in the tanks ranges from 10.64 to 48.65 for small *O. epilurus* in experiment 1, and from 16.99 to 25.84 for large fish in experiment 3. In spite of the relatively high fluctuation in the un-ionised levels at each treatment it was possible to draw simple linear regression curves from these data. The correlation between un-ionised ammonia and weight gains was high (-0.96 and -0.99 for small and large fish respectively). The high correlation with such a fairly high CV might be explained by an additive effect of ammonia.

Overall, it seems that the resistance of sea water acclimated *O. epilurus* to ammonia toxicity is very high. The incipient LC-50 values observed in this study, 2.70 and 2.50mg NH_3N/l for small and large fish, fall within the normal range of LC-50 levels for other fishes. Render and Stickney (1979) reported that the 48hr LC-50 for *O. aureus* is 2.40mg NH_3N/l, which is very close to the values obtained in this study. Meade (1985)

reported that the 96hr LC-50 values range from 0.32 to 3.10mg NH_3N/l, with rainbow trout and channel catfish at the respective extremes. The comparison between 96hr LC-50 and incipient LC-50 is probably valid; thus Hasan and Macintosh (1986) reported that potentially toxic ammonia level may be tolerated by the fish for longer periods once they have survived a certain exposure. Furthermore, Thurston and Russo (1983) reported that the LC-50 values obtained from 12- and 35-day tests with rainbow trout were not appreciably different from those tests of shorter time periods. Thus, it can be concluded that O. spilurus falls in the higher tolerant range for ammonia toxicity.

Colt and Tchobanoglous (1978) used the concept of EC-50 for measuring the effect of ammonia toxicity on fish growth rate. The EC-50 for O. spilurus found in this study was 0.60 and 0.58mg NH_3N/l for small and large fish, respectively. Interestingly, these values are very close to those reported by Colt and Tchobanoglous (1978) for channel catfish, which was 0.52mg NH_3N/l. The reduction in the growth rate of O. spilurus in this study was associated with a similar reduction in the feeding rate. Palanichamy et. al. (1985) also reported reduced food consumption of O. mossambicus with increasing concentrations of diammonium phosphate.

The decrease in the feeding rate at high ammonia levels may be due to loss of appetite. Literature suggests that accumulation of nitrogenous metabolites in the medium affects appetite (Groves and Kogel, 1973). Knights (1985) reported an increase in the fish metabolism (hypermetabolism) under ammonia stress. Hypermetabolism will reduce the metabolic scope. Priede (1985) reported that some fishes regulate their feeding activity simply to keep their metabolic rate within the bounds of the metabolic scope. Therefore, it seems that the reduction in appetite and feeding rate with ammonia stress could be partly due to the self-adjusting of the food consumption by the fish themselves to maintain feeding metabolism within the reduced metabolic scope.

There are indications also that feed utilisation efficiency is affected by the ambient ammonia level. This is demonstrated by the difference in the regression slopes for growth and feeding rates, showing that the reduction in the growth rate is more than that in the feeding rate and that feed utilisation efficiency is accordingly reduced. The reduction in the feed utilisation efficiency with the hypermetabolism reported earlier could be due to a new physiological demand required for ammonia detoxification. For example, Sreeramulu Chetty et. al. (1979) found increased levels of urea during induced ammonia stress in O. mossambicus

which was explained as a detoxification mechanism for blood ammonia.

On the other hand, ammonia stress might reduce feed utilisation efficiency by reducing the energy production systems in the body. For example, Russo and Thurston (1977) reported that ammonia may be an uncoupler of oxidative phosphorylation, and reduced growth may result from the inability of the animal to convert food energy to ATP. Corticosteroid hormones are also released proportionally to sublethal ammonia exposure. These hormones cause a negative nitrogen balance deaminating amino acids. Such deaminated amino acids are not available for protein synthesis essential for growth. In summary, the reduced growth observed at high ammonia levels could be due to any or all of the reduction in feeding rate, higher energy required for ammonia detoxification, and low efficiency of energy production in the fish.

As there is no substantial evidence of the independent rate of H^+ or NH_4^+ in ammonia toxicity in these trials, lowering pH seems to be a very good means for reducing the toxicity of ammonia. The mean EC-50 for the two fish sizes is 0.59mg NH_3-N/l. This concentration of un-ionised ammonia is equivalent to 17 and 82mg NH_4^+-N/l (total ammonia) at pH values of 7.9 and

7.3, respectively at the salinity and temperature conditions used in this experiment. Thus, by lowering the pH 0.6 units the total ammonia concentration can be increased by about 4.85 times without any reduction in the survival or growth rates.

Fish size seems to have no significant effect on ammonia toxicity of C. apilurus ranging from 12 to 200g bw. Thurston et. al. (1983) concluded that ammonia toxicity does not appear to be influenced by the fish size for fathead minnows ranging from 0.1 to 2.3g. Furthermore, Haywood (1983) reported that size difference had no apparent effect on susceptibility to ammonia.

CHAPTER 6: Final Discussion

It was identified at the beginning of this study that the effect of protein intake on both growth and ammonia excretion rates is controlled mainly by three factors: protein quality, protein : energy ratio, and feeding rate. The effect of protein quality was not tested in this study, though an optimum protein source, fish meal, was used.

The results of this study show that O. spilurus can utilise fish meal as a protein source very efficiently. This was demonstrated at the lowest protein intake tested 4.99g/kg/d. With this low protein intake it was possible to record a positive growth rate (SGR = 0.91) with small O. spilurus. Furthermore, protein utilisation for growth was very high. This is demonstrated by the high PER (2.36) and low ammonia-nitrogen excretion rate (6.87% of the nitrogen consumed). This indicates that the majority of the protein consumed was directed towards growth. This efficient protein utilisation is not surprising for an omnivorous fish such as O. spilurus. Pandian and Vivekanandan (1985) reported that herbivorous and omnivorous fishes are capable of digesting proteins as efficiently as the carnivorous. This is achieved by a more intensive proteolytic digestion occurring in the long digestive tracts of herbivorous and omnivorous fishes.

The significance of P:E ratio of the diet and feeding rate in determining the protein intake for growth and ammonia excretion rates was studied. The results of this study show that both growth and ammonia excretion rates are affected by P:E ratio and feeding rate and in fact it was possible to record some similarities between the two. However, some differences were also noticed.

Similarities Between Growth and Ammonia Excretion Rates:

It was noted that the effect of P:E ratio on both growth and ammonia excretion rates is linear, whereas the food ration effect was quadratic. This similarity between growth and ammonia excretion rates is not only qualitative but also quantitative. For example, the maximum feeding rates derived from feeding rate - ammonia excretion rate curves (Chapter 4) were 5.84, 4.68 and 4.28% bw/d which are very close to those values derived from feeding rate - SGR curves (Chapter 3) which were 5.41, 4.97 and 4.60% bw/d for 24.94, 34.44 and 43.35% protein diets, respectively. This means that the maximum food ration for any particular diet can be derived from both growth and ammonia excretion measurements and the values obtained from both estimates are very close.

This similarity between feeding rate-growth curves and feeding rate-ammonia excretion curves raises the possibility that growth and ammonia excretion can be related to each other. By compiling the growth data from Chapter 3 and ammonia excretion data from Chapter 4, it was possible to draw a positive linear relationship with high correlation between SGR and ammonia outputs for all diets. This relationship is summarised in Table 1 and shown in Fig 1. The slopes of these three regressions are statistically different; therefore a common slope can not be computed. This indicates the significant contribution of P:E ratio to this relationship. Nevertheless, this relationship indicates that growth is associated with protein catabolism. Very little information is presented on this relationship. Hambrey (1980) found also a linear relationship with a slope of 0.413 between ammonia excretion and growth rate (SGR x biomass) with rainbow trout.

This similarity between growth and ammonia excretion rates is in agreement with the general concept of protein utilisation for energy in fish. Jobling (1981a) reported that as fish utilise protein as their primary source of energy, rates of nitrogenous metabolism and excretion should reflect general trends in the overall metabolic rate of the fish.

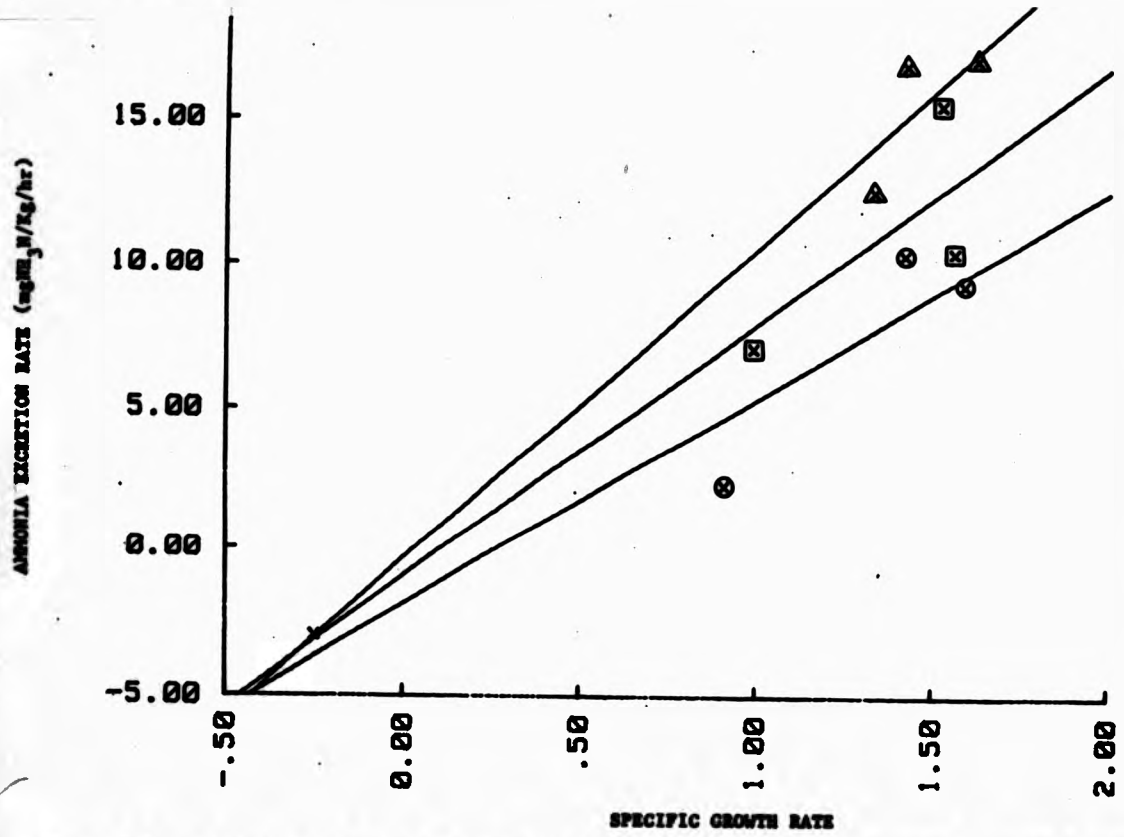


Figure 6.1 Relationship between growth rate (SGR) and ammonia excretion rate for small O.spilurus fed on 3 diets: 24.94% (O), 34.44% (⊠) and 43.35% (△) protein.

Nitrogen Balance v.s. Growth:

In view of this similarity between growth and ammonia excretion rates some scientists suggested the use of a nitrogenous loss measurements approach to differentiate between different feeding regimes. For example, Jobling (1981a) found that the short-term monitoring of nitrogenous excretion can give reliable estimates of endogenous rate of excretion and maintenance requirements and may, therefore, provide a quick assessment of food-growth relationship of fish. Furthermore, Rychly (1980) found that values obtained when working with different groups of fish and when investigating only one group coincided very well. Thus there may also be an advantage in that it is possible to work with only one fish group fed on different diets. In that case genetic differences of the fish can be excluded, which leads to a better reliability of the results.

To estimate the endogenous and maintenance nitrogen requirements Jobling (1981a) suggested formulating a relationship between digestible nitrogen fed and nitrogen retention. This relationship is shown in Fig 2 and 3 for small and large O. spilurus. A linear relationship was found between digestible nitrogen and nitrogen retention as described by Jobling (1981a). The intercept of the Y axis represents the

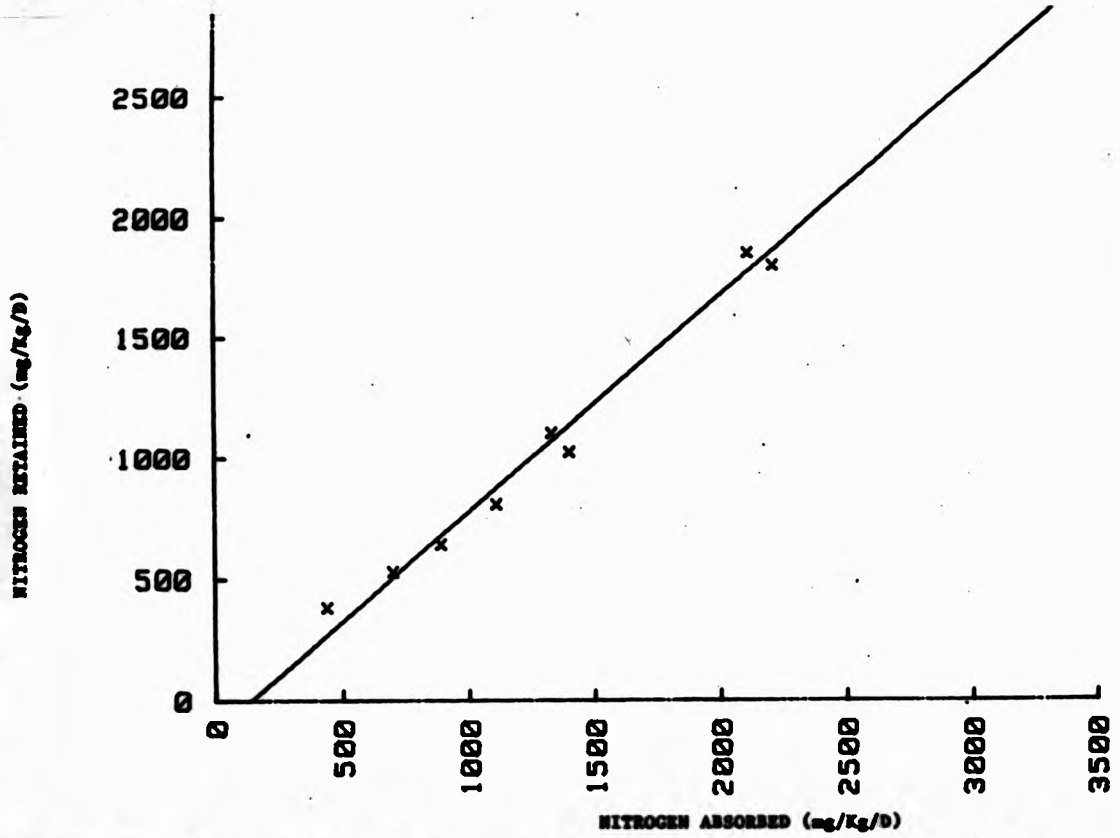


Figure 6.2 Relationship between nitrogen absorbed and nitrogen retained for small O.spilurus.

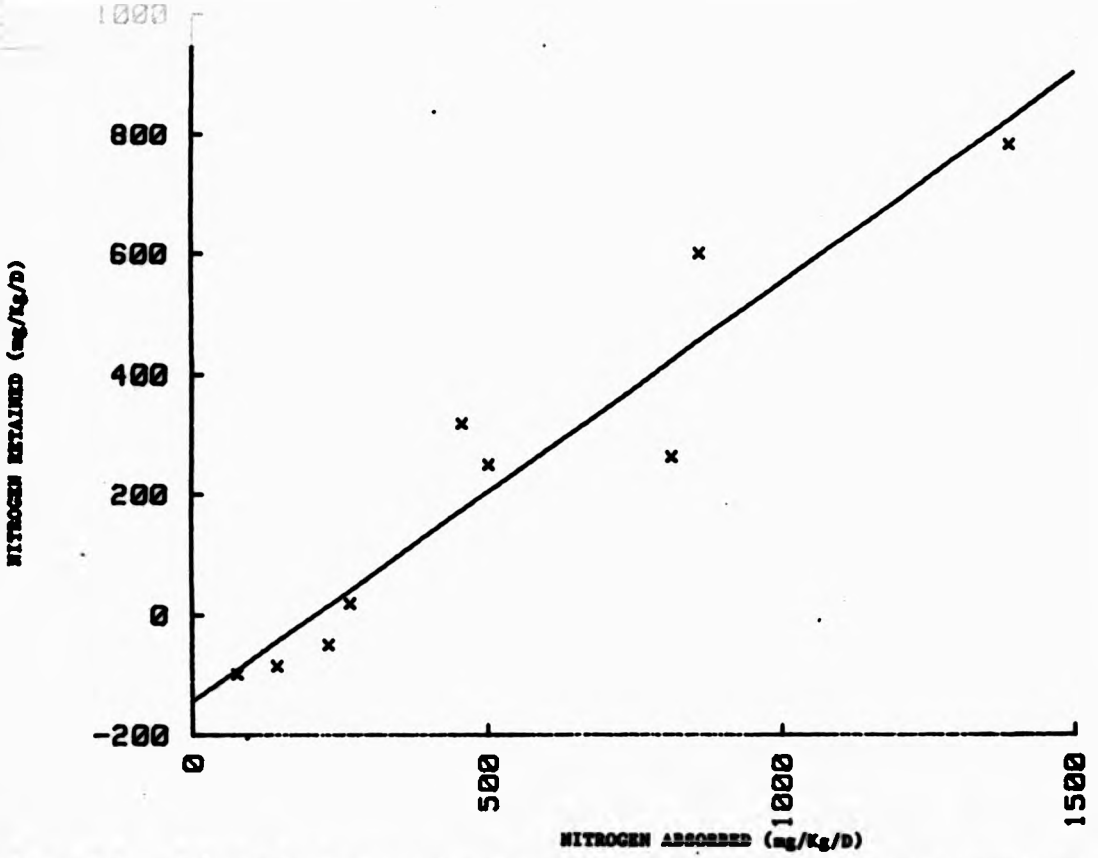


Figure 6.3 Relationship between nitrogen absorbed and nitrogen retained for large O.aquilurus.

endogenous nitrogen excretion and was found to be 122.93 and 144.03mg N/kg/d for small and large O. spilurus, respectively. However, these values are different from those derived from starved fish which were 71.52 and 96.84mg NH_4^+-N/kg/d for small and large O. spilurus, respectively. Jobling (1981a) found also differences in the endogenous nitrogen excretion rates between the two estimates with plaice and he recommended the value derived from the nitrogen absorbed-nitrogen retained curve as the true estimate.

It is interesting to note that these two values derived from the nitrogen absorbed - nitrogen retained curve are higher than the actual ammonia excretion estimates obtained from starved fish. It is worth noting that those fishes were starved for several days (10 days) and their lower ammonia excretion rates might represent an adaptive mechanism for long starvation. Hepher et. al. (1983) working on the effect of starvation on body losses in red tilapia reached the same conclusion. They found that red tilapia lost weight in the first fasting week more than the subsequent weeks. They suggested that some intrinsic mechanisms of adaptation to starvation as low feeding level may therefore be involved, and metabolism is reduced to routine metabolic levels only after a period of such adaptation.

Besides the estimation of the endogenous nitrogen excretion rate the nitrogen absorbed - nitrogen retained curve can provide other valuable information. The maintenance nitrogen requirement can be estimated as the intercept of the X axis when retention (growth) is zero (Jobling, 1981a) and was found to be 136 and 207mg N/kg/d for small and large O. spilurus, respectively. Jobling (1981a) reported a maintenance nitrogen requirement of 150mg N/kg/d for 35.11g plaice at 10°C whereas Rammarine et. al. (1987) reported a value of 303mg N/kg/d for 199.3g Atlantic cod at 14°C.

Although variation in temperature and fish sizes can cause differences in the maintenance nitrogen requirement, it seems that the large difference in the maintenance nitrogen requirement found in the literature is not solely due to variations in temperatures and fish sizes. In this study a maintenance nitrogen requirement of 136mg N/kg/d was estimated for small O. spilurus when ammonia-nitrogen excreted was considered as the only excreted nitrogenous compound. However, when urea-nitrogen was included with the ammonia-nitrogen to form the total excreted nitrogen a different maintenance nitrogen requirement was found (266mg N/kg/d). Similarly, the endogenous nitrogen excreted changed from 122.93 to 236.44 mg N/kg/d when urea-nitrogen was included. It is expected, therefore, that a different

endogenous and maintenance nitrogen requirement will result if other excreted nitrogenous compounds are included to form the total nitrogen excretion rate. Therefore, care must be taken when comparing different estimates from different studies about the "components" of the total nitrogen excretion rate.

For a given nitrogen intake small O. spilurus seems to have higher nitrogen retention than large individuals. For example at 1.0g nitrogen intake small fish will retain 817.75mg N/kg/d whereas large fish will retain 668.73mg N/kg/d. These estimates are based on the assumption that ammonia is the only nitrogenous excreted compound. It is not clear whether this difference is a result of size or it results from the fact that large fishes are non-growing fish. Similarly Gerking (1971) working with blue gill sunfish and Rychly (1980) working with rainbow trout found a negative correlation between body weight and nitrogen retention.

When nitrogen retention is related to nitrogen intake, the nitrogen efficiency can be calculated (Savitz et. al. 1977). When the nitrogen efficiency data obtained from Chapter 4 is compared with those derived from growth and body composition measurements (Chapter 3), 3 interesting observations can be drawn. First, nitrogen efficiency data obtained for all diets

fed at 2% bw/d by the two estimates show approximately the same trend. However, estimates obtained from body composition were always of lower magnitude. Second, 24.94% protein diet fed at all rations also shows approximately the same trend by the two estimates. The balance method yields also a higher estimates. Third, estimates obtained from balance method for medium and high protein diets fed at medium and high food rations show the opposite trend to that obtained by growth and body composition measurements.

According to the balance method nitrogen efficiency increases with increasing dietary protein level and feeding rate, whereas it decreased with the other estimate. Table 3 compares the nitrogen efficiency estimated by both nitrogen balance method and growth and carcass analysis.

This comparison shows that the nitrogen balance method cannot be used, in general terms, as a substitute to normal growth and body composition analysis method. However, it indeed shows a trend that the nitrogen balance method can give a clear indication for comparing different diets fed at low ration or evaluating different food rations for a low protein diet. It must be noted that the values obtained by the nitrogen balance method are usually higher (1-10%) than the

growth estimates. The nitrogen balance method, however, will not give true trends for high protein diets fed at high rations. This is strange since it has been mentioned earlier that growth rate (nitrogen retention) shows a linear relationship with ammonia excretion rate. However, it must be noted that in this linear relationship between growth rate and ammonia excretion rate there was a significant difference between the slopes of the different dietary protein diets. This reflects the significant contribution of the non-protein energy sources to the anabolic growth process.

It must be noted that the nitrogen balance method is based on measurement of nitrogenous excretion, mainly ammonia, which by themselves are a by-product of protein catabolism. On the other hand growth (or nitrogen retention) is an anabolic process. Therefore, care must be taken when comparing catabolic with an anabolic data. Brett (1976) reported that a catabolic expression can not be expected to apply directly to an anabolic process.

Differences Between Growth and Ammonia Excretion Rates:

In spite of the above mentioned similarities there are differences between the two relationships. These differences are noted from the significant difference observed among the slopes of the three linear

regressions developed between growth and ammonia excretion rates. Furthermore, there are also differences in the relative contribution of the independent variables on the dependent variables in the two multiple regression models. While protein percent of the diet was not incorporated as a variable in the growth model, it has a significant contribution in the ammonia excretion model. In order to understand the basis of these differences a comparison between growth and ammonia excretion data is presented.

A positive and fairly good growth rate (SGR = 0.91) was achieved with small O. spilurus fed on a low protein diet (24.94%) at low ration (2% bw/d). This low protein intake yields also a very good PER (2.38). This suggests that the majority of the protein consumed was used for growth. The ammonia excretion measurements support this view. The excreted ammonia-nitrogen represents only 6.87% of the total nitrogen consumed. This very high protein utilisation with such a low protein intake is not surprising. It was shown in Chapter 3 that many nutrition studies show such a result.

The high PER could be a physiological adaptation by the fish to the low protein intake in order to maximise its utilisation. Walton (1985) reported that

amino acids are preferentially required for protein synthesis, therefore animals must exercise some control over catabolic pathways especially when dietary supplies are restricted. With such a high PER the energy required for growth was obviously obtained from the non-protein energy sources. However, because of the limited energy supply (97 Kcal/kg/d) with this low feeding rate no further growth was possible.

Increasing the protein content of the diet by 9.50% and feeding at the same low feeding rate (2% bw/d) results in an improvement in the SGR from 0.91 to 0.99. In other words increasing protein intake by 38.08% results in increasing the growth by only 8.79%. This is probably because part of the extra protein was used for energy purposes to replace the reduction in the dextrin in the diet. PER measurements support this view and it is reduced by 23.53%.

Ammonia excretion measurements also support this view as the excretion rate increased by 212.13%. However, the very sharp increase in the ammonia output suggests that the amount of protein catabolised is more than the amount added. Nevertheless, with this reduction in PER and the increase in protein catabolism, it was possible to record 8.79% extra growth. This extra growth could be due to more metabolisable energy

content with high protein diet. Tacon and Cowey (1985) reported that diets with higher protein contents tends to have higher metabolizable energy content also. It was concluded also in Chapter 3 that the three experimental diets are not iso-calorific in terms of their metabolizable energy content.

A further increase in the protein content of the diet by 8.91% and feeding at the same low feeding rate (2% bw/d) results in a significant improvement in the SGR from 0.99 to 1.33. In other words, increasing protein intake by 25.83% results in increasing the growth by 34.34%. It should be realised that part of the extra protein should replace the reduction in dextrin and, therefore, protein utilisation for energy increases. This is demonstrated by the increase in ammonia output by 75.95%. However, with this increase in protein catabolism the PER increases also by 10.44%. This means that the high protein catabolism was associated also with high growth rate. This suggests that PER measurements do not necessarily mean high protein utilisation for growth and subsequently low ammonia output.

Although it is realised that the metabolizable energy content of this high protein diet (43.35%) is high, it does not seem that this will explain the

significant increase in the growth rate achieved with this feeding regime. It is noted that increasing protein level from 24.94% to 34.44% led to an increase in the growth of only 8.79%, while increasing it from 34.44% to 43.35% increases the growth by 34.34%. This means that there is an extra 25.55% in the growth rate with 43.35% protein diet. This extra growth seems to result from high protein catabolism as noted from the ammonia excretion measurements. This view was proposed also by Jackson and Capper (1982) who suggested that energy provided by protein catabolism leads to improved growth at high protein levels. This could probably explain also the very sharp increase in ammonia excretion rate observed with 34.44% protein diet. It suggests that there is a high demand for protein catabolism, however, the limited protein intake with this diet did not provide a sufficient protein for catabolism. Therefore, only little extra growth was recorded.

Increasing protein intake by 15.11% from 8.67 to 9.98g/kg/d with feeding a low protein diet at 4% bw/d results in increasing the growth rate by only 6.77%. In fact this insignificant increase in the growth rate was noticed with all higher protein intake. For example, increasing protein intake 200% from 8.67 to 26.01g/kg/d led to only 21.80% increase in the SGR from 1.33 to

1.62. This lead to an important conclusion that is the insignificance of P:E ratio of the diet at high feeding rates suggests that the energy supplied with these food rations (194 and 290 Kcal/kg/d) is adequate for optimum growth. In other words, optimum growth can proceed with a fairly wide range of P:E ratios (51 to 90mg protein/Kcal) provided that the minimum dietary protein and energy requirements are met. It further demonstrates the significant contribution of feeding rate only in supplying the required nutrients and energy for growth. The effect of food ration itself seems not to be important. For example, with 43.35% protein diet the growth rate increased only 6.77% by increasing the feeding rate 100% from 2 to 4% bw/d.

Under such high feeding rates the relative contribution of protein as an energy source was clearly noticed with PER and ammonia output results. Unlike growth there were significant differences in the PER and ammonia excretion rates. The general trend observed at both feeding rates was that the higher the P:E ratio the lower the PER and subsequently the higher the ammonia output. This is probably because that protein replaces the reduction in carbohydrate of high protein diets. This demonstrates the significance of P:E ratio of the diet on ammonia excretion rate even at high feeding rates.

It is interesting to note that the proportion of ammonia-nitrogen output relative to nitrogen intake for all diets was almost constant at 15.71% +/- 1.15% and 8.91% +/- 1.19% for 4 and 6% bw/d feeding rates, respectively. This suggests that even with such a high energy intake still there is a need to catabolize some protein for energy. In other words, for optimum growth there is a need for minimum dietary protein catabolism. The fixed proportion of ammonia-nitrogen excretion is probably a reflection of the increase in the protein content of the diet which replaces carbohydrates. The reduction in the proportion of ammonia-nitrogen output observed with increasing the feeding rate could be partly due to incomplete consumption of the food supplied and partly because of protein savings by the high energy consumed at 6% bw/d.

High feeding rate (6% bw/d) seems to have a significant contribution in lowering the magnitude of ammonia excretion rate. This is particularly true for low and medium protein diets. The reason for this reduction is not clear, whether it represents protein savings by the high non-protein energy consumed or a protein (amino acids) loss due to high protein intake. As discussed in Chapter 4 this observation seems to be a result of both high protein and energy intakes with high feeding rate. It seems that feeding rate has an

important contribution on this reduction in ammonia-nitrogen excretion rate.

In summary, it seems that the differences between growth and ammonia excretion rates are due to:

(1) P:E ratio, which seems to have a different effect on growth from that on ammonia output. It seems that the P:E ratio is significant to growth only at low feeding rate (limited energy supply), while it is relatively unimportant for growth at medium and high feeding rates (with adequate energy supply). On the other hand, P:E ratio is important for ammonia output at all feeding rates.

(2) Protein-energy seems to have a different contribution on growth from the non-protein energy.

Protein Savings:

One of the objectives of this study was to clarify whether it is possible to define optimum dietary protein intake for growth with the lowest possible ammonia excretion rate. In other words, whether it is possible to show the protein sparing effect by reducing ammonia output. Protein sparing has been found in nutrition studies where the fish growth rate is the major

parameter measured. Takeuchi et. al. (1978) found an improvement in weight gain and food conversion with 35% protein and 15-20% lipid. Similarly Jauncey and Ross (1982) have shown that it is possible to reduce the protein content of the diet for 10-40g O. aureus X O. niloticus hybrids from 40 to 30% with little reduction in growth performance and with improved protein utilisation, provided that the diets contained 12% lipid.

However, this clear protein saving has not been demonstrated clearly by ammonia excretion rate measurements. If protein is saved by lipid or carbohydrate, then the reduction in protein catabolism is expected to result in a concomittent reduction in ammonia excretion. However, there are insufficient data in the literature to show this relationship. For example, Beamish and Thomas (1984) found that total nitrogen excretion (ammonia + urea) associated with each of the dietary protein levels 49 and 35%, tended to be slightly lower when dietary lipid and energy : protein ratio were high. Leid and Bratten (1984) found that with iso-caloric diets higher ammonia was excreted with low protein diets (28.3 PE:TE) than with medium diets (38.3 and 48.0 PE:TE). In this study lipid was incorporated at fixed concentrations for all diets. Therefore, it is not possible to detect protein saving

by lipid. However, the lipid level was selected (10%) to optimise protein utilisation.

Contrary to the above study Kaushik and Teles (1985) studying the protein sparing effect of carbohydrates succeeded in demonstrating a reduction in the ammonia excretion with increasing the digestible carbohydrate (Gelatinised Starch) for 50g rainbow trout. However, they stated that looking at the nitrogen budget, it was clear that ammonia-nitrogen alone does not amount for all the difference between absorbed and retained nitrogen. This study seems to be the only one available that shows protein saving by ammonia measurement.

Other available studies (Cowey, 1975; Ogino et. al. 1976; Rychly, 1980) did not show any protein saving effect by starch for rainbow trout. The difference between these studies and that of Kaushik and Teles (1985) was in the digestibility of starch. The more digestible the starch, the more saving it yields and subsequently the less ammonia excreted. In this experiment dextrin was used as a carbohydrate source. According to Jackson and Capper (1982) the digestibility of dextrin is 95% in T Zilli. Assuming a similarity between T Zilli and O. spilurus in dextrin digestibility this probably indicates that there is a limited scope

for improving protein savings by incorporating more digestible carbohydrates in the diets of O. spilurus. The very high dextrin digestibility, and in turn protein savings, was already noticed with the low proportion of ammonia-nitrogen excreted (6.86 - 21.67% of total nitrogen intake) and the high PER's recorded in this study.

Selection of Optimum Protein Intake for Growth, Ammonia Output, and Feed Utilisation Effiency:

The above mentioned differences between growth and ammonia excretion rates contributed significantly on the selection of an optimum protein intake for both. For example, the optimum protein intake for growth was selected in Chapter 3 as 8.76g/kg/d. This protein intake is provided by feeding a high protein diet (43.35%) at low feeding rate (2% bw/d). This feeding regime will necessarily result in a high ammonia excretion rate because of an inadequate supply of non-protein energy with such a low feeding rate.

Lowering ammonia output can be achieved by using the opposite feeding regime with a low protein diet fed at high ration. This is mainly because lowering P:E ratio will provide more non-protein energy source that will substitute the protein in supplying the energy required for growth. However, with such a low protein

diet a higher feeding rate is required for two reasons: first, to provide the protein requirement for both growth and metabolism, and second, to balance the difference between protein energy and non-protein energy.

The results of this study show that the optimum growth rate can also be achieved with low protein diet fed at high ration. For example, by using a low protein diet (24.94%) fed at high feeding rate (6% bw/d) it was possible to record a SGR of 1.59 for small O. spilurus. The growth rate is even higher by 19.55% than that supported by a high protein diet fed at low ration. With this extra growth the ammonia excretion rate decreases by 25.70%. Therefore, it seems that it is possible to lower ammonia excretion rate significantly by feeding a low protein diet at high ration with even an improved growth.

However, with such a feeding strategy there is a substantial decrease in the protein and overall feed utilisation efficiencies. For example, feeding a high protein diet (43.35%) at low ration (2% bw/d) results in a FCR of 1.09, whereas the low protein diet (24.94%) fed at high ration (6% bw/d) results in a FCR of 2.82, an increase of 158.72%. Similarly PER decreased by 32.34%. This reduction in the feed utilisation efficiency could

be partly due to incomplete food consumption, and partly due to higher gut evacuation rate. Pandian and Vivekanandan (1985) reported tht the gastric evacuation rate increases in proportion to the feeding rate.

In summary, it seems that there is no conflict in defining an optimum protein intake for both growth and ammonia output. This can be achieved by feeding a diet with low P:E ratio at high feeding rate. However, the conflict comes in defining the optimum protein intake for both ammonia output and feed utilisation efficiency.

In view of these results it seems that providing the optimum protein requirement by feeding a high protein diet at low ration is the recommended feeding strategy in terms of growth rate, PER, and FCR. Therefore, this feeding strategy can be used under culture conditions where food supply is the most limiting factor for production. On the other hand, the low protein diet fed at high ration could be recommended under culture conditions where water supply and/or treatment is the most limiting factor for production.

Effect of Ammonia on Growth Rate:

It has been shown in Chapter 5 that the high ambient ammonia levels decreases fish growth rate. It was concluded also that this reduction in the growth

rate is due to reduction in food consumption, higher maintenance requirement due to ammonia detoxification; and low efficiency of energy production in the fish. The first two factors would probably be the major cause of growth reduction at low ammonia levels, whereas the last factor seems to be responsible for fish death at high ammonia levels.

It should be noted that the reduction in growth and feeding rates with high ammonia concentrations was calculated in Chapter 5 on a percentage basis. This was mainly because of practical limitations in experimental methodology. In order to have a more general idea about the effect of ammonia toxicity on growth - protein intake relationships the following calculations were made and presented in Fig 4.

The growth curve at 0.00mg NH_3N/l is the same growth - protein intake curve developed in Chapter 3 for small O. spilurus. This curve was used as a control. The curves from 0.1 to 0.4mg NH_3N/l were calculated as a percentage from the control curve using the growth - ammonia linear regression equation developed in Chapter 5 for small O. spilurus. This comparison shows that the defined optimum growth rate (0.55g/fish/d) can be achieved at a protein intake of 0.9, 1.1, 1.3, and 1.6% bw/d at 0.0, 0.1, 0.2, and 0.3mg NH_3N/l, respectively.

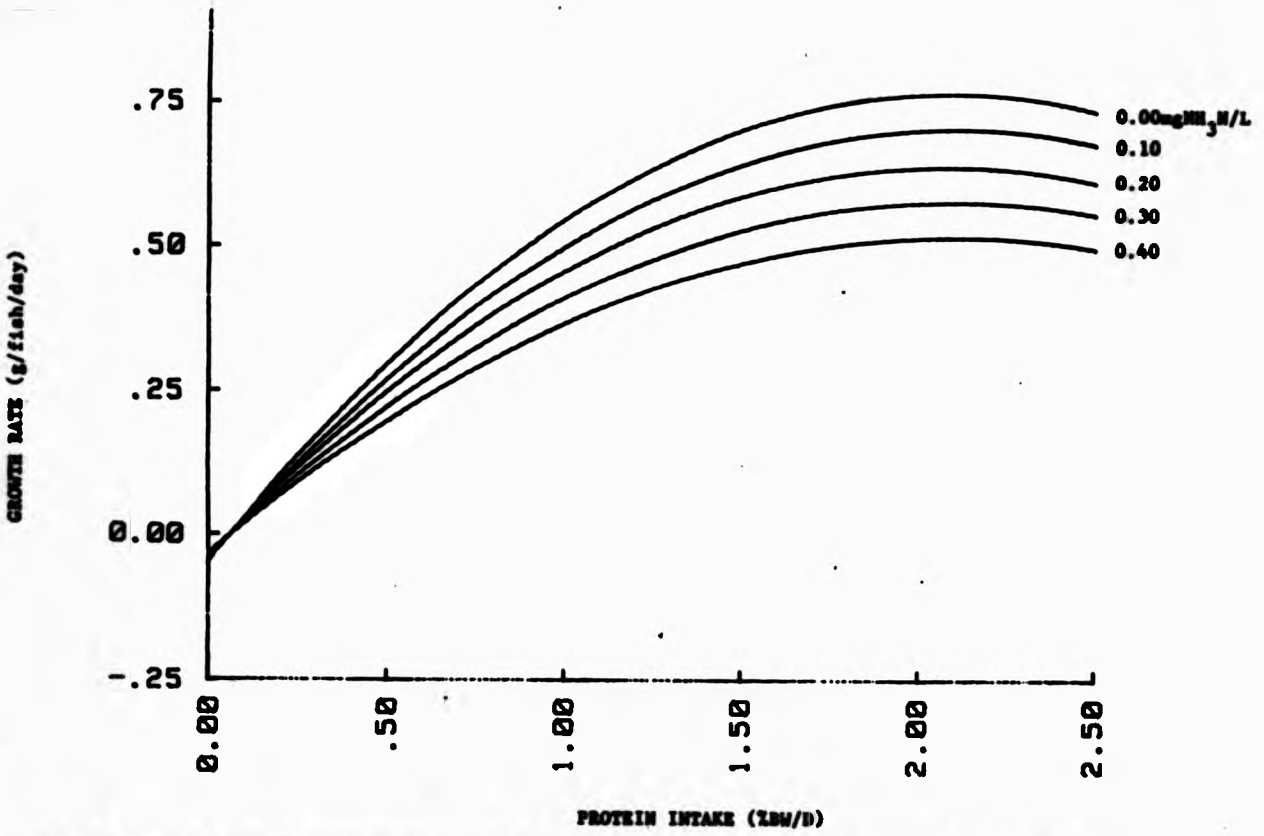


Figure 6.4 Effect of un-ionized ammonia level on protein intake-growth rate relationship for small *O.spilurus*.

This shows that at higher ammonia levels, higher protein intakes are required to produce optimum growth. At 0.4mg NH_3N/l and higher concentrations it becomes impossible to obtain the optimum growth rate at any level of protein intake. The higher protein intake required at higher ammonia levels is probably due to both higher maintenance metabolism for ammonia detoxification and reduction in the energy production rates and deamination of amino acids described earlier by Russo and Thurston (1977).

In view of these results it seems that feeding a high protein diet at low ration is the best feeding strategy for supplying the dietary protein requirement for growth under ammonia stress conditions. This is mainly because of two reasons: first, ammonia stress reduces feeding rate, therefore, it would be advisable to provide all nutrient requirements concentrated in small rations. Second, ammonia stress reduces feed utilisation efficiency, therefore, it would be advisable to use a very efficient energy source, such as protein, to balance this reduction.

It should be noted, however, that the actual food eaten as % bw/d is somehow below the optimum as compared to the results obtained from the feeding experiment (Chapter 3). Food eaten by the small control fish in

ammonia toxicity experiment 1 is 1.67% bw/d, whereas the maximum feeding rate for approximately the same high protein diet is 4.77% bw/d. The reason for this low feeding rate in the ammonia toxicity trials is probably due to the satiation feeding regime used in this study. It has been experienced that satiation feeding with tilapia is difficult. The fish eat slowly from the water column and the tank bottom. In this study feeding was stopped once the fish stopped eating. This low feeding rate would not be likely to change the basic ammonia toxicity effect on protein intake - growth rate relationships shown in Fig 4. However, since low feed efficiency ratios are expected with low feeding rates, it might be possible to obtain higher growth rates with higher feeding rates under ammonia stress. If this is true then the optimum growth rate might be achieved even at higher ammonia levels than 0.3mg NH_3N/l. In other words the protein intake - growth rate curves developed at different ammonia levels might shift upwards with higher feeding rates. However, this requires further experimentation.

Relationship Between Ammonia Excretion and Ammonia Toxicity:

A multiple linear regression equation was developed in chapter 4 for calculating the ammonia outputs provided that feeding rate and protein content

of the diet are known. From these different feeding regimes more than one was identified for supplying the optimum protein intake for growth. These different feeding regimes result in ammonia production rates ranging approximately from 220 to 300mg NH_4^+-N/kg/d. These production rates can be used to calculate the water requirements and the filter sizes in recirculating systems. For example, Speece (1973) provided a mathematical formula for calculating the required flow rate based on ammonia production rate as follows:

$$Q/V = \frac{W_a/V}{E_q}$$

where, Q/V = water flow rate per unit mass of fish

W_a/V = ammonia produced per unit mass of fish

E_q = effluent quality desired in terms of ammonia concentration

Similarly, Rogers and Klemetson (1985) provided another formula for calculating the specific filter surface areas as follows:

$$\text{Specific surface area} = \frac{\text{Ammonia Production Rate}}{\text{Nitrification Rate}}$$

They gave a nitrification rate of 2.63g/m^2/d for rotating biological contactor. The data collected in this study on ammonia production rates can be applied directly to these two formulas. Ambient ammonia level can be selected according to the requirement of the fish

farm. At a fixed ambient ammonia level there is a simple positive linear relationship between ammonia production rate and water (or filter) requirements. For example, within the above mentioned production range it would appear that using the protein intake that produces 220mg NH_4^+-N/kg/d will save 36.36% of the water (or filter) requirements in the fish farm. This protein intake was provided by feeding a low protein diet (24.94%) at high ration (6% bw/d).

Since ammonia excretion rate fluctuates considerably during the 24hr period, it might be advisable to consider the peak ammonia excretion rates rather than concentrating on the average values only. The ammonia excretion data collected on small *O. spilurus* in Chapter 4 show that feeding rate has the greatest effect on the daily variation, while P:E ratio of the diet has only a minor effect, mainly at low ration. Feeding at 2 and 6% bw/d results in a daily variation of 110-150% between the average and the peak values. However, at 4% bw/d the difference between the average and the peak values ranges from 45-59%. This information is particularly important for marine recirculating systems where the biofilter efficiency depends largely on the ammonia level.

Rogers and Klemeston (1985) reported that ammonia removal in the biofilters is highest when steady state ammonia levels are maintained. Although nitrification is a concentration-dependent process, the biological nature of the bacterial cell growth might result in a "lag" present in the response and, therefore, there may be an appreciable delay (Muir, 1982). This might be the reason for the reduced nitrification efficiency with a wide variation in the ammonia levels. In this case, the advantage of using the medium feeding rate (4% bw/d) is clear in increasing the biofilter efficiency.

It should be noted that the ammonia excretion and oxygen consumption rates were calculated in chapter 4 on the basis of fish biomass (mg/Kg fish/D). In earlier studies Liao et. al. (1972) suggested to relate these parameters to the feed input (mg/Kg feed/D). However, it was concluded from chapter 4 that, for a more accurate estimate, there is a need to differentiate between the dietary protein level and feeding rate. A better estimate would be available from the multiple regression models developed in chapter 4 for ammonia excretion and oxygen consumption rates. Table 6.4 summarises some of these estimates as calculated from those models for small O. mykiss.

Significance of Results Obtained in this Study on the Mariculture of O. spilurus:

Generally, the results obtained in this study reveal that the intensive mariculture of O. spilurus has a good potential. First, dietary nutritional requirements do not seem to be significantly changed in sea water environment. The results indicated that neither maintenance nor maximum food ration was affected significantly by salinity. For example, starved O. spilurus for 8 weeks (36.44g bw) lost about 0.09g/fish/d during this study. This value is quite similar to results observed with red tilapia starved for 8 weeks in fresh water which lost 0.08 and 0.09g/fish/d for 15.20 and 17.57g fish, respectively (Hepher et. al., 1983). Jurss et. al. (1984) concluded also that the maintenance ration of O. mossambicus remains the same regardless of salinity.

Regarding maximum food ration there seems to be no data available in the literature on the effect of salinity on food intake in tilapia. However, Balarin and Haller (1982) presented a simple equation for estimation of feeding rate for different size groups of tilapia fed 35-40% protein diets in fresh water. By using their formula a fish of similar size to small O. spilurus used in experiment 1 in this study will consume 4.41% food bw/d. This value is quite close to the

maximum food ration for 34.44 and 43.36% protein diets obtained in this study which were 4.97 and 4.66% bw/d. This indicates that O. spilurus are able to consume a high ration and gain high growth even in sea water conditions.

Protein requirement also does not seem to be increased by salinity. In fact the recommended dietary protein requirement in this study seems lower than most of the published values. This subject has been discussed in detail in Chapter 3.

Ammonia toxicity does not appear to be increased in sea water. The incipient LC-50 and EC-50 are quite comparable to other published values for hardy fishes such as channel catfish. This result is interesting because it might be expected that the ammonia stress would add an extra load to salinity stress which would result eventually in poor performance of the fish. It should be realised that both ammonia toxicity and salinity acclimation induce several physiological modifications in the fish body. For example, Lloyd and Orr (1969) reported that rainbow trout exposed to sublethal ammonia levels show an increase in urine volume. Eddy (1981) reported that this high urine flow rate could result from an increase in branchial permeability or from an increase in the drinking water.

Both mechanisms lead to increased water retention in fish body tissues. It should be noted that this work was carried out in fresh water.

However, in sea water the problem of water absorption does not exist because the fish actually are facing a problem of dessication and therefore they are drinking sea water. Thus, Colt and Armstrong (1981) reported that the high water permeability induced by ammonia is probably more important in fresh water because in this environment aquatic species are hyperosmotic.

Salinity acclimation also increases the cellular amino acid level. This elevation helps the fish in the maintenance of the cellular tonicity during exposure to hyperosmotic media (Schmidt-Nielsen, 1979). Sashikala et. al. (1985) reported also that the free amino acid content was elevated in all the tissues of O. mossambicus at ambient ammonia stress. Furthermore, Dabrowska and Wasow (1986) found that the total free amino acids in common carp exposed to ammonia to increase in both brain and muscle. These free amino acids can bind with ammonia to form amino acid amides. Therefore, it seems that this physiological adaptation is required at both salinity and ammonia stress.

In conclusion, there are certain physiological mechanisms that are induced by salinity stress. These adaptations are required also at ammonia stress. This could explain the good performance of sea water acclimated O. spilurus to ammonia stress in this study.

Size Effect:

The major drawback in this study was the failure of the large O. spilurus to grow. This made quantifying optimum protein requirements for growth and ammonia output impossible for large O. spilurus. Under these circumstances and in order to overcome this drawback the best available option is probably to predict the size effect in view of the available information in the literature, based on the results obtained with small fishes in this study.

It seems unlikely that the basic trends of the biological relationships between protein intake, ammonia excretion and growth rates determined for small O. spilurus in this study are going to change with fish size at least in their qualitative terms. For example, the order of significance of protein percent of the diet and feeding rate and their mode of actions with the sparing effect of protein energy with non-protein energy are not likely to change. This is simply because there is nothing to support such changes. However, changes in

fish size would result in quantitative changes in growth rate, feeding rate and metabolic rate. Brett (1979) reported that as size increases maximum ration (R_{max}) falls rapidly; maintenance ration (R_{main}) also decreases but at a slower rate than R_{max}. This result in a convergence, so that scope of growth (G_{scope}) diminishes with size. An old mature fish is mainly eating for maintenance and gonad development; somatic growth is almost terminated. The diminution of growth promoting hormone that must accompany advanced age and size results in a decreased demand for food such that R_{max} approaches R_{main}.

The reduction in the feeding rate results in a similar reduction in metabolic rate and subsequently ammonia excretion rate. This reduction in growth, feeding and metabolic rates is generally described by an exponential equation as follows:

$$M = aW^{b}$$

where, M = fish activity (growth, feeding metabolic) rate

W = fish weight

a = constant

b = weight exponent

Huisman (1986) reported that extensive research proved the value "a" is highly variable depending on: temperature, activity and species. The relation of fish

weight to maximum meal size was quantified by Elliott (1975) to be proportional to $W^{0.75}$ for brown trout. Huisman (1986) reported that the weight exponent "b" for metabolic rate is rather fixed at 0.8. Jobling (1981a) reported that although such values have been quoted relating nitrogenous excretion to body size some workers have found both higher (Iwata, 1970) and lower (Gerking, 1955) values to be applicable.

In order to develop such a quantitative measurement for the size effect on growth and feeding rates for O. spilurus in sea water the data obtained from this study were compiled with data provided by Al-Ahmad et. al. (1986) on the growth rate of 2 size groups of O. spilurus in sea water. This comparison is probably valid in view of the similarities between diets and other culture conditions. This is shown in Table 6.5. The feeding rate used for small O. spilurus was calculated from the multiple linear regression equation developed in Chapter 3 for a 40% protein diet. After natural log transformation the slopes of growth and feeding rates were found to be -0.23 and -0.32, respectively (Table 6.6). This gives weight exponents of 0.77 and 0.68 for growth and feeding rates, respectively. This indicates that growth rate declines faster than feeding rate. This conclusion is in agreement with that reported by Brett (1979) who stated

that size has a greater restricting effect on growth rate than on metabolic rate, a difference which is accounted for declining conversion efficiency with size.

It seems that there are no data available on growth - size relationship for other tilapia species. Therefore, no comparison can be made between fresh and sea water growth rates. On the other hand, Balarin and Haller (1982) gave a feeding formula with a slope of -0.457 for 35-40% protein pellets fed to O. niloticus. Melard and Phillippart (1980) gave another formula but with a different slope (-0.580) for 23-26% protein diets with O. niloticus also. The slope obtained in this study is larger than these two (-0.68). Although the large slope obtained with O. spilurus suggests that feeding rate declines faster in sea water than in fresh water, it should be realised also that differences in protein digestibilities, temperature, fish size have also a significant contribution on these slopes.

The major intention here was to have some quantitative measures for estimating growth and feeding rates for O. spilurus in sea water. However, because of the unavailability of ammonia excretion or oxygen consumption estimates for large O. spilurus no such a size-metabolic (ammonia excretion) rate relationship can be calculated. It is expected, however, that the

Table 6.1. Relationship between growth rate, SGR (X) and ammonia excretion rate, NH_4^+-N mg/Kg/hr(Y) for small O.spilurus

Diet	b_o	b_1	r	R^2
24.94	-1.879	7.233	0.95	0.91
34.44	-0.924	8.861	0.96	0.92
43.35	-0.321	10.880	0.99	0.96

Table 6.2. Relationship between nitrogen absorbed, mg/Kg/D(X) and nitrogen retained mg/Kg/D(Y) based on ammonia as the only excreted nitrogen.

Fish Size	b_o	b_1	St. Error of Est.	r	R^2
Small	-122.93	0.901	79.89	0.99	0.99
Small*	-236.44	0.889	104.96	0.99	0.98
Large	-144.03	0.698	105.22	0.95	0.90

* Based on ammonia+urea measurements.

Table 6.3. Comparison between nitrogen efficiency estimated by nitrogen balance method and growth and carcass analysis for small O.spilurus.

Feeding Rate %BW/D	Dietary Protein %	Nitrogen intake g/Kg/D	Nitrogen efficiency(%) (Balance method; ammonia+urea)	Nitrogen efficiency(%) (Balance method; ammonia only)	Nitrogen efficiency (%) (growth +carcass)
2	24.94	0.80	80.91	87.54	68.91
	34.44	1.10	56.06	75.54	48.57
	43.35	1.39	61.62	72.86	47.75
4	24.94	1.60	60.79	71.97	60.67
	34.44	2.20	64.36	73.39	43.57
	43.35	2.77	76.79	81.67	26.24
6	24.94	2.39	63.03	83.17	64.14
	34.44	3.31	77.58	88.09	31.28
	43.35	4.16	84.80	87.68	20.12

Table 6.4 Ammonia excretion (gN/Kgfish/D) and oxygen consumption (g/Kgfish/D) rates for small O.spilurus calculated from the multiple regression models developed in Chapter 4.

Feeding Rate (%BW/D)		Protein % 20	30	40
2	Ammonia	1.001	5.431	9.861
	Oxygen	273.41	270.51	267.61
4	Ammonia	7.951	12.381	16.811
	Oxygen	386.07	380.27	374.47
6	Ammonia	5.981	10.411	14.841
	Oxygen	498.73	490.03	481.33

Table 6.5 Growth and feeding rates for different size groups of O.spilurus in sea water fed 40% Protein diet.

Body Weight (g)	SGR	Feeding Rate %BW/D	Source
43.64	1.33	3*	This study
99.20	0.98	2	Al-Ahmad et. al. (1986)
349.50	0.78	1.5	Al-Ahmad et. al. (1986)

* Estimated for 40% protein diet from the multiple linear regression equation formulated in Chapter 3 for small fish.

Table 6.6 Body weight-growth rate and body weight-feeding rate relationships for O.spilurus in sea water fed 40% protein diet.

Parameter	b_0	b_1	r^2
Growth rate (SGR)	1.06	-0.23	-0.99
Feeding rate (%BW/D)	2.26	-0.32	-0.97

metabolic rate exponent will approximately follow the feeding rate exponent rather than the growth rate exponent. This is mainly because of the increase in the maintenance requirements with increasing fish size.

Future Directions:

Results obtained in this study on the growth rate of O. spilurus as affected by dietary protein intake and ammonia toxicity are consistent with other data in literature on tilapia and other fishes. Therefore, general trends for these relationships can be drawn for planning purposes. On the other hand, little data seems to be available on the effect of protein intake - ammonia excretion relationship. In this study the protein intake was spread as much as possible in order to define the major trends of this relationship. It is not clear whether these trends defined in this study are tilapia specific or can be applied also to other fishes. The decline in total ammonia output observed with high feeding rate needs to be explained. It was concluded that feeding rate is important for the appearances of this decline, thus it is interesting to study the effect of feeding frequency on this relationship.

Experimentation with ammonia excretion with such a wide range of feeding regimes necessitates modifying some of the experimental procedures (eg stocking

density), which results in difficulty in applying the standard statistical analytical procedure. Thus, there is a need to optimise the experimental methodology, particularly the fish activity level and ambiant oxygen level, to standardise fish metabolic rate, while maintaining the same number of fish for all feeding regimes. It would be advisable to consider other parameters such as removal of uneaten food and faeces in any future tank design.

Nevertheless, the results of this study confirm the original aim set for this project; that is, engineering an intensive aquaculture enterprise by manipulating protein intake. Feeding a high protein diet at low ration appears to be an efficient procedure for intensive aquaculture with high ambient ammonia level. This is mainly because of the likely reduction in the food consumption at high ammonia level, which necessitates providing all the nutrient requirements concentrated in small rations. Optimum growth can be achieved with sub-satiation feedings if the daily nutrients and energy requirements are fulfilled with these rations (See Chapter 3).

Furthermore, high ambient ammonia levels reduces the feed utilisation efficiency. Therefore, it would be advisable to use highly digestible ingredients under

such conditions. High protein diets are usually considered as high digestible, high metabolisable diets.

However, for practical application of the data collected in this study it would be advisable to consider the following two criteria:

(1) In this study a very digestible carbohydrate (dextrin) was used. Thus, it may be possible that by using a less digestible carbohydrate, as is the case in most practical diets, the high growth rate observed with low protein intake will be adversely affected. Therefore, there is a need to confirm this result with a more typical carbohydrate source. If fish growth is adversely affected, then increasing the digestibility of the diet by incorporating more lipid and/or increasing carbohydrate digestibility, for example by gelatinisation (See: Kaushik and Teles, 1985), can be applied.

(2) The protein intake-growth relationship developed at different ambient ammonia levels, as shown in Chapter 6, needs to be confirmed by a practical data. In view of the reduction in the food consumption rate at high ammonia levels this is

likely to be achieved by feeding high protein diets, probably higher than 43.35%.

If these two relationships are proved to be true using practical diets, it seems that using a high protein diet fed at low ration will be advantageous with respect to growth rate, FCR, PER, and ammonia toxicity. Since feeding rate is the most important factor determining oxygen consumption (Chapter 4) and other water quality parameters such as chemical oxygen demand, nitrite, nitrate, and carbon dioxide (See: Cole and Boyd, 1986), there is an added advantage from this feeding strategy.

Therefore, in intensive fish culture at high ammonia levels it appears that the only disadvantage of this feeding regime is the high ammonia output. Two approaches may be taken to overcome this problem. First, an engineering approach where, for example, periodic flushing of the system (after meals) may be used to lower the excreted ammonia level, thus taking advantage of the short peak duration of ammonia excretion rate observed with this feeding regime (See: Chapter 4).

Second, a nutritional approach, where increasing the digestibility of the diet by increasing lipid and/or

more digestible carbohydrate, as mentioned earlier, may be applied. Furthermore, the low proportion of ammonia-nitrogen excreted with respect to total nitrogen consumed (as was observed in Chapter 4) and was explained to be due to higher feeding frequency) may be used. It is likely that it is possible to lower the ammonia output by distributing the feed ration more frequently than 3 times per day. This feeding strategy is particularly suited to tilapia which are small, frequent meal eaters.

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