DETERMINATION OF THE OPTIMUM ENVIRONMENTAL REQUIREMENTS OF JUVENILE MARINE FISH: THE DEVELOPMENT OF A PROTOCOL

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DEDICATED TO MY FAMILY

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Abstract

The spotted grunter, *Pomadasys commersonnii*, has been identified as a candidate species for mariculture in South Africa due to its high market demand and apparent biological suitability for culture. In common with most other potential mariculture species the spotted grunter has an estuarine juvenile phase. In this respect, the difficulties encountered in determining the optimum environmental requirements of spotted grunter are applicable to the majority of other potential mariculture species. Due the variability of estuarine habitats determination of the optimum environmental requirements of estuarine species under laboratory conditions are a prerequisite to subsequent evaluation of aquaculture potential. Therefore, using the spotted grunter as a representative of a typical potential mariculture species, the aim of this study was to contribute to the protocol for evaluating the environmental requirements of potential aquaculture species.

The growth of fish is dependent on the relationship between food intake, metabolism and environmental factors. In this relationship, environmental factors do not act on growth *per se*, but rather act through metabolism on growth. Consequently, the environmental factors affecting the growth of a fish species can be classified into functional categories according to their respective influence on metabolic processes. Five functional categories are recognised, namely: controlling, limiting, masking, directive and lethal factors. In this study, the functional categories were sequenced to develop a theoretical protocol for determining the optimum environmental requirements of potential mariculture species under artificial conditions. It was hypothesised that the correct sequence for experimentally determining the optimum environmental requirements of a species should correspond to theoretical protocol.

The hypothesis was tested from the experimental investigation into the individual effects of temperature, salinity, light intensity, photoperiod and food intake on growth performance of juvenile spotted grunter. The investigations quantified the relative effects of controlling, masking, directive and limiting factors on growth performance. The results of the studies were statistically compared to obtain a ranking of the effects of the environmental factors (e.g. temperature, salinity, etc.) on growth performance of juvenile spotted grunter. The statistical ranking facilitated the formulation of a second protocol for sequentially determining the optimum environmental requirements of a species. The second protocol was derived purely from the experimental data. Based on the corroboration between the theoretical protocol and that formulated from the experimental investigations, the hypothesis was accepted.

Confirmation of the protocol for examining the effects of controlling, limiting, masking and directive factors provided a basis for the development of a preliminary experimental sequence for determining the optimum environmental requirements of juvenile marine fish. In the development of the experimental sequence, the protocol was interpreted in conjunction with the experimental methods used to determine the effects of environmental factors on the growth of juvenile spotted grunter. The experimental sequence provides a logical frame work within which the optimum environmental requirements of other marine fish species can be determined. In addition, evaluation of growth performance by this method provides a basis for comparison of the mariculture potential between species.

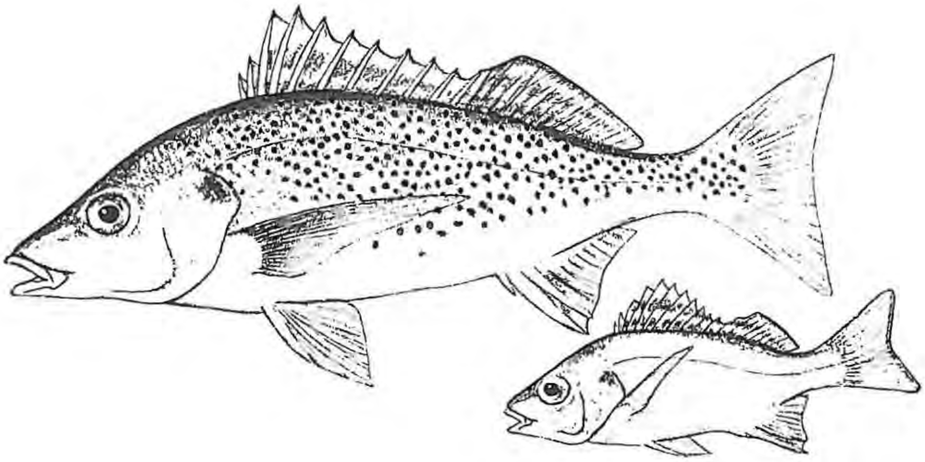

Plate 1.1: Adult (680mm)(top) and juvenile (62mm) phases of the spotted grunter, *Pomadasys commersonnii* (Lacepede, 1802). Reproduced with kind permission of Phil and Elaine Heemstra, and the J.L.B. Smith Institute of Ichthyology.

Chapter 1

General Introduction

The aquaculture production of any species is governed by its economic viability. Therefore the biological factors that directly contribute to this, namely survival, growth rate and food conversion should be among the fundamental criteria for assessing the mariculture potential of any species. However, growth performance is a summary measure of a diverse spectrum of complex interactions between an organism and its environment. The effects of environmental factors act at many levels, including biochemical, cytological and physiological, and via a multiplicity of metabolic pathways (Brett, 1979). Consequently, any estimate of growth of fishes is also a reflection of the prevailing environmental conditions under which these are measured. Even though growth performance is inherently biased by the effects of environmental factors, it is nonetheless the only meaningful biological measure for comparison between species. Since the effect of environmental factors on growth performance cannot be removed and environmental requirements are species specific, these must somehow be 'standardized' before candidate mariculture species can be compared on an equal and realistic basis.

Maximum growth is the only biological value where the compromising effects of environmental factors on growth performance are negated. An estimate of maximum growth, therefore, represents the only unbiased biological measure for comparison between candidate aquaculture species. Maximum growth can, however, only be achieved under optimum environmental conditions. In an optimum environment metabolic processes are optimized. Consequently, all activities related to catabolism, including growth, are maximised. Therefore, any evaluation of the culture potential of a candidate mariculture species should begin with the determination of the optimum environmental requirements.

Traditionally the environmental requirements of fish have been derived from observations of their natural environment (Person-Le Ruyet, 1990). The fundamental environmental requirements, such as salinity and pH, are extrapolated from field observation and simulated in the captive environment. This suite of environmental conditions then acts as a basis upon which economically important criteria, such as growth performance and optimum ration size, are assessed (Chua & Teng, 1982; Harris & Cook, 1995). This approach is flawed at a fundamental level as it assumes that fish are found under optimum environmental conditions in their natural environment. In addition, for many potential mariculture species experimentation on this basis is not possible as the data pertaining to the distribution of marine fish is often not accurate nor complete enough to precisely define their environmental requirements. Many marine fish have nursery areas with environmental conditions which differ significantly from those to which the adults are exposed. The latter is particularly pertinent to the majority of potential mariculture food fish. From a review of all the marine species which are currently being considered for aquaculture, it was established that over 75% of these species are euryhaline and utilize estuaries as nursery areas (Appendix 1). Therefore the determination of the environmental requirements of the majority of mariculture species is complicated by the variability of their natural habitat.

Estuaries are dynamic systems typified by uneven and rapid fluctuations in water currents, salinity, turbidity, temperature and dissolved oxygen (Potter *et al.*, 1990). The extreme and complex variation of the environmental factors in estuaries, coupled with the difficulties of accurately determining fish distribution within these systems, makes it impossible to estimate even the most fundamental environmental requirements from field observation. Consequently, the traditional approach to determining the optimum environmental requirements and subsequent assessment of growth performance provides an almost random evaluation of the mariculture potential of candidate species. This provides no scientific basis for comparison between species. The evaluation of potential mariculture species must, therefore, be performed under laboratory conditions. Information derived from observation of the natural environment can only be considered as indicative of environmental tolerances rather than providing a clue to the environmental requirements. Data on environmental tolerances are, nevertheless, fundamental for developing the technology for artificial culture of a species as these ranges define the limits upon on which laboratory determination of optimum environmental requirements can be based. The inherent limitations of laboratory studies, such as restrictions in number of experimental aquaria, necessitate a step-wise approach to determining the optimum environmental requirements of a species. However, the environmental requirements of a species encompasses a multitude of interacting factors which all affect growth to some extent (Brett, 1979). In addition, the degree to which individual environmental factors affect growth and interact with one another varies from species to species. Consequently, before the complete suite of optimum environmental requirements can be defined, it is necessary to first quantify the independent effect of each environmental factor. This is essentially a simplistic approach as it ignores interactions between factors and by doing so the accuracy of the study can be jeopardized. This can be alleviated to a certain extent by ensuring that the sequence of experimentation is correct and conducted under precise, well-controlled conditions (Fry, 1971). Surprisingly, at the present time there is no recognised sequential protocol for the determining the environmental requirements of any species. Since there is no protocol for accurately determining the optimum environmental conditions for the culture of a species under artificial conditions, essentially no scientific basis for comparison of mariculture potential between candidate species exists.

At present there is no finfish mariculture in southern Africa. Attempts to culture rainbow trout (*Oncorhynchus mykiss*) in seawater have not proven successful (Hecht & Britz, 1993). The proposed developments of salmon (*Salmo salar*) farming in Saldanha Bay, put forward in 1992, have also yet to materialize (Hecht & Britz, 1993). The high cost of production and competition from the Norwegian salmon industry has, so far, proved to be detrimental to these industries. This suggests that the development of finfish mariculture in South Africa will have to rely on novel species and the creation of niche markets in order to succeed as an export industry. Since translocation of exotic species has generally proven to be detrimental to the environment (de Moor & Bruton, 1988), the culture of indigenous species would appear to be the natural choice.

The potential for finfish mariculture in South Africa was first conceptualized by Safriel and Bruton (1984) when they included turbot (*Scophthalmus maximus*) as a high priority candidate species in a list of potential aquaculture species. Ten species of indigenous marine fish, including spotted grunter (*Pomadasys commersonnii*), were also listed, but these were considered to be non-priority species. In a later comment on the status of mariculture in South Africa, turbot was still the primary candidate for mariculture. However, the indigenous galjoen (*Dichistius capensis*) and the grey mullet (*Mugil cephalus*) were also included in the list of species under consideration (Genade, 1985). Subsequent research into the mariculture potential of finfish has been confined to indigenous species. Assessment of the aquaculture potential of South African marine candidate species has varied from research on artificial propagation to growth trials and behavioural studies. For example, galjoen *Dichistius capensis* (Dichistidae) and two sparid species *Chrysoblephus puniceus* and *Cheimerius nufar* have been successfully spawned in captivity (van der Lingen, 1986; Garrat, 1991). Large scale rearing of the larvae of two other sparids, red roman *Chrysoblephus laticeps* and carpenter *Argyrozona argyrozona*, has also been attempted (Davis, 1996). Growth trials have been conducted on white steenbras *Lithognathus lithognathus* (Harris *et al.*, 1991) and spotted grunter *Pomadasys commersonnii* (Bussiahn, 1992; Deacon & Hecht, 1996). Additional studies of behaviour in relation to environmental factors have also been conducted on the latter species (Deacon & Hecht, 1995; Burton, 1995).

With the exception of spotted grunter, all assessment of South African potential mariculture species has been limited to preliminary investigation. This is in spite of the recommendations that many of them are suitable candidates for mariculture (van der Lingen, 1986; Garrat, 1991; Harris *et al.*, 1991). Since no set criteria have been used to measure mariculture potential, the results of these studies are inconclusive with regard to the economic viability of any of these species. Ideally, further research effort should be conducted in such a manner that the performance of all potential candidate species is quantified on criteria that allow direct comparison between each other. This will at least provide development agencies or the private sector with an appropriate list of comparable alternatives.

Although the spotted grunter has long been considered a candidate for mariculture on the basis of its high market price, a number of other factors contributed to its recent selection for further research. From a marketing perspective this species represents an ideal aquaculture species as it is a popular eating fish with no reliable source of supply. In a survey of restaurants serving fish in major centres in South Africa it was established that spotted grunter ranked amongst the three best table fish in this trade (Hecht, pers. comm.). The species is only recognised as a recreational angling species and may not be sold (Government Gazette No. 14192, Sea Fishery Act No 12 of 1988 as amended in 1992, regulation 48, sections: 1-3). Passing of this act was prompted by the increasing pressure placed on natural stocks by estuarine and rock and surf anglers (Van der Elst, 1988). The spotted grunter is regarded as a premier angling species in South Africa (Van der Westhuisen & Marais, 1977; Van der Elst, 1988). As there is no legitimate natural fishery for this species, supply is currently limited to the illegal selling to restaurants. In addition, a natural fishery for spotted grunter is unlikely to develop as it only occurs in estuaries and the shallow subtidal zone and has not been recorded in trawl catches. The absence of a natural fishery for a species is important to developing an aquaculture industry as the high cost of hatchery production cannot compete with the lower cost of fishing natural stocks.

From a biological perspective the spotted grunter possesses many of the traits that have proven to be desirable in aquaculture species. The distribution of spotted grunter encompasses both temperate and tropical waters of the east coast of southern Africa (Van der Elst, 1988). This wide distribution reflects a high eurythermal capability in this species as water temperatures in these regions vary between 12 and 32°C. Although *P. commersonnii* is considered to be an Indo-pacific species (Van der Elst, 1988), the distribution data on spotted grunter indicates that it may, rather, be endemic to the south east coast of southern Africa and possibly Madagascar. Within this distribution it is most commonly found off sandy beaches or in estuaries (Wallace, 1975a; Wallace *et al.*, 1984). The species is estuarine dependent (Whitfield, 1990) and is highly euryhaline, surviving in salinities of 0-70 ‰ (Whitfield *et al.*, 1981). The spotted grunter is also tolerant of turbidity levels in excess of 200 NTU (Cyrus, 1988; Ter Morshuizen *et al.*, in press).

Spawning occurs during late winter at sea (Wallace, 1975b), although the precise location of spawning has yet to be determined. The juveniles (<50 mm total length; Plate 1.1) recruit into the estuaries of Natal and the Eastern Cape between July and December (Wallace, 1975b; Beckley, 1984). Post spawning adults (Plate 1.i) also enter the estuaries at about this time (Wallace, 1975a), where they feed principally on crustaceans and molluscs (Marais, 1984; Van der Westhuizen & Marais, 1977; Hecht & van der Lingen, 1992). The juveniles remain in the estuaries for at least one year before they return to the sea, where they remain until reaching sexual maturity at an age of approximately two to three years and 400mm TL (Wallace, 1975b; Wallace and Schleyer, 1979). During this period they grow from ca. 160 mm to 400mm TL (Wallace and Schleyer, 1979). *Pomadasys commersonnii* attains a maximum weight of 10kg (Van der Elst, 1988).

The spotted grunter is considered a candidate species for mariculture due to its high market demand and apparent biological suitability for culture. In common with many other potential mariculture species the spotted grunter has an estuarine juvenile phase (Appendix 1). In this respect, the difficulties encountered in determining the optimum environmental requirements of spotted grunter are applicable to the majority of other potential mariculture species. The aim of this study was to contribute to the protocol for evaluating the environmental requirements of potential aquaculture species and so provide a basis for comparison between species. Implicit to the study was the examination of the responses of juvenile spotted grunter, *Pomadasys commersonnii*, to environmental factors.

Fundamentally, environmental factors act on the metabolism and activity of fish (Fry, 1971; Brett, 1979; Jobling, 1981; Morgan & Iwama, 1991). Although these terms appear to be interrelated, or similar, they represent two entirely different levels of effect. Metabolism, or more specifically catabolism, refers to the biochemical reactions which yield the energy utilized by an organism (Fry, 1971). The purposes to which this energy is invested are all termed activities (Fry, 1971). Activities, therefore, include all aspects of life including movement, excretion, reproduction and growth. Since the aim of determining the optimum environmental requirements of fish is to maximise growth, the primary interest is directed towards those factors which promote this activity.

The interaction between food intake and metabolism is the fundamental "driving force" behind fish growth (Brett, 1979; Jobling, 1983). Growth rate increases or decreases as a function of food intake and metabolism (Elliot, 1975a, Grayton & Beamish, 1977). Within this relationship food intake is a potential limiting factor as any restriction of food intake directly affects the chain of metabolism. The limiting effect of food intake, therefore, involves independent and dependent states which become operational at a particular level. Generally growth rate increases with increasing ration from a minimum negative value at zero ration to a maximum at the point of maximum food intake (Elliot, 1975a; Wurtsbaugh & Davis, 1977; Chua & Teng, 1982). Above maximum food intake, growth rate reaches a plateau where it is independent of any increase in ration size. However, maximum food intake and metabolism are dependent on the effect of environmental factors (Brett, 1979). Therefore, when food intake is not a limiting, factor maximum growth is dependent on the relationship between metabolism and environmental factors. In this relationship environmental factors do not act on growth *per se*, but rather act through metabolism on growth (Fry, 1947; 1971; Brett, 1979).

In defining the environment of a fish, the individual elements (i.e. temperature, light, food) can be broadly dicotimised into abiotic and biotic factors. Abiotic factors are then generally classified into five simple categories, following Fry's (1947, 1971) elaboration of Blackman's (1905) classical concept of limiting factors. The various factors are designated to these categories according to their respective influence on metabolic processes, namely:

1). **Controlling factors**, which govern the rates of reaction of metabolic processes (e.g. temperature, pH).

2). **Limiting factors**, which restrict the supply or removal of metabolites (e.g. oxygen).

3). **Masking factors**, which modify or have a mitigating effect on an environmental factor through some regulatory device (e.g. increased tolerance to heavy metal toxicity derived from the presence of salinity).

4). **Directive factors**, which cue or signal the animal to select or respond to particular characteristics of the environment (e.g. photoperiod induced gonadal maturation).

5). **Lethal factors**, which cause a breakdown of metabolic functioning leading to death (e.g high temperatures or salinities).

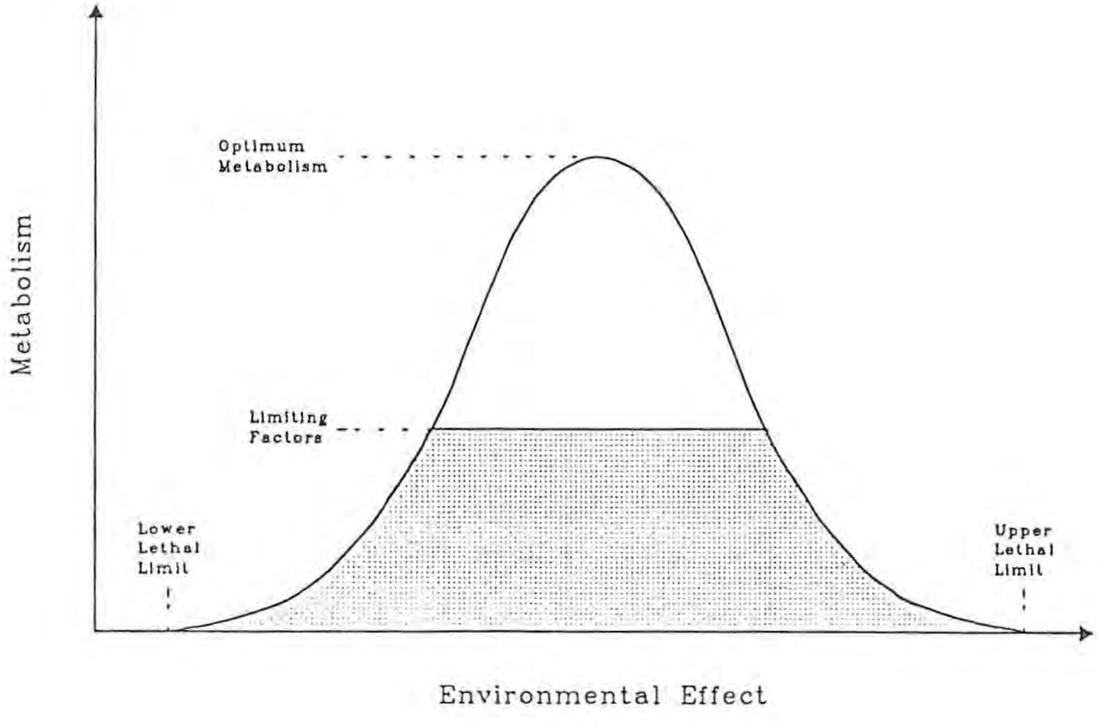

Figure 1.1: A hypothetical model of the effect environmental factors on fish metabolism (solid line). Environmental effect in this model refers to any combination of environmental factors. The shaded area defines the level at which metabolism is dependent on the effects of limiting factors.

The effects of these functional categories are best contextualized in a hypothetical curve of the effect of environmental factors on metabolism (Figure 1.1). For the purposes of this discussion, environmental effect (on the *X* axis) refers to any combination of environmental factors. Metabolism only occurs within the bounds set by the lethal limits of the respective environmental factors (Fig 1.1). Beyond the lethal limits there is an irreversible breakdown of metabolic processes which ultimately lead to death. Therefore, **lethal factors** do not enter into further consideration in the experimental determination of the optimum environmental requirements. As mentioned previously, environmental tolerance data obtained from observation of the natural environment is used as a basis on which the optimum environmental requirements are determined experimentally.

Within the lethal limits, metabolism increases or decreases in response to any one of a combination of environmental factors (Fig 1.1). An increase in metabolism represents optimization of the environmental factors. Maximum metabolic rate, therefore, corresponds to the optimum suite of environmental conditions (Fig 1.1). However, the increase of metabolic rate above a certain level, the shaded area in Fig 1.1, is dependent on the fulfillment of the **limiting factors**. **Limiting factors**, such as oxygen or ammonia, restrict metabolic processes even if all other environmental factors are optimal. Therefore, unless the critical limiting factors are not exceeded, metabolism cannot be maximised. This indicates that the levels at which limiting factors become operational should be the first aspect to be investigated in the sequential determination of the optimum environmental requirements of a species.

Assuming that the critical levels of limiting factors are exceeded, metabolism is independent of limiting factors (Fry, 1971, Brett, 1979). The reasons for this phenomenon will be addressed at a later stage. Rate of metabolism is then dependent on the interaction of **controlling, masking** and **directive** factors (represented as the unshaded area in Fig 1.1). Metabolic processes are essentially biochemical reactions. In fish, the molecular activation of metabolites and enzyme systems and, therefore, the rate of the biochemical reactions are governed by certain environmental factors, such as temperature and pH (Fry, 1971; Brett, 1979). The upper limit of metabolic processes are ultimately defined by the point where the biochemical reactions are optimized (Brett, 1971; Crawshaw, 1979; Jobling, 1981). However, metabolism is a combination of physiological and metabolic processes. The two processes require coordination in order to maximise metabolism. **Directive factors**, therefore, act as a cue for a particular physiological response (Fry, 1971; Schwassmann, 1971). At this point it should be emphasised that the effects of individual environmental factors may fall into one or more of the functional categories at any one time. Therefore an environmental factor, such as temperature, may act as both a **controlling** and a **directive** factor. Physiological processes also demand regulation of the internal environment. A constant expenditure of energy is required to maintain the internal milieu. However, the energy expenditure is dependent on the difference between the external environment and the internal requirements (Woo & Fung, 1981; Morgan & Iwama, 1991). Theoretically the smaller the difference between the internal and external environment, the lower the energy expenditure (Potts, 1954; Febry & Lutz, 1987; Watanabe *et al.*, 1989). Under these conditions the energy saving may **mask** the effect of other environmental factors on metabolism. For example, metabolism at low temperature and in an isosmotic salinity may be closer to optimal than that in high salinity and optimal temperature. Therefore, in this hypothetical example, salinity acts as a masking factor on temperature.

The interactions between controlling, masking and directive factors are highly complex. However, the determination of the optimum environmental conditions for a species requires interpretation of the effects of controlling, masking and directive factors into an experimental sequence. It can be argued that if the biochemical processes are not maximised in the first place then optimum metabolism cannot be attained. This implies that an investigation of the effect of controlling factors should follow the determination of the limiting factors. Masking factors have a compromising effect on metabolism and, therefore, interpretation of data pertaining to metabolism. Masking factors, therefore, need to be investigated before directive factors. As directive factors moderate metabolism through both physiological and behavioural means, accurate interpretation of the effect of directive factors requires optimization of all other factors. The first step in defining the optimum environmental requirements of a species is, therefore, to identify the environmental factors that may influence metabolism and classify them according to the functional categories. As limiting factors affect metabolism by interfering with the supply or removal of metabolites, the environmental factors that contribute to this category apply to all fish. From the literature it was established that the environmental factors which limit fish growth are ration size, dissolved oxygen, ammonia and nitrite. The effect of ration size on growth has been discussed previously, however; the pattern of growth is similar for all limiting factors (Stewart *et al.*, 1967; Alderson, 1979; Chiba, 1988).

Essentially the growth response of fish relative to the concentration of the limiting factor involves dependent and independent states. To use the effect of oxygen as an example, it has been found that as oxygen concentration increases from the lower lethal limit, growth increases almost exponentially (Stewart *et al.*, 1967; Chiba, 1988). However, as the concentration is increased, further growth rate declines until it eventually reaches a plateau of maximum growth (Stewart *et al.*, 1967; Chiba, 1988). Growth is, therefore, at a stable maximum from a critical concentration to the saturation point (Stewart *et al.*, 1967; Chiba, 1988). Provided oxygen concentration is maintained within this range, growth is independent of oxygen concentration (Brett, 1979). The critical concentration at which growth becomes independent of dissolved oxygen varies from species to species. However, maximum growth is always observed at 100% saturation. For toxic metabolites, such as ammonia and nitrite, a similar pattern of growth is observed, although in reverse. At low concentrations toxic metabolites have no effect on growth, but from a critical concentration their effect increases with increasing concentration. From this it can be surmised that limiting factors evoke an 'all or nothing response' as they are governed by critical threshold levels of tolerance and therefore their effect on growth is negligible provided they are maintained at appropriate levels (Fry, 1971; Brett, 1979). Since the appropriate levels correspond to the maximum or minimum concentrations of the respective factors, there is no need for optimization of limiting factors. Therefore, with the exception of ration size, the effect of limiting factors did not enter into further consideration in this study. The effect of ration size was investigated as it is a critical factor in the accurate determination of the environmental requirements of any species (Jobling, 1983).

Assuming the critical levels of the limiting factors are met, environmental factors influence metabolism by acting through controlling, masking and directive factors. The major environmental factors that contribute to the respective functional categories were identified from studies of the natural history of juvenile spotted grunter as well as ecological studies of South African east coast estuaries. The spotted grunter is classified as estuarine dependent, as the juveniles of this species are found only in estuaries (Whitfield, 1990). Southern African estuaries are highly unpredictable environments due to the variable volume and erratic nature of rainfall received in the sub-continent (Whitfield, 1990). For example, in temporarily open estuarine systems, which are a feature of the south-east African coastline (Potter *et al.*, 1990), salinity may fluctuate between 0 - 114‰ depending on the amount of freshwater inflow. Turbidity levels and temperature may also vary according to season and between different estuarine systems. Juvenile grunter have been recorded in turbidities of 0-350 NTU (Cyrus, 1988; Ter Morshuizen *et al.*, in press) and temperatures ranging between 12-32°C (Wooldridge & Bailey, 1982; Ter Morshuizen *et al.*, in press). Since changes in turbidity are accompanied by a concomitant change in light intensity (Alabaster & Lloyd, 1980), juvenile grunter are also subject to natural light intensity variation of between 0 and $3.65 \times 10^{-2} \mu E.sec\text{-}1.cm^2$. Changes in light intensity are also dependent on photoperiod. In the Sundays River Estuary (33°43'S, 25°51'E), the most southerly distribution of juvenile grunter (Beckley, 1984), the photoperiod cycle varies from 10L:14D in winter to 14L:10D in summer. Therefore, under natural environmental conditions, juvenile spotted grunter are exposed to changing temperature, salinity, light intensity and photoperiod.

The effect of temperature on fish is predominantly manifested through its control of the activation of metabolites and enzyme systems (Fry, 1971). Temperature is, therefore, considered to be the principal controlling factor of metabolic processes (Fry, 1971; Brett, 1979). However, under non-optimal environmental conditions masking factors have a compensatory effect on metabolic processes (Brett, 1979). Juvenile spotted grunter are capable of surviving in salinities ranging from 0-70‰. *P. commersonnii* is an efficient osmoregulator (Bussiahn, 1992). This involves internal ion regulation with the active transport of ions requiring some energy expenditure. As energy expenditure varies according to external salinity concentration (Woo & Fung, 1981; Morgan & Iwama, 1991), salinity acts as a masking factor on growth (Brett, 1979). Photoperiod and light intensity are important components in the complex of cues that synchronize the endogenous physiological and activity rhythms of fish (Fry, 1971; Schwassmann, 1971; Reynolds & Casterlin, 1976). Photoperiod and light intensity, therefore, act as directive factors on the metabolism of fish (Fry, 1971; Brett, 1979).

In contextualizing the effects of the respective functional categories, a theoretical sequential protocol for the experimental investigation of environmental requirements was developed. The sequence is as follows:

limiting factors → controlling factors → masking factors → directive factors.

Therefore, the sequence for the determination of the environmental requirements of juvenile spotted grunter would be:

ration size →	temperature →	salinity →	photoperiod/light intensity
(limiting factor)	(controlling factor)	(masking factor)	(directive factors).

However, if food intake is not restricted, the effect of limiting factors can be eliminated from the protocol. In addition, preliminary investigation of the effect of salinity on the growth of juvenile grunter was completed by Bussiahn (1992) before the current study.

The order of investigation was, therefore, altered to the following sequence:

temperature → photoperiod → light intensity → feeding frequency → ration size → salinity.

The effect of each environmental factor on growth performance was determined individually under controlled conditions where only the factor under investigation was varied. All other environmental factors were kept constant at levels which were optimal or within ranges of environmental tolerances. The effect of ration size was included in the investigation to test the assumption that food intake was not restricted when testing the effect of the respective controlling, directive and masking factors. Since food intake is primarily dependent on feeding regime (Grayton & Beamish, 1977), the effect of feeding frequency on food intake was also investigated. Other biotic factors that may restrict food intake, such as competition and dominance between individuals (Brett, 1979), were not investigated within the scope of this study. However, the effects of these environmental factors were considered when interpreting results. An investigation into the effect of salinity was repeated under 'optimum' environmental conditions for comparison with the conclusions reached by Bussiahn (1992).

In this study it was hypothesised that the sequence for experimentally determining the optimum environmental requirements of a species should correspond to the following theoretical protocol:

limiting factors → controlling factors → masking factors → directive factors.

The experimental investigation into the individual effects of temperature, salinity, light intensity, photoperiod and food intake on growth performance of juvenile spotted grunter facilitated the determination of the relative effects of controlling, masking, directive and limiting factors. Statistical comparison of the effects of the environmental factors (temperature, salinity, etc.) on growth performance, facilitates the formulation of a experimentally based protocol for sequentially determining the optimum environmental requirements of a species. On the basis of comparison between the theoretical model and the experimental model, the hypothesis can be accepted or rejected. It was, therefore, also hypothesised that temperature, salinity, light intensity, photoperiod and food intake would affect the growth of juvenile *P. commersonnii*. The effects of these factors on growth performance are contextualized in the respective chapters.

This study begins with a general description of the methods and methodologies employed in the experimental determination of the individual effect of environmental factors on growth performance of juvenile spotted grunter (chapter 2). In the following chapters (3-5), the effects of temperature, light and salinity on growth performance of juvenile spotted grunter are described and discussed. Chapters 6 and 7 report the investigations into the effects of feeding regime, including feeding frequency, digestion and ration size. In Chapter 8 the overall effects of environmental factors on growth of juvenile spotted grunter are statistically compared and ranked according to their effect. A sequential protocol for determining the optimum environmental conditions based on the results of the experimental data is then developed. In Chapter 9, a comparison of the theoretical and experimental models for determining environmental requirements is undertaken. Based on this comparison a preliminary protocol for the sequential determination of the optimum environmental requirements of potential mariculture species is developed. Chapter 9 concludes with recommendations for the hatchery rearing of spotted grunter and an assessment of maximum growth.

Chapter 2

General Methods

Accurate measurement of the growth performance of juvenile spotted grunter was fundamental to determining the environmental requirements of juvenile spotted grunter. All experimentation was conducted under laboratory conditions at the Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, on fish captured from the wild. The experimental procedures and culture systems used to monitor growth as well as the methods employed for the capture, acclimation and feeding of the experimental fish are described in this chapter. A contextualization of the theoretical and practical aspects of monitoring growth and statistical analyses of growth data is also undertaken.

Collection and acclimitisation of experimental fish

Juvenile grunter were caught in the Great Fish River Estuary (33° 30'S 27° 07'E) with a 12m x 3m x 15mm mesh seine net. From there they were transported in 200 L plastic containers, filled with 150 L estuarine water supplied with oxygen, to the Department of Ichthyology and Fisheries Sciences marine fish laboratory in Port Alfred. Here the fish were gradually acclimitised from natural conditions to those in the holding facility. Juvenile spotted grunter were generally captured at salinities of 5-12‰, and were acclimated to the higher salinity (32-35‰) of the laboratory seawater at a rate of 2‰ every 30 minutes. The temperature of the natural water ranged between 16-25°C but normally only differed from the laboratory water by 2-4°C. However, the rate used for acclimation to differences in salinity allowed adequate time for any adjustment to temperature differences as it exceeded the recommended rate of 2°C every 10 minutes. Survival of the juvenile grunter after capture varied from 40-85% depending on the ambient water temperature in the estuary and the degree to which fish were exposed to silt during capture. Although in some cases initial mortality after capture was high, survival beyond 48 hours was generally greater than 90%. During acclimatisation to captive conditions the fish were kept in 300 L rectangular tanks linked to a 4000 L partially recirculating seawater system exposed to natural light and ambient temperatures. The fish were acclimatised for a minimum of two weeks before they were used for experimentation. During this time they were fed minced pilchard (*Sardinops sagax*) in excess of their requirements, three times daily.

The fish were then transported to the laboratories in Grahamstown where further acclimatisation to the experimental conditions was undertaken. The fish were transferred directly from their transport containers to the experimental system. Acclimatisation to the temperature or salinity of the water in the experimental system was not necessary as this was adjusted to equal that of the Port Alfred facility. The fish were size sorted and eight to ten fish were stocked in each of several 40 L experimental aquaria. The water temperature of the experimental system was then gradually increased to within the zone of final preference (24 - 25°C) for this species (Deacon & Hecht, 1995). The fish were fed finely chopped pilchard at least three times daily until they became conditioned to accepting food, after which they were weaned onto a semi-moist pelleted diet. The fish were acclimatised to respective experimental treatments for a minimum of two weeks.

Experimental diet

The adoption of a standard diet for experimentation on the environmental requirements of juvenile spotted grunter was considered essential for the prevention of any growth variation stemming from diet related differences. Early attempts to wean juvenile grunter onto a commercial dry diet proved unsuccessful. Although the fish would repeatedly inhale dry pellets, they would not swallow them. It was concluded from this behaviour that the texture of a dry pellet is inappropriate for this species. However, a semi-moist pelleted diet proved to be highly palatable and was well accepted by juvenile spotted grunter. Formulation of the diet was based on the protein and fat requirements of other juvenile marine fish (Table 2.1). Table 2.2 summarises the

the proximate concentration of the semi-moist pelleted diet used for feeding juvenile spotted grunter during the experimental period. The diet, by weight, consisted of fishmeal (42%), pregelatinised starch (19.9%), marine oil (4.4%), sunflower oil (0.5%), a vitamin and mineral premix (3.6%) and water (11.9%). Homogenised fillets of pilchard were added at 17.2% by weight to increase palatability. All ingredients were thoroughly mixed by hand before extrusion through a pelletizer with a 1mm die. The length of the pellets was varied according to the size of the fish. The diet was stored frozen at -29 ± 2°C. Fresh food was prepared every two weeks during the experiments.

Table 2.1: The dietary protein and fat requirements of some juvenile marine fish.

FISH SPECIES	FISH SIZE (g)	PROTEIN LEVEL (%)	FAT LEVEL (%)	SOURCE
Sciaenops ocellatus	46-77	35-44		Daniels & Robinson, 1986
	2.0	40-45		Serrano *et al.*, 1992
			6-10	Ellis & Reigh, 1991
Scopthalmus maximus		50-60		Person-Le Ruyet *et al.*, 1991
			6	Bromley, 1980
Dicentrarchus labrax	31-57	50		Hidalgo & Alliot, 1988
Seriola quinqueradiata		57		Shimeno *et al.*, 1985
Pagrus major	1.6	52		Takeuchi *et al.*, 1991
		50		
Sparus aurata		61		Sabaut & Luquet, 1973
			6	Kalgeropoulas *et al.*, 1992
Gadus morhua	173-318	54		Lie *et al.*, 1988

Table 2.2: The proximate concentration of the semi-moist pelleted diet used for feeding juvenile spotted grunter during the experimental period.

Crude protein	36.30%
Crude fat	3.54%
Ash	16.77%
Moisture	27.7%
Gross energy	18.1878 MJ/kg

Experimental culture system

Most of the experiments were conducted in 40 L glass aquaria, linked to a 2000 L seawater recirculation system. The seawater used to fill the system was obtained from Port Alfred. The aquaria were illuminated by 91cm Biolux fluorescent tubes providing a light intensity of 1.95x10 μE.sec^{-1}.cm^{2}. Photoperiod was controlled by means of a time switch. Filtered seawater was circulated through the tanks at a rate of 60 L/h and water was aerated by means of airstones connected to an oil free rotary blower. Outflow water from the experimental tanks drained into a 350L settlement tank after which it was passed through a series of biological and mechanical filters. Biological filtration was affected in two serially linked 70 L filter boxes and a tower trickle filter (2m x 0.15m) filled with finely shredded plastic (Fig 2.1). Water exiting the biological filters was mechanically filtered through aquarium floss and a crushed shell buffer tank (70 L) before reaching the sump from where it was recirculated by a submersible pump (Fig 2.1). Water destined for the culture tanks was passed through a pressure filter capable of filtering to 10μm and a Ultraviolet sterilizer (Fig 2.1). The excess delivery from the pump was directed to either the trickle filter (described above), or to a protein skimmer situated in the settlement tank (Fig 2.1). The water temperature was maintained within the thermal preferendum of the species (Deacon & Hecht, 1995), at 24.8 ± 0.3°C by means of a thermostatically controlled 3kW heating element located in the sump (Fig 2.1).

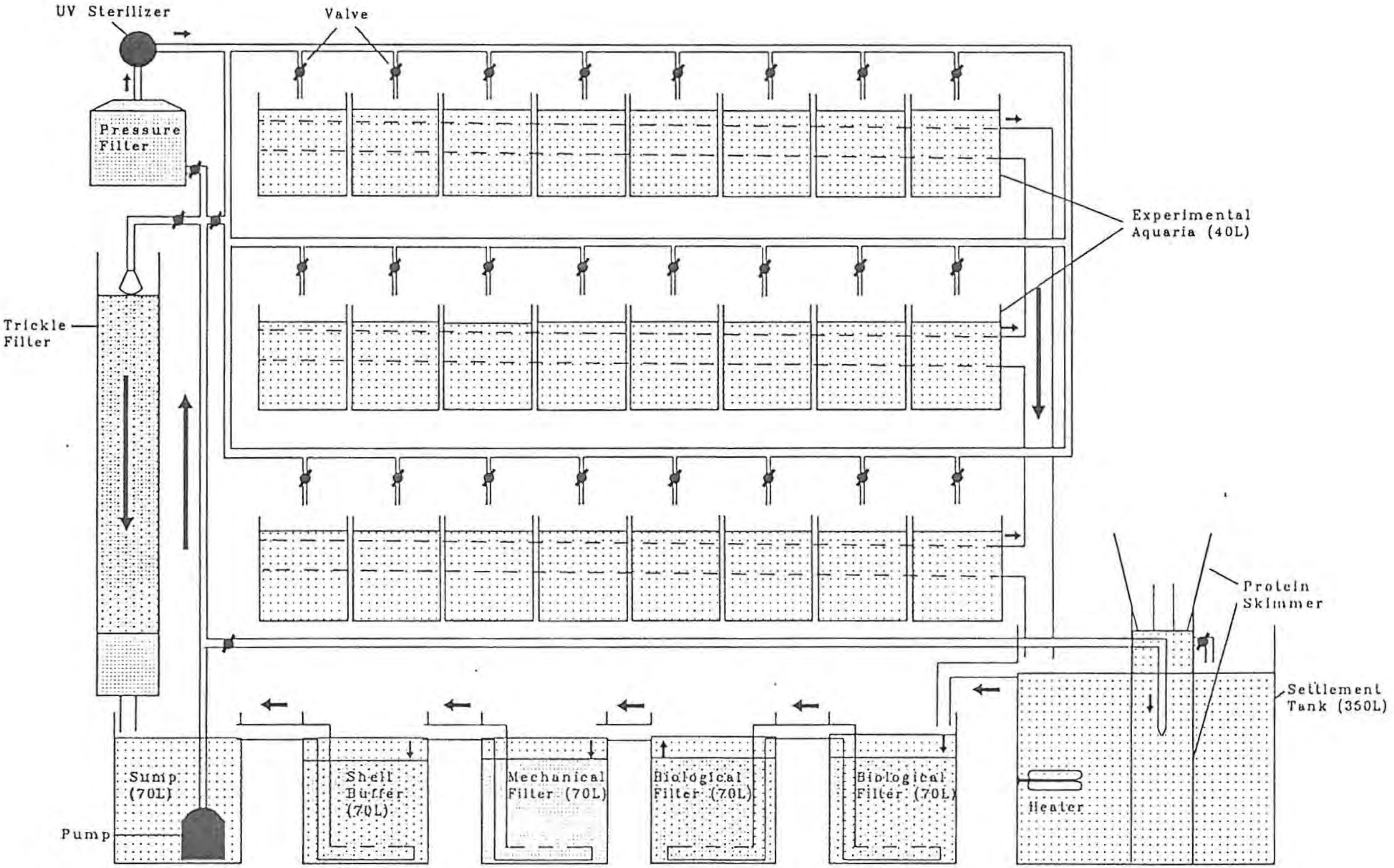

Figure 2.1: The experimental system used in the determination of the optimum environmental requirements of juvenile spotted grunter. The arrows indicate direction of water flow.

Due to the requirements of the temperature and salinity experiments, two other culture systems were constructed for these studies. These are described in the respective chapters.

Experimental procedure

General maintenance and cleaning of the experimental system was undertaken prior to each experiment. These activities included removal of algal and diatomaceous growth from surfaces of the aquaria and cleaning of filters. Any accumulated sludge was siphoned from the settlement tanks and filters. Mechanical filters were also changed or rinsed. A water change of ± 20% with dark-matured seawater was conducted at this time.

Each of the tanks was stocked with 8 fish which had been acclimatised to the respective experimental conditions for 14 days. To improve statistical treatment of data all fish were individually marked (Brett, 1979), under anaesthesia, by clipping off the tips of different combinations of dorsal and anal fin spines. The fish were anaesthetised by placing them in a 0.02% solution of 2-phenoxyethanol (Deacon *et al.*, 1997). Although it is known that anaesthesia effects the growth of some fish species, the growth rate of spotted grunter is not affected by regular anaesthesia with 2-phenoxyethanol (Deacon *et al.*, 1997). During the experiments standard length and weight of each fish were measured weekly, under anaesthesia. The fish were starved for 36 hrs prior to measurement to ensure complete evacuation of the gut and to prevent the fish from choking on regurgitated food. During the weighing and measuring procedure, the fish were transferred to a 10 L container filled with 4 L of seawater, freshly drawn from the recirculation system, to which the anaesthetic at a concentration of 0.2ml/L was added. The fish were weighed to 0.01 g and standard length was measured to the nearest millimeter, after which they were placed in their respective tanks to recover. Water quality parameters, such as ammonia, nitrite, temperature and salinity, in the experimental system were monitored daily. Salinity and dissolved oxygen levels were kept constant at 35‰ and 100% saturation, respectively.

Statistical procedures

The growth (increase in grams per week) of individual fish in the replicates of each treatment were compared by one-way ANOVA. The condition factor (C) was calculated from weight (W) and length (L) using the relationship

$$C = W/L^b$$

where b = 2.71, which was determined from a length/weight regression, $y = 2.71x - 109.52$ ($r^2 = 0.97$, n = 498), of wild fish for the size range (2.5 - 10.0g). Food conversion ratio (FCR) was calculated according to the equation:

$$FCR = \text{Dry food fed /wet weight gain}$$

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

$$SGR = [(\ln W_1 - \ln W_0)/t] \times 100$$

where t is time in days, W_1 = weight at time t and W_0 = initial weight.

Additional comparison of weight gain between treatments was achieved by an analysis of covariance (F-Test), for which the data were linearly transformed using the natural Log of individual weight. Specific Growth Rate, Condition Factor and Food Conversion Ratio were compared using a one-way ANOVA and differences among means were tested by applying Tukey's Multiple Range Analysis. Equality of variances among treatments were confirmed with a Bartlett's Test (Zar, 1984).

Fish size is an important factor influencing the growth of fish (Elliot, 1975b; Brett & Shelbourne, 1975; Brett, 1979). Generally, maximum growth rate of fish decreases with increasing size (Elliot, 1975b; Brett & Shelbourne, 1975; Brett, 1979). Initial growth is almost exponential and is generally linear. However, as size increases growth rate moderates and the relationship between maximum growth and size becomes curvilinear. In species which have distinct larval and juvenile phases, the size-growth continuum is punctuated by short periods of variable growth corresponding to metamorphosis (Brett, 1979).

The average initial length of juvenile spotted grunter used in experiments to determine the independent effect of environmental factors on growth, varied between 50 and 68mm. This variation is not considered to have had any compromising effect on maximum growth as fish of this size are still in the exponential phase of growth. Furthermore, all major differentiation, such as metamorphosis, has occurred before juvenile spotted grunter reach this size.

Chapter 3

Thermal resistance and the effect of temperature

Introduction

Temperature has consistently been identified as the primary abiotic factor controlling key physiological, biochemical and life history processes in fish (Beitinger & Fitzpatrick 1979). The control that temperature exerts on rates of reaction, through its influence on the activity of metabolites, ultimately determines the effect of all other environmental variables. A knowledge of the interaction of a fish and its thermal environment is, therefore, of fundamental importance to the study of environmental requirements. Since it also directly affects growth and survival of fish, it is essential information for the aquaculturalist.

The responses of fish to temperature are most often described in terms of the thermal limits, optimum temperature and preferred temperature. The zone of thermal **tolerance** of a species is defined by the incipient lethal temperature (ILT) range within which at least 50% of the population could survive indefinitely (Jobling, 1981). Beyond this range lies the zone of **resistance** where there is a progressive increase in thermal stress (Elliot, 1981). Both the thermal tolerance and thermal resistance of fish are strongly influenced by acclimation temperature (Jobling, 1981). Indication of thermal stress begins with abnormal behaviour, such as cessation of feeding, and terminates with metabolic disfunction and ultimately death depending on exposure time to the stress (Elliot, 1981). The extreme measure of thermal resistance is the critical thermal maximum (CTMax) which represents both a method and a parameter (Becker & Genoway, 1979). CTMax and the upper incipient lethal temperature (UILT) are often confused as they both appear to measure maximum thermal tolerance. However, the methods of estimating these values illustrate the fundamental differences between them. Determination of the CTMax requires a progressive upward change from acclimation to exposure temperatures until a physical

disorganisation response is observed. On the other hand, estimation of the upper incipient lethal temperature (UILT) requires an **abrupt change** of temperature upwards from acclimation to exposure until a **lethal response occurs** (Becker & Genoway, 1979).

Within the limits of thermal tolerance fish, when provided a choice, ultimately congregate at and occupy a narrow range of temperatures referred to as the zone of final thermal preferendum or preferred temperature (Reynolds & Casterlin, 1979). The selected range of temperature is species specific and ultimately independent of previous thermal history (Fry, 1947; Jobling 1981). This behaviour is significant as numerous studies on the thermal responses of fish have shown that the zone of final thermal preferendum closely approximates the temperature at which their physiological functions, including growth, are optimised (Brett, 1971; Crawshaw, 1979; Jobling 1981; Britz & Hecht, 1987). Furthermore, a strong correlation between the final preferendum and optimum growth has led to the conclusion that temperature preference studies provide a rapid technique for establishing the optimal temperature for rearing that species (Jobling, 1981). Methods for determining the temperature preference include temperature gradient tanks, electronic shuttle boxes and body core temperature telemetry and calorimetry (McCauley, 1977). A number of temperature preference studies have, however, exhibited experimental bias, such as an 'end of tank' effect (Badenhuisen, 1967). Non-thermal environmental factors such as season (Barans & Tubb, 1973), light intensity (Reynolds *et al.*, 1977), age (McCauley & Huggins, 1979) and biotic interactions (Beitinger & Magnuson, 1975) have also been shown to influence thermoselective behaviour. Consequently estimates of the zone of final thermal preferendum often require validation through growth trials. In these experiments survival, growth and food conversion are measured above and below the final thermal preferendum. This is time consuming and requires a high number of experimental animals.

Strong positive correlations have also been found between the CTMax, and the final thermal preferendum (Jobling, 1981; Tsuchida, 1995). Regression analyses between the CTMax and the final thermal preferendum of 14 species of Japanese marine fish yielded a correlation coefficient of 0.973 (Tsuchida, 1995). On the strength of the good correlation between lethal temperature and CTMax, and the final preferendum, it has been suggested that determination of the CTMax may provide a rapid means of assessing the final thermal preferendum (Tsuchida, 1995). An indirect estimate of the optimum temperature for growth could then be obtained from the final thermal preferendum. Although Jobling (1981) was the first to recognise the potential of this method, it was not considered precise enough to give an accurate estimate of the final thermal preferendum. The main criticism was the inconsistency of the methods, in particular the heating rate, used to determine the CTMax.

The CTMax was originally defined as the temperature at which fish lose equilibrium when subjected to a progressive heating of the water at a rate of 1°C min^{-1} from acclimation temperature (Jobling, 1981). This rate is no longer considered appropriate as it results in a lag between deep-body temperature and test temperatures (Stevens & Fry, 1974, Becker & Genoway, 1979). Therefore the higher the heating rate the higher the estimate of CTMax (Becker & Genoway, 1979). In their evaluation of methods for determining CTMax, Becker and Genoway (1979) recommended a heating rate of 18°C h^{-1} (0.3°C min^{-1}). This moderate rate was considered optimal as deep-body temperature parallelled test temperatures, but at the same time did not allow for compensation by acclimation. In recent studies of CTMax a heating rate of 5°C h^{-1} has been adopted (Tsuchida, 1995; Kita *et al.*, 1996). This rate is presumably based on Becker and Genoway's (1979) study which showed no significant difference between a heating rate of 6 and 18°C h^{-1}. Nonetheless, the methodology for determining CTMax now appears to be more or less standardised which suggests that this may be a reliable criteria for calculating the final preferendum. Furthermore, independent evaluation of the final thermal preferendum by the CTMax method and temperature preference experiments may eliminate the need for growth studies when establishing thermal optima.

In an investigation into the thermoregulatory behaviour of juvenile *Pomadasys commersonnii*, Deacon and Hecht (1995) found the zone of final temperature preference of this species to lie between 24 and 25°C. In this study (*op cit.*) supplementary temperature preference experiments were also conducted to the test the effect of potential biases such as variation in salinity, social interaction, acclimation temperature and 'end of tank' effect. Although these factors were found to have no effect on temperature preference, it is still unknown whether the zone of final thermal preferenda correlates with the optimum temperature for growth in this species. The aim of this study was, therefore, to establish the optimum temperature for growth in juvenile spotted grunter and to test the hypothesis of whether thermal resistance data can be used to accurately predict the zone of final thermal preferendum and to confirm that this corresponds with the growth optimum.

Materials and methods

Temperature growth trial

The effect of temperature on growth was conducted in 50 L glass aquaria. The experimental setup included two replicates at each of five temperatures with independent filtration for each tank. All tanks contained an *in situ* type filter (18 L) filled with crushed shell, through which water was circulated at a rate of 60 L/h by means of an airlift system (Fig 3.1). Ten fish were used per replicate. Initial length and weight of the fish were 68 ± 6.7mm and 14.62 ± 2.79g. The temperature treatments differed by increments of 2°C within a range of 4°C above and below the thermal preferendum of 24.5°C (Deacon & Hecht 1995). The tanks were heated with 300 Watt thermostatically controlled heaters. Average temperature and standard deviation for the treatments were 20.5 ± 0.1°C, 22.6 ± 0.2°C, 24.6 ± 0.3°C, 26.3 ± 0.4°C and 28.5 ± 0.5°C, respectively. Water quality parameters were monitored daily and these remained optimal throughout the experiment with nitrite levels remaining below 0.1 mg/L and unionised ammonia remaining below 0.02 mg/L. Photoperiod was controlled to a 12L:12D cycle. Salinity was kept constant at 35‰, with the addition of distilled water when necessary. The fish were fed to satiation twice daily. The experiment was conducted over a period of 8 weeks.

Determination of thermal resistance

The thermal resistance of juvenile spotted grunter was evaluated on the basis of a CTMax test (Becker & Genoway, 1979). A total of 100 fish (SL: 55.3 ± 2.4mm and weight: 3.71 ± 0.92g) were divided into four groups of 25 fish and acclimated to temperatures of 20 ± 0.2, 24 ± 0.3, 27 ± 0.5 and 30 ± 1°C, respectively. The fish were acclimated for three weeks prior to use in CTMax experiments. During this time fish were fed to satiation three times a day on a semi-moist pelleted diet. Photoperiod was maintained on 12L:12D cycle and salinity was kept constant at 35‰.

For the CTMax tests five fish were taken at random from the respective acclimation temperature and transferred to a 30 L test tank containing seawater of the acclimation temperature. The fish were starved for 24 hrs prior to experimentation. The test tank was immersed in a temperature moderating water bath to prevent excessive heat loss during the experiment. Airstones were positioned in the test tank to create water movement and maintain dissolved oxygen at saturation level. The fish were left undisturbed for a minimum of 1 hr to acclimatize to conditions in the test tank. Water temperature was then progressively increased by the addition of heated (60°C) seawater. The stream of heated seawater was positioned so as to fall directly into the plume of the central airstone. This ensured the rapid distribution of heated water throughout the test tank within 2 seconds. Temperature was simultaneously measured in the centre and edges of the tank. The CTMax for each fish was recorded as the temperature at which loss of equilibrium occurred. The fish were then returned to water at the respective acclimation temperature to recover. CTMax tests at each acclimation temperature were performed at two rates of increase, 0.3 and 1°C min^{-1}, respectively. All tests were performed in duplicate (n = 10 fish).

Statisical analyses

Statistical analyses of the growth data was conducted according to the methods described in Chapter 2. CTMax values for individual fish in each of the replicates, acclimation temperature and heating rate were compared using a one-way ANOVA. CTMax for each acclimation temperature and rate was then calculated as the arithmetic mean from observation of 10 fish. Linear regression analyses was applied to establish the relationship between acclimation temperature and CTMax for the respective heating rates of 0.3 and 1.0°C min^{-1}. Slope of the regressions were compared by analysis of covariance (F-test).

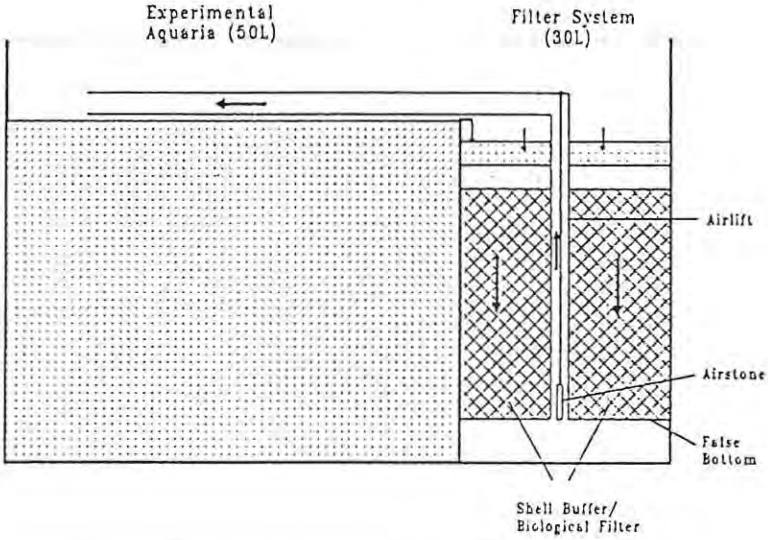

Figure 3.1: The experimental unit used to establish the effect of temperature on the growth of juvenile spotted grunter (*Pomadays commersonnii*). The arrows indicate direction of water flow.

Results

Effect of temperature on growth rate

Temperature was found to have a highly significant ($P<0.01$) effect on growth, condition factor and food conversion of juvenile spotted grunter (Table 3.1). Growth, expressed as weight gain over time, was found to increase rapidly from the lowest temperature (20.5°C) to 24.5°C (Fig 3.1). Above 24.5°C there was no significant improvement in weight gain (Fig 3.2) or Specific Growth Rate (Table 3.2). The highest condition factor of the fish was recorded at 22.5°C, although it did not differ significantly from the condition of fish at 24.5°C (Table 3.2). The condition of the fish was, however, significantly lower at the lower and upper extremes of the temperature range (Table 3.2). Food conversion ratios were similar at all temperatures except at 20.5°C, where a significantly ($P<0.01$) higher FCR was recorded (Table 3.1 & 3.2).

Table 3.1: Summary of one-way ANOVA tests of the effect of temperature on the growth performance of juvenile spotted grunter, *Pomadasys commersonnii*.

FACTOR	F-RATIO	D.F.	SIG. LEVEL
Temperature: 20.5, 22.5, 24.5, 26.5 & 28.5°C			
Condition factor	12.070	4	0.0000
Food conversion ratio	45.722	4	0.0004
Specific growth rate	7.937	4	0.0000

Table 3.2: Tukey's Multiple Range Analyses' of the effect of temperature on the condition factor, food conversion and growth of juvenile spotted grunter, *Pomadasys commersonnii*. Superscript letters (a,b & c) indicate homogenous groupings of means. Temperature treatments that share any common letter are not significantly different from one another ($P<0.05$)

Growth parameter	Temperature (°C)	Tukey HSD Average
Condition factor	20.5	0.65065^{ab}
	22.5	0.66873^{c}
	24.5	0.66070^{bc}
	26.5	0.64368^{a}
	28.5	0.64328^{a}
Food conversion ratio (FCR)	20.5	2.46495^{a}
	22.5	1.58635^{b}
	24.5	1.46273^{b}
	26.5	1.51714^{b}
	28.5	0.64328^{b}
Specific growth rate (SGR)	20.5	0.71823^{a}
	22.5	1.22994^{a}
	24.5	4.46579^{b}
	26.5	4.73737^{b}
	28.5	5.31598^{b}

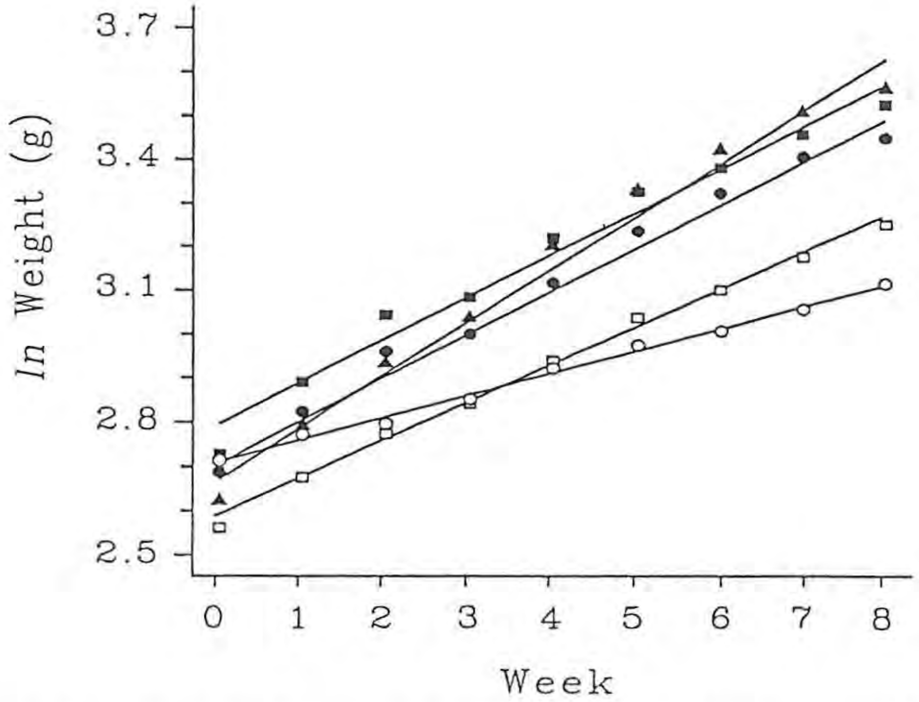

Figure 3.2: The effect of temperature on the weight gain of juvenile spotted grunter, *Pomadasys commersonnii*. Each datum for 20.5 °C (○), 22.5 °C (□), 24.5 °C (■), 26.5 °C (●) and 28.5 °C (▲) is the mean of *ln* weight (g).

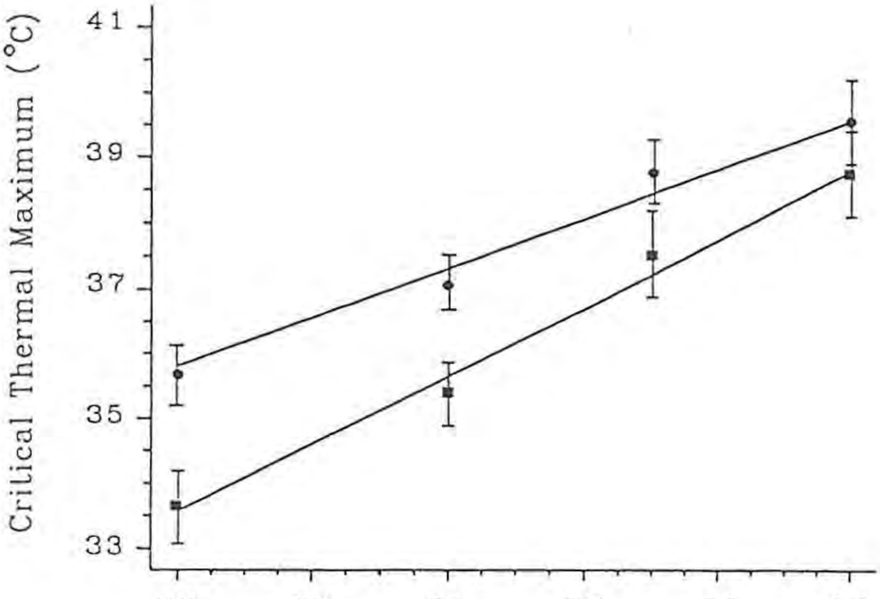

Figure 3.3: The effect of acclimation temperature and heating rates of 0.3 (■) and 1.0 °C min^{-1} (●) on the critical thermal maximum (CTMax) of juvenile spotted grunter, *Pomadasys commersonnii*. Each datum is the arithmetic mean of 10 fish and the vertical bars are standard deviations.

Thermal resistance

The CTMax of juvenile spotted grunter was significantly affected by acclimation temperature and heating rate ($P<0.05$) (Fig 3.3). Increasing acclimation temperature resulted in significantly higher ($P<0.05$) values of CTMax at both heating rates (0.3 and 1°C min^{-1})(Fig 3.2). The relationship between acclimation temperature and CTMax was found to be linear regardless of heating rate (Fig 3.2). However, the slopes of the linear regressions of acclimation temperature and CTMax for 0.3 and 1°C min^{-1} were found to be significantly different. Values of CTMax determined at a heating rate of 1.0°C min^{-1} at each acclimation temperature were significantly higher than those determined at a rate of 0.3°C min^{-1} ($P<0.05$).

Discussion

The effect of temperature is predominantly manifested through its control of the rate of chemical reactions (Fry, 1971). As fish are obligate poikliotherms, their metabolism is temperature dependent, with ambient water temperature controlling molecular activation of the metabolites and enzyme systems. As temperature is the principal factor controlling metabolism, and therefore activity, investigation into this environmental factor must precede all others. The optimum temperature for growth is thought to correspond to the temperature at which all physiological and biochemical reactions are optimal. Determination of the optimum temperature for growth, therefore, serves as a basis upon which the effects of all other factors can be examined.

The metabolic and physiological activities of juvenile spotted grunter were undoubtedly regulated by temperature. Optimum growth, condition and feed conversion were observed at 24.5°C. At 20.5°C the growth and condition of juvenile grunter was 38.4% and 2.7% lower than at 24.5°C, respectively. Food conversion was 59.3% higher at 20.5°C than at 24.5°C. At 22.5°C growth was slightly, though not significantly better than at 20.5°C although condition factor and feed conversion were significantly better. Above the optimum temperature there were no further improvements in growth or food conversion, but there was a significant loss in condition. This suggests that the high temperature tolerance of grunter is incurred at high metabolic cost. However, the highest temperature tested in this experiment was not sufficiently high enough to be detrimental to growth. It is concluded that juvenile grunter should be successfully reared in water temperatures of 24-28°C, with optimum growth at 24.5°C.

The optimum temperature for growth of 24.5°C for juvenile grunter fell within the zone of final preference 24-25°C determined by Deacon and Hecht (1995). These results provide further confirmation that observation of behavioural thermoregulation is a valid method of establishing the zone of final thermal preference and therefore the optimum temperature for growth. Furthermore, knowledge of both the optimum temperature for growth and the final preferendum provides an ideal base to assess alternative methods of determining the final preferendum.

Tsuchida (1995) suggested that determination of the CTMax provides a feasible alternative to temperature preference studies for determining the final thermal preferendum. Correlation of the CTMax with the final preferenda of 14 marine fish species resulted in a highly significant relationship between these responses. The correlation describes a linear functions as follows:

$$\mathrm{CTMax} = 0.741\ \mathrm{FP} + 17.104\ (r = 0.973)\ \text{(Tsuchida, 1995).}$$

On the strength of this correlation it was implied that the final preferendum could be calculated from an estimate of the CTMax (Tsuchida, 1995). As the relationship between acclimation temperature and CTMax is linear, it is not clear from Tsuchida's (1995) discussion how a single value of CTMax parameters would be obtained for substitution into the relationship. Substitution of random values of CTMax into Tsuchida's (1995) relationship will provide a range of temperatures which may or may not correspond with the final preferendum.

The critical thermal maximum of juvenile spotted grunter was found to be highly dependent on acclimation temperature irrespective of whether the heating rate was 0.3 or 1.0°C min^{-1}. An increase in acclimation temperature resulted in a linear increase in CTMax. This relationship is consistent with the response observed in other species (Becker & Genoway, 1979; Jobling, 1981; Tsuchida, 1995; Kita *et al.*, 1996). Since there is no point of inflection, at least within the zone of tolerance, it is not possible to determine a finite value for CTMax. Furthermore, the CTMax at any one acclimation temperature is dependent on the heating rate. In juvenile spotted grunter the CTMax of fish acclimated to 24°C was 1.65°C higher at a rate of 1°C min^{-1} than that at 0.3°C min^{-1}. In addition, the respective regressions of acclimation versus CTMax for the two rates (0.3°C and 1.0°C min^{-1}) were significantly different. Therefore the error between the different heating rates is also inconsistent. Ultimately the difference in CTMax in relation to acclimation temperature and heating rate prevents any reliable calculation of final preferendum. For example, in juvenile spotted grunter acclimated to 20°C, the mean CTMax values determined for heating rates of 0.3 and 1.0°C.min^{-1} were 33.6 and 35.8°C, respectively. The mean CTMax values for fish acclimated to 30°C were 38.7 and 39.5°C for heating rates of 0.3 and 1.0°C.min^{-1}, respectively. If these CTMax values are substituted into Tsuchida's (1995) equation the estimate of the final thermal preferendum could range between 22.3 and 30.2°C depending on acclimation temperature and heating rate. However, temperature preference studies and growth trials indicate the final preferendum for juvenile spotted grunter to be 24.5°C (Deacon & Hecht, 1995; 1996).

In this study juvenile spotted grunter were acclimated to four different temperatures including one (24°C) which corresponded to the final preferendum. In an attempt to establish how Tsuchida (1995) calculated the final preferendum from CTMax data, the respective CTMax values determined at each acclimation temperature and heating rate for juvenile spotted grunter were consecutively substituted into the relationship. It was found that only the CTMax value determined from fish acclimated to 24°C at a heating rate of 0.3°C min^{-1} correlated with the final preferendum. Substitution of this value into the relationship resulted in a calculated final preferendum of 25.03°C. This agrees well with the final preferendum (24 - 25°C) determined from studies of behavioural thermoregulation and the growth optimum of 24.5°C.

Kita *et al.* (1996) observed a similar relationship between acclimation temperature, CTMax and final preferendum in rock fish, *Sebasticus marmoratus*. However, the CTMax values determined at an acclimation temperature of 20°C for *Sebasticus marmoratus* were compared with those calculated from a final preferendum value of 20.7°C (Kita *et al.*, 1996). Perhaps comparison of CTMax values at 20.7°C, determined from the regression of these parameters against acclimation, would have given a more accurate estimate of the final preferendum. In any case, accurate calculation of the final preferendum from CTMax and/or lethal temperature data seems to require an independent estimate of the final preferendum. Using a preliminary estimate of final preferendum determined from a behavioural study, CTMax can be determined experimentally from fish acclimated to a temperature which corresponds to the theoretical final preferendum. Alternatively the corresponding CTMax and lethal temperature values can be obtained from regression of acclimation versus CTMax and/or lethal temperature. The accuracy of the final preferendum from both approaches can be validated by comparison of experimental and calculated estimates of CTMax and lethal temperature or by back calculation of the final preferendum.

In juvenile spotted grunter a significant difference was found between CTMax at the two heating rates (0.3 and 1.0°C min^{-1}). However, an accurate estimate of CTMax can only be obtained by using an appropriate heating rate. Becker and Genoway (1979) suggested that CTMax should be determined at moderate heating rates to prevent discrepancies between deep body and exposure temperature. Higher values of CTMax at the faster heating rate (1.0°C min^{-1}) in juvenile grunter may indicate a lag between deep body and exposure temperature, thereby supporting this observation. It has also been observed that at rapid heating rates, slight variation in measurement of CTMax endpoints (i.e. defining the point of loss of equilibrium) result in differences of CTMax which may exceed 2°C (Becker & Genoway, 1979). This can create errors in the regression analyses and result in unrealistic values of CTMax. In testing the effect of different heating rates (1, 6, 18, 30 and 60°C h^{-1}) on juvenile coho salmon (*Oncorhynchus kisutch*) and pumpkinseed sunfish (*Lepomis gibbosus*), Becker and Genoway (1979) recommended a heating rate of 18°C h^{-1} (0.3°C min^{-1}). This rate was considered optimal as deep-body temperature parallelled test temperatures, but at the same time did not allow for compensation by acclimation. The use of a heating rate of 0.3°C min^{-1} for determination of CTMax for juvenile grunter provided an accurate estimate of the final preferendum. Kita *et al.* (1996) also obtained good estimates of CTMax for *Sebasticus marmoratus* using a heating rate of 5°C h^{-1}. It is therefore concluded that heating rates of 0.1 - 0.3°C min^{-1} should be appropriate for determining CTMax values for confirmation of the final preferendum.

To summarize, optimum growth, condition and food conversion of spotted grunter were observed at 24.5°C. This corresponded with the zone of thermal preference (24 - 25°C) determined by observation of thermoregulatory behaviour (Deacon & Hecht, 1995). An evaluation of the CTMax method of determining the final preferendum (Tsuchida, 1995) revealed that this approach relies on a preliminary value of the final preferendum for further data analyses. The CTMax method does not therefore constitute an independent method of determining the final preferendum. However, if used in conjunction with temperature preference studies, CTMax data could provide confirmation of the final preferendum on purely physiological criteria. The determination of CTMax is not subject to the same experimental biases as studies of behavioural thermoregulation. However, the accuracy of CTMax data is dependent on the use of an appropriate heating rate. The combination of studies of behavioural thermoregulation and the CTMax method could eliminate the need for growth experiments testing the effect of temperature.

Chapter 4

The effect of photoperiod and light intensity

Introduction

Light has been implicated as a major environmental factor influencing the endogenous cycles of metabolism and activity in fish and other organisms (Fry, 1971). Photoperiod and light intensity are important components in the complex of environmental cues or "zeitgebers" which synchronise the physiological and activity rhythms of fish (Schwassmann, 1971). Primarily they control the periodicity of endogenous rhythms, such as gonadal maturation (Hanyu & Razani, 1985), but may also directly influence the behaviour of fish. The presence of cyclic diel activity patterns have been recognised in both freshwater and marine fish species (Reynolds & Casterlin, 1976; Reynolds, 1977). The dominant factor influencing daily activity pattern is the imposed light-dark regime (Schwassmann, 1971), while the locomotor activity observed within this pattern is determined by the ambient light intensity (Reynolds & Casterlin, 1976). Since the energy requirement of fish is directly related to activity, an understanding of the effect of photoperiod and light intensity is critical when striving to maximise growth of fish under culture conditions.

The role of photoperiod on reproductive cycles has been extensively studied (Hanyu & Razani, 1985). However, its effect on growth is not fully understood (Tandler & Helps, 1985). While many authors recommend a continuous light regime for the rearing of marine fish larvae (Tandler & Helps, 1985, Duray & Kohno, 1988), little is known of the effect of photoperiod on the growth of juvenile fish. Research into its effect on juvenile freshwater and anadromous fish suggests that the response is species specific. In their study of juvenile (1.5g) *Perca fluvescens*, Hu *et al.* (1976) found that growth rate was nearly three times greater with 16h than with 8h light. Similarly, Gross *et al.* (1965) found that the growth and food conversion efficiency of *Lepomis cyanellus* were higher at 16L:8D than 8L:16D. Meske (1985) examined the effect of photoperiod on the survival and growth of three freshwater species, common carp (*Cyprinus carpio*), wels (*Siluris glanis*) and eel (*Anguilla anguilla*). Eel were found to grow best under a light regime of 12L:12D, but no photoperiod effect was recorded for carp and wels, of which the latter is a nocturnal feeder. The growth of juvenile walleye (*Stizostedion vitreum*), another nocturnal predator, was also found to be independent of photoperiod (Hu *et al.*, 1976). Juvenile African catfish (*Clarias gariepinus*) are negatively phototaxic and have been found to display maximum growth under conditions of continual darkness (Britz & Pienaar, 1992).

It is generally accepted that marine fish larvae are positively phototaxic and that feeding is greatly facilitated by high light intensities. As they are visual hunters, acuity of vision and searching volume are governed by a threshold in light intensity below which feeding and spontaneous swimming do not occur (Blaxter, 1968; 1973; Tandler & Mason, 1983). However, the scotopic (dim-light) vision of most marine teleosts develops during their metamorphosis from larvae to juveniles (Hu *et al.*, 1991). This may be accompanied by a change of feeding behaviour. For example, Marlivae (1981) found that the larvae of soft sculpin (*Gilbertia sigalutes*) fed visually, but after metamorphosis shifted from visual to "distant touch" feeding behaviour and from twilight to nocturnal feeding. Research on *Sparus (Rhabdosargus) sarba* indicates that scotopic visual acuity develops progressively during the early juvenile stage (Hu *et al.*, 1991). Young juvenile *Sparus (Rhabdosargus) sarba* (TL: 12-15mm) were unable to feed under dark conditions, whereas larger juveniles (TL: 30-40mm) actively fed during this period. Unfortunately there is no record of the light intensity under which the experiment was performed.

The spotted grunter has been described as an estuarine dependent species as its life cycle is characterised by a marine larval phase followed by a wholly estuarine juvenile phase (Whitfield, 1990). As the larvae are pelagic it can be assumed that they, like most other marine fish larvae, are visual predators and are therefore photopositive. Juvenile grunter, on the other hand, feed on benthic invertebrates (L. Makesela, Department of Ichthyology and Fisheries Science, Rhodes University, pers. comm) and may not necessarily be visual predators, especially when considering the variation in turbidity of the estuaries in which they occur. Since turbidity drastically reduces the penetration of light into water, individuals living in very turbid systems survive under a very different light intensity regime compared to those in clear estuaries. This suggests that light intensity is not an important factor in the survival of juvenile spotted grunter. The indifference of juvenile grunter (78-104mm) to various levels of turbidity supports this theory (Cyrus, 1984). However, it is still uncertain whether fish respond to turbidity *per se* or to the associated reduction in light intensity (Cyrus, 1984).

Under artificial conditions juvenile grunter are most active under high light intensities (Deacon & Hecht, 1995), whereas larger grunter (50-2390g) are most active at night (du Preez *et al.*, 1986). This would suggest that development in this species may be accompanied by a shift from positive to negative phototaxis. In other species which inhabit turbid waters, for example the walleye (*Stizostedian vitreum*), a gradual shift from positive to negative phototaxis is observed as larvae develop into juveniles (Bulkowski & Mead, 1983). Larval walleye of between 1 and 3 weeks old show a marked preference for a high light intensity (7800 lux), whereas juveniles of 35 to 40mm actively seek shelter from light. Light intensity preference studies performed on juvenile spotted grunter indicate a similar response to that of walleye. Burton (1995) found that juvenile grunter consistently selected the darkest area of a light gradient, even when there were only slight differences in light intensity. However, it remains to be established whether light intensity preference is a reliable indicator of a species' light intensity requirements.

Overall, the effects of photoperiod and light intensity on growth, in marine as well as freshwater fish, indicate that knowledge of the natural history and ecology is vital to understand a species' response to light. Stomach content analyses of juvenile spotted grunter captured from its natural environment suggest that it feeds both during the day and night (A. Paterson, Department of Ichthyology and Fisheries Sciences, Rhodes University, pers. comm.), while under captive conditions juvenile grunter were found to be most active during the light phase, remaining almost stationary at night (Deacon & Hecht, 1995). This is contrary to the pattern of activity observed in larger (50-2390g) spotted grunter (du Preez *et al.*, 1986). The conflicting nature of these observations reveals no clue to the light requirements of this species or the importance of light as an environmental factor.

Considering the powerful directive nature of photoperiod and light intensity, this investigation of these two components of light was undertaken to establish their influence on the growth performance of juvenile spotted grunter and to determine the conditions which promote maximum growth and survival.

Material and Methods

Photoperiod growth trial

The 40 L experimental tanks were arranged on three tiers with three tanks per tier. Each tank was stocked with eight fish. Initial length and weight of the fish were 105.2 ± 9.6mm and 16.18 ± 4.72g, respectively. Photoperiod in each tier was controlled by a separate time switch set to 16L:8D, 12L:12D and 8L:16D respectively. Each tier was sealed on five sides with black polyethylene sheeting (250 micron) and thick black curtains were hung in front. This excluded all extraneous light and prevented light filtering between the tiers. Each compartment was lit by a single Biolux fluorescent tube (3ft) providing a light intensity of 1500 lux at water surface. During the dark period light intensity was <2 lux. Water quality parameters remained optimal throughout the experimental period. The growth trial was conducted over a period of six weeks. Fish were fed three times daily to satiation on the standard semi-moist pelleted diet during the hours of light.

Light intensity growth trial

The tanks were arranged on three tiers with three tanks per tier. Each tank was stocked with eight fish. Initial length and weight of the fish were 98 ± 6.7mm and 14.62 ± 2.79g. Light intensity in each tier was controlled at 6.31×10^{-3} (high), 1.95×10^{-3} (intermediate) and 2.32×10^{-4}

$\mu E.sec^{-1}.cm^2$ (low), respectively. This was achieved by altering the number of fluorescent tubes (Osgram^R 1m Biolux) per tier. Sections of the tubes were shielded to ensure homogeneity of light intensity to each tank. Light intensity was measured in quanta.sec^{-1}.cm^2 with a Biospherical Instruments QSL-100 Laboratory Quantum Scalar Irradiance Meter. Quanta.sec^{-1}.cm^2 were converted to microeinsteins/second/cm^2 ($\mu E.sec^{-1}.cm^2$) by dividing by 6.022×10^{17} (QSL-100 Instruction Manual). Biolux^R fluorescent tubes deliver light in the wavelength range of 520-750nm. Each tier was sealed on five sides with black polyethylene sheeting (250 micron) and thick black curtains were hung in front. This excluded all extraneous light and prevented light filtering between the tiers. Photoperiod was set to a 14L:10D cycle with the dark period light intensity falling to below 2 lux in all tiers. Water quality parameters remained optimal throughout the experimental period, with nitrite levels remaining below 0.1 mg/L and unionised ammonia remaining below 0.02 mg/L. The experiment was conducted over a period of 8 weeks. The fish were fed to satiation three times daily on the standard semi-moist pelleted diet during the hours of light.

Results

Effect of photoperiod on growth rate

Modified photoperiod had a subtle influence on the growth performance of juvenile *P. commersonnii*. Weight gain at daylengths of 8, 12 and 16 hours were not significantly different, although a slightly lower weight gain was observed in fish kept under the 8hr light regime (Fig 4.1). This trend was also reflected in the specific growth rate of fish in this treatment (Table 4.1 & 4.2). However, the fish kept at 12L:12D had a significantly ($P < 0.01$) higher condition factor than those kept at longer or shorter photoperiods (Table 4.1 & 4.2). Photoperiod had no significant ($P > 0.05$) effect on FCR (Table 4.1 & 4.2).

Table 4.1: Summary of one-way ANOVA tests of the effect of photoperiod on the growth performance of juvenile spotted grunter, *Pomadasys commersonnii*.

FACTOR	F-RATIO	D.F.	SIG. LEVEL
Photoperiod: 8L:16D, 12L:12D & 16L:16D			
Condition factor	17.947	2	0.0000
Food conversion ratio	0.999	2	0.4222
Specific growth rate	0.596	2	0.5518

Table 4.2: Tukey's Multiple Range Analyses' of the effect of photoperiod on the condition factor, food conversion and growth of juvenile spotted grunter, *Pomadasys commersonnii*. Superscripts (a & b) indicate homogenous grouping of means. Photoperiod treatments which share any common superscript are not significantly different from one another at $P<0.05$.

Growth parameter	Photoperiod (Hrs)	Tukey HSD Average
Condition factor	8L:16D	0.62842^{a}
	12L:12D	0.65637^{b}
	16L:8D	0.62842^{a}
Food conversion ratio (FCR)	8L:16D	1.60995^{a}
	12L:12D	1.84874^{a}
	16L:8D	1.89989^{a}
Specific growth rate (SGR)	8L:16D	1.35774^{a}
	12L:12D	1.42029^{a}
	16L:8D	1.45633^{a}

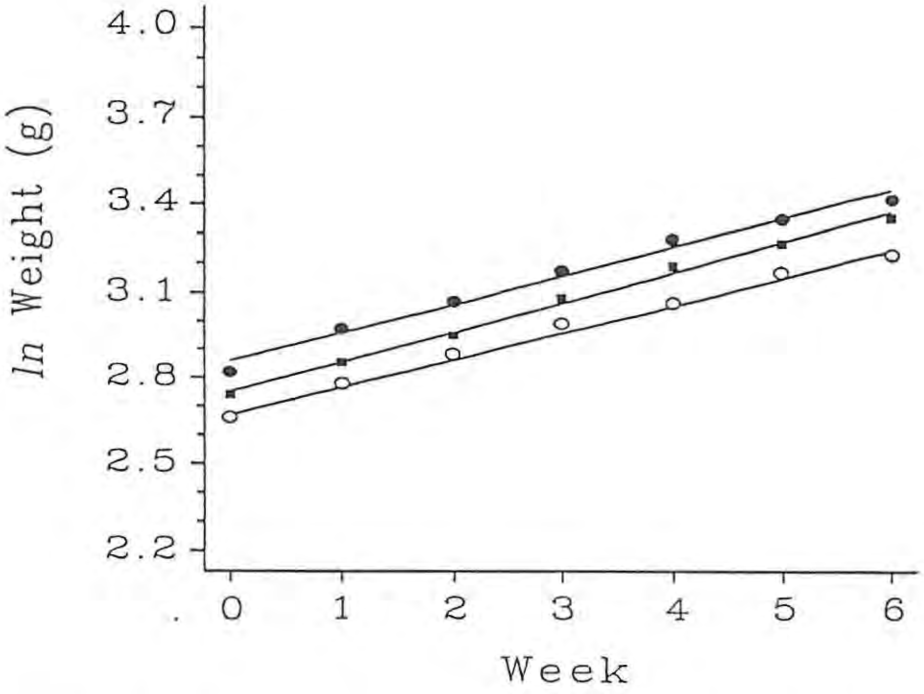

Figure 4.1: The effect of photoperiod on the weight gain of juvenile spotted grunter, *Pomadasys commersonnii.* Each datum for 8L:16D (○), 12L:12D (■) and 16L:8D (●) is the mean of *ln* weight (g).

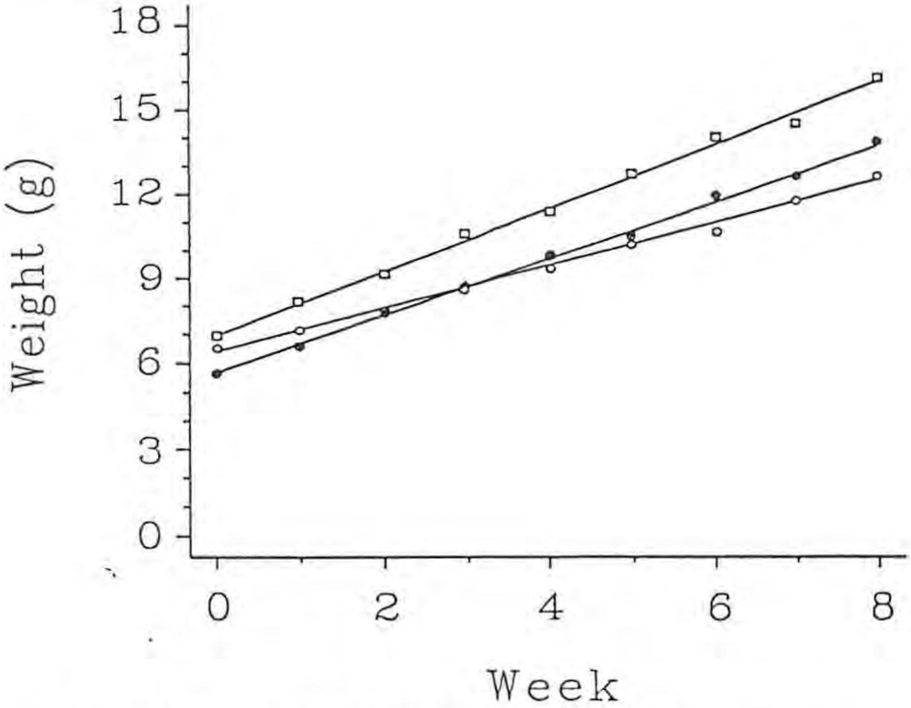

Figure 4.2: The effect of high, $6.31\text{x}10^{-4}\mu\text{E.sec}^{-1}.\text{cm}^{2}$ (●); intermediate, $1.95\text{x}10^{-3}\mu\text{E.sec}^{-1}.\text{cm}^{2}$ (■) and low ,$2.32\text{x}10^{-4}\mu\text{E.sec}^{-1}.\text{cm}^{2}$ (○) light intensity on the weight gain of juvenile spotted grunter, *Pomadasys commersonnii.*

Effect of light intensity on growth

Light intensity was found to have a significant effect on the growth and survival of juvenile spotted grunter. Mortality of fish exposed to the lowest light intensity (2.32×10^{-4} μE.sec^{-1}.cm^{2}) was significantly higher than at either the intermediate ($1.95\times10^{-3}$$\mu$E.sec^{-1}.cm^{2}) or high ($6.31\times10^{-3}$$\mu$E.sec^{-1}.cm^{2}) light intensities (Table 4.3 & 4.4). Comparison between growth rates at each of the treatments indicated that the growth of fish in the lowest light intensity was significantly lower than that of fish under intermediate and high light intensity (Table 4.3, 4.4 & Fig 4.2). In addition, the rate at which the fish consumed food was significantly lower at the lowest light intensity than that observed in the higher light intensities (Table 4.3 & 4.4). Surprisingly, this did not affect food conversion ratio, which was similar in all treatments (Table 4.3 & 4.4). However, protein efficiency ratio of fish at the low light intensity was poorer than at the other intensities, although not significantly so (Table 4.3 & 4.4). On the other hand, the condition factor of fish at both high and low light intensity was similar, although both were significantly lower than that of fish under an intermediate light intensity (Table 4.3 & 4.4).

Table 4.3: Summary of one-way ANOVA tests of the effect of light intensity on the growth performance of juvenile spotted grunter, *Pomadasys commersonnii*.

FACTOR	F-RATIO	D.F.	SIG. LEVEL
Light intensity: 2.32×10^{-4}, 1.95×10^{-3} & 6.31×10^{-3} μE.sec^{-1}.cm^{2}			
Condition factor	17.264	2	0.0000
Food consumption	7.330	2	0.0016
Food conversion ratio	0.810	2	0.4511
Mortality	5.333	2	0.0467
Protein efficiency ratio	2.553	2	0.0891
Specific growth rate	3.366	2	0.0356

Table 4.4: Tukey's Multiple Range Analyses' of the effect of light intensity on the condition factor, food consumption, food conversion, survival, growth and protein efficiency ratio of juvenile spotted grunter, *Pomadasys commersonnii*. Supersripts (a & b) indicate homogenous grouping of means. Light intensity treatments which share any common superscript are not significantly different from one another at $P<0.05$.

Growth parameter	**Light intensity** (μE.sec^{-1}.cm^{2})	**Tukey HSD Average**
Condition factor	$2.32\text{x}10^{-4}$	0.85268^{a}
	$1.95\text{x}10^{-3}$	0.93107^{b}
	$6.31\text{x}10^{-3}$	0.86317^{a}
Food consumption	$2.32\text{x}10^{-4}$	5.75348^{a}
	$1.95\text{x}10^{-3}$	8.25614^{b}
	$6.31\text{x}10^{-3}$	8.38961^{b}
Food conversion ratio (FCR)	$2.32\text{x}10^{-4}$	2.17232^{a}
	$1.95\text{x}10^{-3}$	2.11646^{a}
	$6.31\text{x}10^{-3}$	2.77211^{a}
Mortality	$2.32\text{x}10^{-4}$	20.8333^{a}
	$1.95\text{x}10^{-3}$	4.16667^{b}
	$6.31\text{x}10^{-3}$	4.16667^{b}
Protein efficiency ratio (PER)	$2.32\text{x}10^{-4}$	1.53929^{a}
	$1.95\text{x}10^{-3}$	1.96814^{a}
	$6.31\text{x}10^{-3}$	1.32526^{a}
Specific growth rate (SGR)	$2.32\text{x}10^{-4}$	1.46036^{a}
	$1.95\text{x}10^{-3}$	1.85460^{b}
	$6.31\text{x}10^{-3}$	1.85822^{b}

Discussion

Photoperiod had a varied effect on the measured growth performance parameters of juvenile spotted grunter. Growth rate was not significantly affected by the manipulation of photoperiod. However, fish kept under a 12L:12D regime showed a significantly higher condition to those under extended or protracted photoperiods. The combination of a slightly depressed growth rate and the lower condition of fish kept under 8L:16D suggests that juvenile grunter are predominantly diurnal. The equally low condition of fish kept under the extended photoperiod (16L:8D) might suggest that the feeding regime, and therefore ration, was not sufficient to compensate for the associated increase in activity. The slight, although not significant, improvement in food conversion ratio lends some support to this observation. On the other hand, Kiyono and Hirano (1981) found that, even when fed *ad libitum*, juvenile black porgy (*Mylio macrocephalus*) did not consume more food under a 24 hour light photoperiod. Assuming that the additional food is either not consumed, or is channelled into non-productive energy by juvenile marine fish under extended photoperiods, it is concluded that there is no advantage in growing juvenile grunter under modified photoperiods. This finding is consistent with research on the effect of extended photoperiod on the growth of other juvenile marine fish (Fuchs, 1978, Kiyono & Hirano, 1981).

Most of the research into the effect of light intensity on the growth of marine fish has been undertaken on larvae. However, there is a paucity of knowledge with regard to its effect on later life-history stages. Light intensity has been shown to have a profound effect on other fish, in particular salmonids (Kwain, 1975; Mitchell, 1986 in Stefansson *et al.*, 1993). Besides its effect on smoltification, both high and low light intensities have been found to have a negative effect on the growth of some species. For example, under dim conditions (<0.2 lux) growth rate of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*) were significantly reduced (Kwain, 1975). On the other hand, Mitchell (1986 in Stefansson *et al.*, 1993) found reduced growth in juvenile Atlantic salmon (*Salmo salar*) at 300 lux, whereas intensities between

0.3 and 30 lux did not significantly affect growth. However, in contrast to Mitchell's (1986 **in** Stefansson et. al., 1993) observations, a later study found that even the highest light intensity (715 lux) did not reduce growth or appear stressful to the fish (Stefansson *et al.*, 1993). This discrepancy was thought to arise as a result of variation in environmental conditions, such as water temperature, fish size and pretreatment history, between the two experiments. This suggests that the effects of light intensity are sensitive to interaction between environmental factors. In this study on juvenile spotted grunter, key environmental factors, such as water temperature, photoperiod, salinity and feeding frequency, were kept constant at the optimum levels throughout the experiment (Bussiahn, 1992; Deacon & Hecht, 1996; Deacon & Hecht, in press).

Another potential source of discrepancy between light intensity studies is derived from the manner in which light intensity is measured. The use of photometric units, in particular lux, for recording light intensity is not strictly accurate as they only measure the relative illuminance of white light and therefore ignore other visible incident wavelengths (Weinberg, 1976). Since the sensitivity of an animal to light is ultimately dependent on the wavelengths its visual pigments absorb, measurements of intensity should be conducted within the relevant range. In fish, age related changes in retina structure, such as the reduction in certain cone photoreceptors, have been shown to coincide with changes in feeding behaviour (Loew *et al.*, 1993). Loss of near ultraviolet (UV) retinal cone receptors containing a peak absorbance of around 400nm in larval and juvenile perch (*Perca flavescens*) is concomitant with a change in feeding behaviour from that of a surface planktivore to a demersal omnivore. Adult perch have no UV sensitive cones and instead have retinal pigments with peak absorbances around 550 and 680nm, respectively (Cameron, 1982 in Loew *et al.*, 1993). A similar change has also been observed between juvenile and adult brown trout, *Salmo trutta* (Bowmaker & Kuntz, 1987). However, in adult trout the number of UV cones is reduced but not totally absent. Prey capture studies on juvenile perch show that visual detection, recognition, location and capture of natural food can only occur in the presence of near-UV light (Loew *et al.*, 1993). This suggests that the effect of light intensity on any particular species is a function of the wavelength spectrum provided by the light source and the age of the animals. The visual spectrum of fish ranges between 385 and 700nm with most fish having maximum spectral absorbance at around 500nm (Hobson *et al.*, 1981; Huang, 1989; Loew *et al.* 1993). The light source used in this experiment on juvenile spotted grunter provided a wavelength spectrum in the range of 520-750nm with peaks at around 540 and 620nm. This spectrum encompasses most of the wavelength range to which fish visual pigments have been found to be most sensitive. However, the shorter wavelengths were not provided by this light source. In fish, such as perch (*Perca fluviatilis*) and the black porgy (*Acanthopagrus schlegeli*), which commonly inhabit poor photic conditions additional peak spectral absorbances also occur in the 550-680nm ranges (Cameron, 1982; Huang, 1989). Since juvenile spotted grunter in the size range used in this experiment are found in highly turbid water it is likely that they also possess retinal pigments required for sight under poor photic conditions. However, this is not known for certain. Nonetheless, as all light intensity treatments were subject to the same wavelength spectrum, any differences in growth performance could not be the result of differences in available visible light. However, it seems that the effects of light intensity should be interpreted in conjunction with light wavelength spectra, especially when conducting research under artificial light. Furthermore, light intensity measurements should measure the incident light energy within this range to establish the actual effect of intensity. Measurements of quanta (in $\mu E.sec^{-1}.cm^{2}$) seem the most suitable as quantum sensors usually measure the total number of quanta in light ranging from 350-700nm (Weinberg, 1976).

Despite the use of a limited, and possibly not ideal, wavelength spectrum, light intensity still had a significant effect on the survival and growth performance of juvenile spotted grunter. Very low light intensity proved to be detrimental to feeding, growth, condition factor and mortality. This is surprising as in its natural environment the spotted grunter is commonly found inhabiting highly turbid conditions (Ter Morshuizen *et al.*, in press). In addition, observation of grunter in various light intensity gradients indicated that grunter were sensitive to, and able to discriminate even slight (> 1 lux) changes in intensity (Burton, 1995). In all gradients grunter consistently selected the darkest region (Burton, 1995). From their studies of silver carp (*Hypophthalmichthys molitrix*) Radenko and Alimov (1992) suggested that the preferred light intensity of larval carp corresponded well with the optimum light intensity for growth. Our observations, however, indicate the contrary as fish at the preferred (lowest) light intensity showed the poorest performance. In addition, there was a significantly higher mortality under low light conditions.

The detrimental effect of low light intensity on fish is most often thought to be caused by a reduction in feeding efficiency, especially amongst species which are visual predators. For example, the reduced growth rate of rainbow trout *O. mykiss* at 0.2 lux was attributed to the fish being unable to see clearly (Kwain, 1975). Brett and Groot (1963) presented evidence that feeding activity of coho salmon (*O. kisutch*) would be significantly reduced at intensities lower than 0.1 lux. The response of juvenile spotted grunter to low intensity is consistent with the conclusions of Kwain (1975) and Brett and Groot (1963) as food intake was significantly reduced under dim conditions. On the other hand, oxygen consumption studies of larger spotted grunter (50 - 2390g) indicate that this species is most active under low light intensities (du Preez *et al.*, 1986). In addition, large grunter are most frequently caught by anglers at night. This suggests an ontogenic response to light with scotopic visual acuity developing progressively during the juvenile stage. This has been observed in *Sparus (Rhabdosargus) sarba*, where early juveniles (TL: 12-15mm) were unable to feed in the dark, whereas larger juveniles (TL: 30-40 mm) actively fed during this period (Hu *et al.*, 1991). However, the response of juvenile *Sparus (Rhabdosargus) sarba* is similar to that observed in marine fish larvae. Feeding and spontaneous swimming activity in marine larvae is governed by a threshold light intensity below which neither of the activities occur (Blaxter, 1968; 1973; Tandler & Mason, 1983). Since juvenile grunter were able to feed, although not effectively, at the lowest light intensity it is unlikely that the reduced feed intake was the result of poor visual acuity. This suggests that some other factor, possibly alteration in behaviour, was the cause of the reduced growth and higher mortality of juvenile spotted grunter at the low light intensity.

In addition to high mortality rate and poor growth performance, fish kept under dim conditions were in poor condition and many of them exhibited fin erosion and scale damage. Since all fish were not affected similarly and fish at intermediate and high light intensity showed none of these symptoms, it is possible that the poor external condition of these fish was caused by an increase in agonistic behaviour. This, however, could not be confirmed under the current experimental conditions. Inappropriate light intensity has been found to increase aggression and metabolic stress in Atlantic salmon, although for this species it is caused by high light intensity (Mitchell, 1986 **in** Stefansson *et al.*, 1993). For juvenile grunter, high light intensity had no significant effect on growth, food conversion, or mortality, but, condition factor was as low as that of fish kept under dim conditions. This can also be attributed to a change in behaviour. Fish in the highest light intensity exhibited increased swimming activity and generally remained in close proximity to each other which is unusual for this species. Their distribution in the tank was also not random, as they tended to concentrate in the bottom corners of the aquaria. Although these regions had a light intensity which was around 8% lower than the middle of the tank, it is uncertain whether they actively selected this area or if it was a consequence of the tank design. Nonetheless, this behaviour is assumed to be an escape response in an attempt to seek shelter from the high light intensity as it was not observed at the intermediate light intensity. It appears then that the reduced growth of juvenile grunter at both high and low light intensities is caused by changes in behaviour, resulting in increased metabolic stress. From this it can be concluded that the effect of light intensity on growth performance is primarily due to its ability to modify behaviour, as opposed to affecting physiological processes.

Overall, juvenile grunter exhibited optimum survival, growth, food conversion and condition at the intermediate light intensity ($1.95 \times 10^{-3} \mu E.sec^{-1}.cm^{2}$). Significantly lower survival, growth and condition was observed in fish kept under the lowest light intensity ($2.32 \times 10^{-4} \mu E.sec^{-1}.cm^{2}$). The slight depression in growth rate and low condition of fish at a photoperiod of 8L:16D may have, therefore, been due to prolonged exposure to low light conditions rather than an effect of photoperiod. Fish kept under a protracted photoperiod would have been exposed to a light intensity of $>2.32x10^{-4}\mu E.sec^{-1}.cm^2$ for 16hrs a day. This does not, however, affect the conclusion that no advantage is gained in growing juvenile under modified photoperiods. Purely by chance, the light intensity used during the 'day' phase in the photoperiod experiment was at the optimum intensity of $1.95x10^{-3}\mu E.sec^{-1}.cm^2$. Therefore, if there was no photoperiod effect the fish exposed to longer periods (16 hrs) of the optimum light intensity should have exhibited increased growth. This was not observed. Furthermore, fish kept under an extended photoperiod showed a lower condition. This provides further support for a photoperiod effect.

From this investigation it can be concluded that the optimum light conditions required for successfully rearing of juvenile spotted grunter include a natural photoperiod with intermediate light intensities ($\pm$ $1.95x10^{-3}\mu E.sec^{-1}.cm^{2)}$ during the day. There is also an indication that the effect of light intensity on growth and behaviour should be interpreted in conjunction with the wavelength spectrum of the light source. The results of the light intensity growth trial revealed that light intensity preference is not a reliable method of determining the light intensity requirements for all species.

Chapter 5

The effect of reduced salinity on growth, food conversion and protein efficiency ratio

Introduction

The effect of salinity is classified as a masking factor as it modifies metabolism (Brett, 1979). Internal ion regulation requires constant energy expenditure and the energy expended depends on the difference between the external and internal environment (Brett, 1979). Almost all bony fishes are osmoregulators as they maintain their body fluids at a constant osmotic concentration of approximately 12‰ in spite of external concentration changes (Blaber, 1974; Mehl, 1974; Martin 1990). In freshwater, fish plasma has a higher ionic concentration than the surrounding water. Therefore, there is constant osmotic water inflow into the body and loss of ions to the environment (Blaber, 1974; Mehl, 1974; Martin 1990). Fish compensate for this effect by producing large volumes of dilute urine and by actively absorbing ions from the surrounding water. However, if the salinity of the surrounding water exceeds the osmotic concentration of the body fluids the situation is reversed as water is lost from the body and ions accumulate. Marine fish counteract this effect by drinking water which restores the water content. They also possess cells in the gill epithelium which actively eliminate excess salts.

A total of 72 marine fish species are currently being cultured or considered high priority candidate species. A review of the literature on the biology of these species has revealed that over 70% of these species, including spotted grunter, are euryhaline and are either estuarine dependent or utilise estuaries during a particular stage of their life-history (Appendix 1). Given the fact that the bulk of marine aquaculture species are euryhaline, the paucity of published information on the effect of salinity on marine fish growth is surprising. Salinity has proven to have a significant effect on the growth of anadromous species, in particular salmonids (Otto, 1971; McKay & Gjerde, 1985; Morgan & Iwama, 1991) and an understanding of the effect of salinity on euryhaline marine fish may be fundamental for developing the rearing protocols for these species.

Marine fish utilise estuaries for a number of reasons. They are highly productive, sheltered systems with abundant food resources and thus provide ideal nursery areas for juvenile fish. Due to the high energy nature of the surf zone along the east coast of southern Africa, estuaries are the primary nursery areas for many marine fish (Blaber, 1981). For example, *Mugil cephalus* which is estuarine dependent in southern Africa (Whitfield, 1990), use the inshore marine zone as nursery areas in western Australia (Potter *et al.*, 1990). Although estuaries are in many senses ideal nursery areas for juvenile fish, they are generally of lower salinity than seawater. Since seawater has a relatively constant concentration of 35‰, lowered salinity constitues a potential stressor for marine fish. However, the improved growth of some marine fish at intermediate salinities, for example European flounder (*Platichthys flesus*) at 5 and 10‰ (Gutt,1985) and atlantic cod (*Gadus morhua*) at 7 and 14‰ (Lambert *et al.*, 1994), indicates the contrary. The utilization of estuaries may, therefore, also convey a metabolic advantage as a consequence of reduced salinity.

Salinity is thought to act on two independent aspects of physiology. It has been hypothesised that osmoregulatory costs are lowest in an isosmotic environment and the consequent energy savings permit for increased growth (Potts, 1954; Febry & Lutz, 1987; Watanabe *et al.*, 1989; Morgan & Iwama, 1991). The validity of this hypothesis has not been established as the metabolic responses of teleost fish exposed to changes in environmental salinity do not show any consistent patterns (Morgan & Iwama, 1991). The optimal salinity for growth appears to be species specific and differs between life-history stage and season (Gutt, 1985; Morgan & Iwama, 1991; Lambert *et al.*, 1994). A second hypothesis, directly related to osmoregulatory processes, concerns the effect of salinity on digestion. It has been shown that digestibility, particularly of protein, and food conversion efficiency decreases with increasing salinity in marine fish (DeSilva & Perera, 1976; MacLoed, 1977; Ferraris *et al.*, 1986). It has been suggested that the osmoregulatory process of drinking water by marine fish is responsible for increased food motility in the gut (Ferraris *et al.*, 1986). Consequently, the residence time for digestion and adsorption of nutrients is reduced (DeSilva & Perera, 1976; MacLoed, 1977; Ferraris *et al.*, 1986). Furthermore, the drinking of water in marine fish may alter stomach pH (DeSilva & Perera, 1976). This study was designed to test the two hypotheses and to define the optimum salinity for rearing juvenile spotted grunter.

Materials and Methods

The trial to investigate the effect of salinity on growth was conducted in four experimental units. Each unit consisted of three equal sized 40L glass aquaria attached to a 70L biological filter containing shredded plastic and a crushed shell buffer (Fig 5.1). Seawater diluted with distilled water to 5, 12 (isosmotic: Bussiahn 1992) and 25‰, respectively, was used to fill three of the units. The remaining unit was filled with undiluted seawater (35‰). The aquaria were each randomly stocked with 8 individually marked fish which had been acclimated to the respective salinities for 14 days. Fish were marked according to the protocol described in Chapter 2. Initial length and weight of the fish were 50.5 ± 5.6 mm and 3.10 ± 1.14 g. Fish were fed to satiation on the standard semi-moist pelleted diet at each meal. Satiation was defined as the stage when the fish no longer accepted single pellets dropped into the tank. Water quality parameters remained optimal throughout the experimental period with nitrite levels remaining below 0.1mg/L and unionised ammonia remaining below 0.02 mg/L. Water temperature was maintained at optimum temperature for growth of 24.5 ± 0.5°C for the species (Deacon & Hecht, 1996). Photoperiod was controlled to 14L:10D cycle (Deacon & Hecht, 1996). The experiment was run for eight weeks. Standard length (mm) and weight (g) of the fish were measured once weekly.

Figure 5.1: The experimental unit used to determine the effect of salinity on the growth of juvenile spotted grunter.

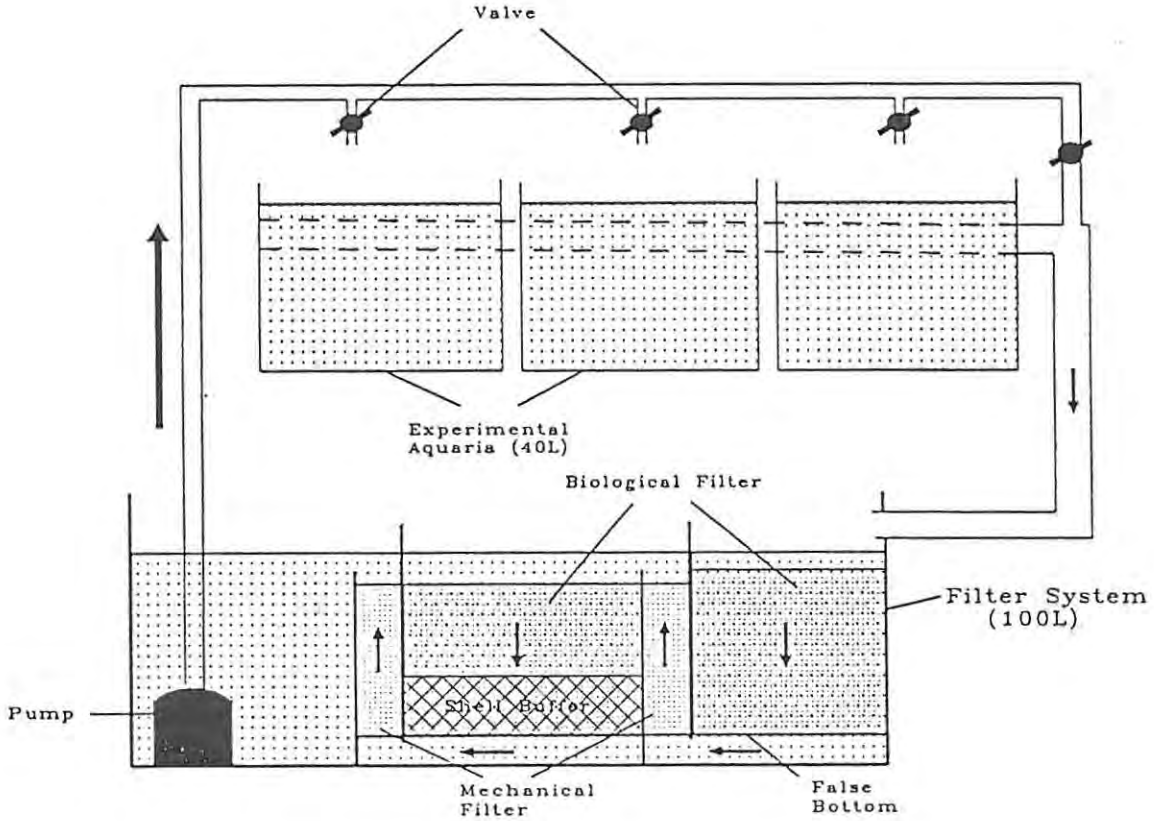

Table 5.1: Summary of one-way ANOVA tests of the effect of salinity on the growth performance of juvenile spotted grunter, *Pomadasys commersonnii*.

FACTOR	F-RATIO	D.F.	SIG. LEVEL
Salinity: 5, 12, 25 and 35‰			
Condition factor	2.396	3	0.0670
Food conversion ratio	8.420	3	0.0003
Mortality	7.000	3	0.0126
Protein efficiency ratio	2.411	3	0.0851
Specific growth rate	3.348	3	0.0187

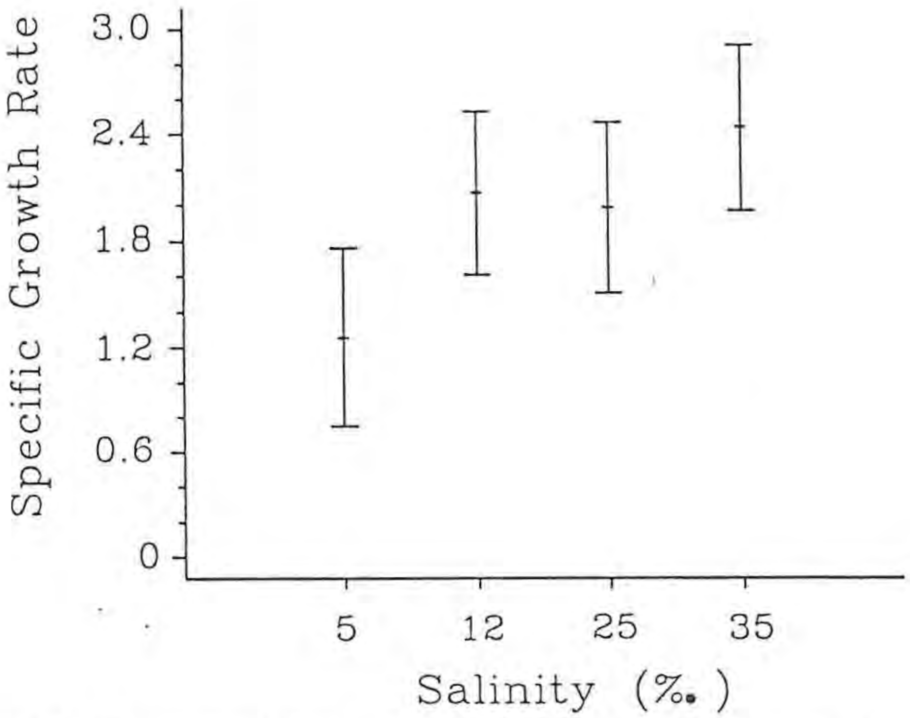

Figure 5.2: Means plot of the effect of salinity on specific growth rate (SGR) of juvenile spotted grunter, *P. commersonnii*. Vertical bars represent 95% confidence intervals.

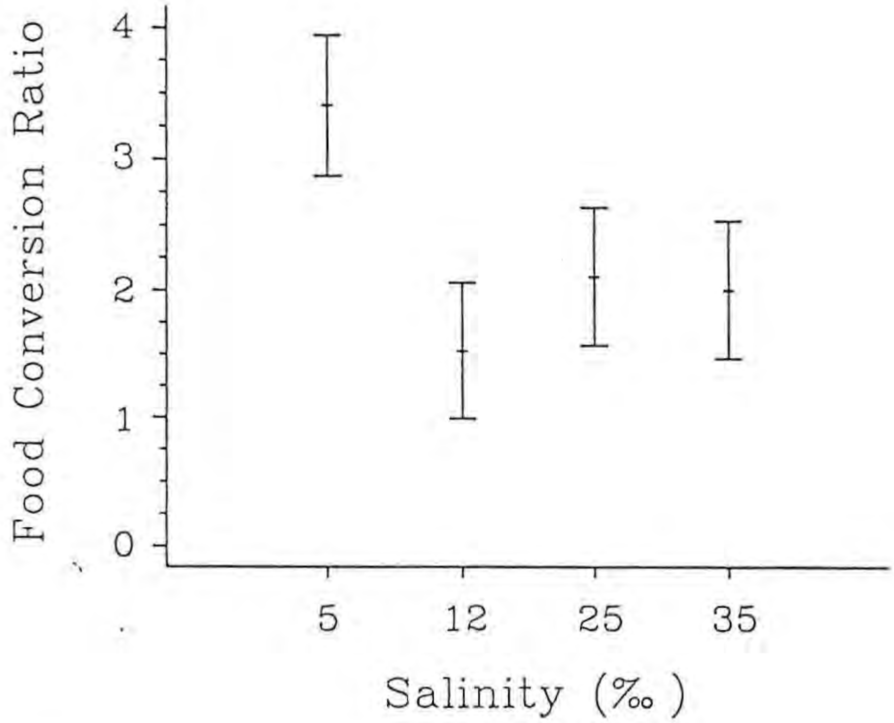

Figure 5.3: Means plot of the effect of salinity on food conversion of juvenile spotted grunter, *P. commersonnii*. Vertical bars represent 95% confidence intervals.

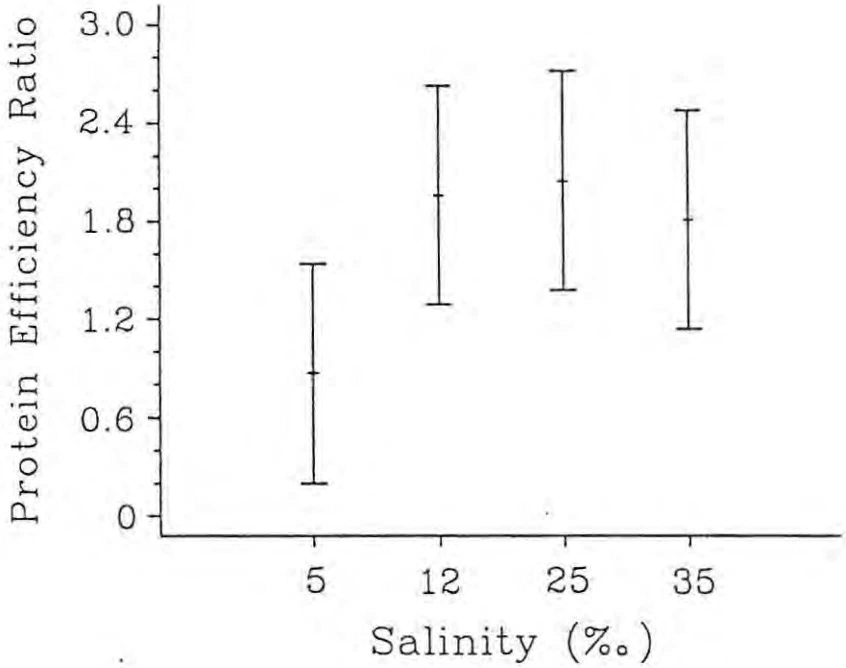

Figure 5.4: Means plot of the effect of salinity on protein efficiency ratio (PER) of juvenile spotted grunter, *P. commersonnii*. Vertical bars represent 95% confidence intervals.

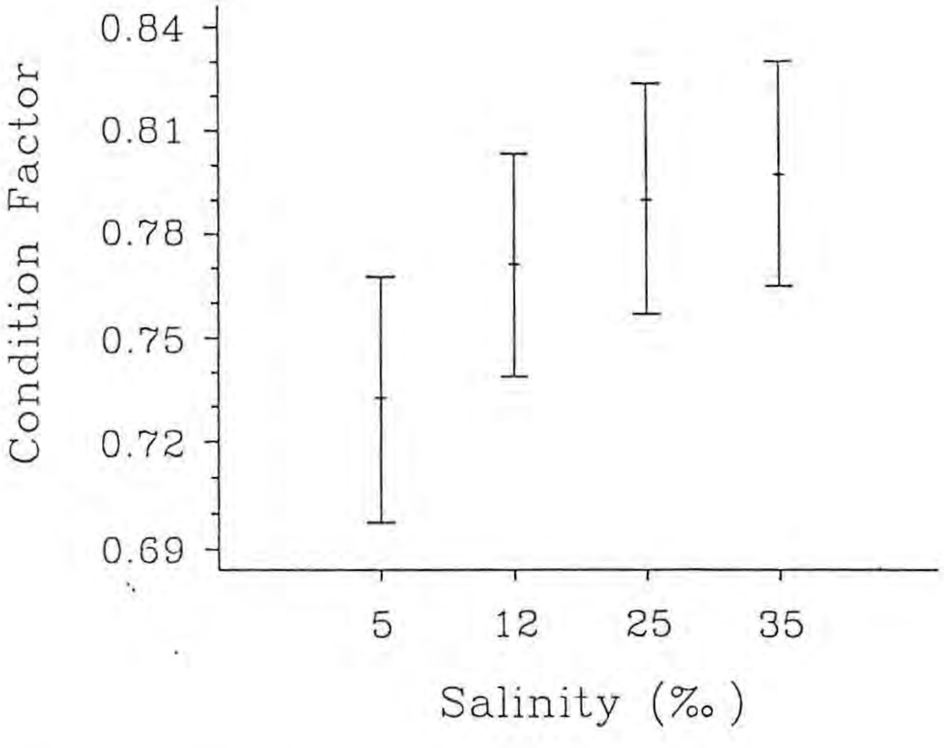

Figure 5.5: Means plot of the effect of salinity on condition factor of juvenile spotted grunter, *P. commersonnii*. Vertical bars represent 95% confidence intervals.

Table 5.2: Tukey's Multiple Range Analyses' of the effect of salinity on the condition factor, food conversion, growth and protein efficiency ratio of juvenile spotted grunter, *Pomadasys commersonnii*. Superscripts (a, b & c) indicate homogeous groupings of means. Salinity treatments which share a common superscript are not significantly different from one another at $P<0.05$.

Growth parameter	Salinity (‰)	Tukey HSD Average
Condition factor	5	0.73257^{a}
	12	0.77113^{a}
	25	0.79024^{a}
	35	0.79754^{a}
Food conversion ratio (FCR)	5	3.40824^{a}
	12	1.52474^{b}
	25	2.01012^{b}
	35	2.00043^{b}
Mortality	5	29.1667^{a}
	12	4.16667^{b}
	25	8.33333^{b}
	35	8.33333^{b}
Protein efficiency ratio (PER)	5	0.87022^{a}
	12	1.95830^{a}
	25	2.04880^{a}
	35	1.80974^{a}
Specific growth rate (SGR)	5	1.25486^{a}
	12	2.06789^{ab}
	25	1.98651^{ab}
	35	2.43592^{b}

Results

Salinity had a significant effect (one-way ANOVA, $P<0.02$) on survival and growth of juvenile spotted grunter (Table 5.1). Mortality was 29.2% in 5‰ compared to 4.2% in isosmotic and 8.3% in 25 and 35‰. The specific growth rate of fish at the lowest salinity (5‰) was significantly lower than that obtained at the isosmotic salinity (12‰) or the higher salinities (25 and 35‰) (Fig 5.2 & Table 5.2). Growth at the higher salinities (25 and 35‰) was not significantly different to that obtained at the isosmotic concentration (12‰). Variation in salinity significantly influenced food conversion ratio ($P<0.05$)(Table 5.1). The food conversion ratio (FCR) of intermediate (12‰) and high salinities (25 to 35‰) was significantly better than at 5‰ (Fig 5.3, Table 5.2). Food conversion ratio (FCR) and protein efficiency ration (PER) of fish kept at isosmotic salinity (12‰) and above (25 and 35‰) were not significantly different (Table 5.4). Protein efficiency ratios of fish at isosmotic (12‰), 25 and 35‰ were also all similar (Fig 5.5). The PER of fish at 5‰ was significantly lower than the rest of the salinities, however, it was not significantly different (Table 5.1). Although not significant, a trend of increased condition factor corresponding to increased salinity was also observed (Fig 5.6). The greatest improvement in condition occurred between 5 and 12‰.

Discussion

In a study of the osmoregulatory capacity of spotted grunter, Bussiahn (1992) determined the isosmotic concentration to be 12‰. The study also illustrated the well developed euryhaline character of this species as blood osmolarity varied by less than 5% over a test range of 5-35‰. Limits of salinity tolerance are defined by the concentrations where a constant blood plasma osmolarity are no longer maintained (Martin, 1990). Disturbance of plasma osmolarity is indicative of disruption of the $Na+$ and $K+$ homeostatic balance which precedes metabolic disfunction and ultimately results in mortality (Woo & Fung, 1981). Euryhaline fish, by definition, are able to maintain blood plasma concentrations at a constant level in ambient salinities above and below the osmotic concentration of blood plasma (Blaber, 1974; Mehl, 1974; Martin 1990). Nordlie (1978), however, noted that extremely euryhaline fish such as *Mugil cephalus* and *Oncorhynchus mykiss* allow their internal concentrations to vary considerably depending on the ambient salinity concentration.

Despite the high osmoregulatory capability of juvenile spotted grunter, the results of the growth trial indicate that salinity has a significant effect on the survival and growth performance of this species. At a salinity of 5‰, which was hypo-osmotic to blood plasma, growth, food conversion and protein efficiency ratio were significantly impaired. Fish below the isosmotic salinity were also generally in poorer condition and exhibited a higher mortality rate than those at higher salinities. As there was no significant change in blood plasma osmolarity between 5 and 12‰ (Bussiahn, 1992), these effects were not caused by physiological disfunction i.e. disruption of the homeostatic balance. This indicates that the stress of hypo-osmotic salinity must be due to some other factor.

The effect of salinity on growth of fish has often been attributed to the metabolic cost of osmotic and ionic regulation. The usual hypothesis proposed is that the energetic cost of ion regulation is lowest in an isosmotic environment, where the ionic gradients between water and blood are minimal, and that this energy saving is substantial enough to increase growth (Morgan & Iwama, 1991). Our data does not support this hypothesis. In an isosmotic concentration the growth of juvenile spotted grunter was not significantly different to that at higher salinities (25 and 35‰). This finding is consistent with many other studies which have failed to show that growth is optimised in an isosmotic environment (Morgan & Iwama, 1991). Lower growth of juvenile spotted grunter at 5‰ may have been caused by an increase in the energetic cost of osmoregulation. However, metabolic data would be required to confirm this.

Table 5.3: Optimum salinity for growth of fresh water and marine fish.

Species	Life History[a]	Optimum Growth Salinity	Reference
Freshwater			
Oreochromis mossambicus (Mozambique tilapia)	Eur	>12‰	Canagaratnam (1959)
Oncorhynchus kisutch (coho salmon)	Anad-Eur	5-10‰	Otto (1971)
Mystus vittatus (freshwater catfish)	Sten	0-2‰	Arunachalam & Reddy (1979)
Ctenopharyngodon idella (grass carp)	Sten	0‰	Kilambi (1980)
Clarias gariepinus (sharptooth catfish)	Sten	0-5‰	Britz & Hecht (1989)
Morone saxatilis (striped bass)	Anad-Eur	7‰	Brown *et al.* (1992)
Oncorhynchus mykiss (steelhead trout)	Anad-Eur	0‰	Morgan & Iwama (1991)
Oncorhynchus tshawytscha (chinook salmon)	Anad-Eur	0‰	Morgan & Iwama (1991)
Marine			
Mugil cephalus (grey mullet)	Eur	20‰	DeSilva & Perera (1976)
Epinephelus tauvina (brown spotted grouper)	Eur	25‰	Akatsu *et al.* (1983)
Dicentrarchus labrax (European seabass)	Eur	25‰	Dendrinos & Thorpe (1985)
Platichthys flesus (European flounder)	Eur	5-15‰	Gutt (1985)
Gadus morhua (Atlantic cod)	Eur	7-14‰	Lambert *et al.* (1994)
Pomadasys commersonnii (spotted grunter)	Eur	12-35‰	this study

[a] Anad, anadromous; Eur, euryhaline; Sten, stenohaline.

Fish that spawn in fresh water, i.e. both stenohaline freshwater and euryhaline (anadromous) species, have better growth rates below isosmotic level (Table 5.3), and as salinity increases growth declines (Otto, 1971, Arunchachalum & Reddy, 1979, Kilambi, 1980, Britz & Hecht, 1989, Morgan & Iwama, 1991, Brown *et al.*, 1992). For marine fish (i.e. those that spawn at sea) the opposite is true. Growth generally increases above the isosmotic concentration (Table 5.3) to a maximum in higher salinity (DeSilva & Perera, 1976, Akatsu *et al.*, 1983, Dendrinos & Thorpe, 1985). Furthermore, the 'optimum' salinities for growth of freshwater and marine species usually fall within the ranges 0-12‰ and 12-35‰, respectively (Table 5.3). Morgan & Iwama (1991) suggested that 'optimum' salinities, which are species specific, possibly reflect adaptation to the environment in which the fish is commonly found (Morgan & Iwama, 1991). In juvenile spotted grunter there is no clearly defined optimum salinity as they grow equally well in salinities ranging from 12-35‰. The absence of an optimum salinity value in spotted grunter implies that the response of a species to salinity is not necessarily related to its natural environment. However, the estuaries which act as the sole nursery areas for juvenile grunter are subject to highly unpredictable salinity changes (Whitfield, 1990). Therefore, the lack of a clear optimum salinity for juvenile spotted grunter could indicate adaptation to an unstable natural environment.

Although the salinity response of many species appears to be a function of adaptation to their natural environment, there are a few exceptions which are contrary to the normal pattern. For example, the euryhaline cichlid *Oreochromis mossambicus*, and many of its hybrids, are either not affected (Jauncy, 1982) or exhibit better growth above isosmotic concentration (Canagaratnam, 1972). Since this species mostly inhabits fresh waters (Skelton, 1993), one would expect the optimum salinity for growth to be below the isosmotic level of 12‰. However, *O. mossambicus* is unusual as it is capable of reproducing in salinities ranging from 0-49‰ (Potts *et al.*, 1967). The life-history of this species, therefore, appears to be largely independent of salinity as it can successfully complete its life cycle above and below isosmotic concentration. Nonetheless, the performance of the offspring is influenced by the ambient salinity at the time of spawning. Watanabe *et al.* (1989) found that offspring of florida red tilapia spawned in seawater exhibited better growth and survival than those spawned in brackish water when cultured under marine conditions. This indicates that even in this species complex there adaptation to a saline environment. Also contrary to expectations are two marine species, Atlantic cod (*Gadus morhua*) and European flounder (*Platichthys flesus*), as they exhibit highest growth at salinities below isosmotic concentration (Gutt, 1985; Lambert *et al.*, 1994). However, in both species the optimum salinity range, 5-15‰ for *P. flesus* (Gutt, 1985) and 7-14‰ for *G. morhua* (Lambert *et al.*, 1994), encompasses a range which extends above and below the isosmotic concentration. This is unusual as the physiological demands of ion osmoregulation in a medium that is hypo-osmotic to blood concentration are quite different from those in a hyper-osmotic environment (Evans, 1984). The significance of this observation is, however, not fully understood.

Since the mechanisms of osmotic regulation are essentially similar amongst teleost fish (Evans, 1984), the discrepancies between the salinity responses of different species, habits and life history stages are not fully explained by differences in ion-osmoregulation capability (Morgan & Iwama, 1991). Febry and Lutz (1987) suggest that these responses are influenced by physiological processes in addition to the energy required for ion-osmoregulation. In Atlantic salmon (*Salmo salar*) the changes in the metabolic capacity of isolated gill and kidney tissues exposed to different salinities did not account for the magnitude of change seen in overall metabolism (McCormick *et al.*, 1989). Furthermore, osmoregulation may also have secondary effects which are not directly related to ion exchange between blood and water. For example, acclimation to different salinity may cause changes in blood haemoglobin content (Guernsey & Poluhowhich, 1975; Woo & Fung, 1981) and changes in hormone levels (Avella *et al.*, 1990). It has also been hypothesised that modified drinking rates resulting from changes in salinity (Conte, 1969) affect stomach evacuation rate, rate of food movement through the intestine, pH of the gastric fluid, food breakdown and consequently adsorption efficiency (DeSilva & Perera, 1976; MacLoed, 1977; Ferraris *et al.*, 1986). The pattern of food conversion and protein efficiency ratio observed in juvenile spotted grunter did not support this hypothesis as both factors were negatively affected at lower salinity levels (5‰), but were similar at the isosmotic concentration (12‰) and above (25 and 35‰). Since other marine fish, such as *Dicentrarchus labrax*, also have best food conversion efficiencies between isosmotic and 35‰ (Dendrinos & Thorpe, 1985), it appears that absorption and digestion is not compromised by the drinking rate as salinity increases.

The differences in food conversion efficiencies at different salinities follow a pattern that is similar to the growth response of juvenile spotted grunter to salinity. This pattern is consistent with that observed for Atlantic cod (*Gadus morhua*), European flounder (*Platichthys flesus*), European bass (*Dicentrarchus labrax*), freshwater catfish (*Mystus vittatus*), coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*O. mykiss*)(Otto, 1971, MacLoed, 1977; Arunachalam & Reddy, 1979; Dendrinos & Thorpe, 1985; Gutt, 1985; Lambert *et al.*, 1994). In all these species, food conversion efficiency is maximised at a species specific optimum salinity which is dependent on life history. The optimum salinity for food conversion corresponds strongly with that of growth. Therefore, there is further evidence in support of the hypothesis that species adapt to a particular environmental salinity according to their respective life-history (Morgan & Iwama, 1991).

In summary, growth performance and survival of juvenile spotted grunter was optimum in a salinity range of 12-35‰ even though there was little variation in blood osmolarity between 5 and 35‰. Growth was not enhanced in an isosmotic salinity even though this concentration theoretically has the lowest energetic demands for ion osmoregulation. Food and protein conversion were also not compromised at higher salinities although it has been hypothesised that digestion efficiency would be affected by increased drinking rates at higher salinities. Therefore, from an aquaculture perspective, spotted grunter would be most successfully reared in salinities ranging from 12‰ to full strength seawater (35‰).

Chapter 6

The effect of feeding frequency on gut evacuation and its implications for food intake and growth

Introduction

Food is the most important biotic factor controlling the growth of fish (Brett, 1979; Jobling, 1983). Under intensive culture conditions it is also the one factor over which the aquaculturalist has absolute control. As food requires the highest economic investment in the intensive rearing of fish, its efficient utilisation directly affects economic yields. Efficient use of food is dependent on the provision of a nutritionally balanced diet under the correct feeding regime.

Under artificial rearing conditions, marine fish are gradually weaned from *ad libitum* feeding on live prey to periodic provision of a dry or semi-moist diet. To take full advantage of the rapid growth rate of juvenile fish under culture conditions it is essential to ensure that the ration is adequate to support maximal growth and that the frequency of feeding facilitates the most efficient use of that ration, without comprising survival and economic viability. Increased feeding frequency has been reported to enhance growth of juvenile fishes (Ishiwata, 1969 a,b; Andrews & Page, 1975; Chua & Teng, 1978). However, at higher than optimal frequencies the increase in growth performance is negligible. High feeding frequency can also lead to excess lipid accumulation (Chua & Teng, 1978; Kayano *et al.*, 1993).

Optimal feeding frequency is normally estimated on a trial and error basis by observing growth under different feeding strategies (Andrews & Page, 1975; Grayton & Beamish, 1977; Chua & Teng, 1978, Kayano *et al.*, 1993). The optimal feeding frequency is the one which supports highest growth and best food conversion ratio (Chua & Teng, 1978, Kayano *et al.*, 1993). On the other hand, sub-optimum feeding frequencies are defined by good food conversion ratios but lower growth rates (Chua & Teng, 1978). Although this method is practical, an estimate of optimum feeding frequency may also be obtained by examining gut evacuation rate. Optimal feeding frequency should ideally combine efficient digestion of the food and maximum food intake with a minimum deprivation time. A relationship between gastric evacuation and appetite is now well established (Brett, 1971; Grove *et al.*, 1978). Intake of food is proportional to the fullness of the stomach and maximum consumption is achieved when the stomach is empty. Fullness of the stomach is in turn related to the rate of digestion (Brett, 1971; Grove *et al.*, 1978). Since appetite dictates frequency of feeding, it follows that data on gut passage time may be used to predict optimum feeding frequency. This relationship has yet to be examined, although potentially it provides an accurate and rapid technique of establishing optimum feeding frequency for fish species.

The aim of the present study was to establish the optimal feeding frequency for growth and efficient food utilisation in juvenile spotted grunter and to test the hypothesis of a relationship between gut evacuation time and optimal feeding frequency.

Materials and methods

The effect of feeding frequency on growth

The investigation into the effect of feeding frequency on growth was conducted in 40 L glass aquaria linked to the experimental recirculating system (Chapter 2). Feeding frequencies of 1, 2, 3, 4 and 5 times daily were allocated randomly amongst aquaria, with each frequency represented in triplicate. The aquaria were each randomly stocked with 8 individually marked fish. Fish were marked according the protocol described in Chapter 2. Initial length and weight of the fish was 54 ± 4.5mm and 3.98 ± 1.23g. Fish were fed to satiation on the standard semi-moist pelleted diet at each meal.

Satiation was defined as the stage when the fish no longer accepted single pellets dropped into the tank. Water quality parameters remained optimal throughout the experimental period with nitrite levels remaining below 0.1mg/L and unionised ammonia remaining below 0.02 mg/L. Water temperature was maintained at optimum temperature for growth of 24.5 ± 0.5°C for the species (Deacon & Hecht, 1996). Photoperiod was controlled to 14L:10D cycle. Salinity was kept constant at 35‰, with the addition of distilled water when necessary. The experiment was run for eight weeks. Standard length (mm) and weight (g) of the fish were measured once weekly.

Total gut passage time

Fish were divided into groups of ten and randomly allocated to 40 L aquaria then acclimated to feeding frequencies of 1, 3 and 5 times daily for a period of two weeks. Food marked with an inert dye (1% Chromic oxide) was substituted for the normal diet at one of the feeding intervals. The marked food was of the same proximate composition as the standard semi-moist pelleted diet, except for the chromic oxide marker. At hourly intervals faecal matter was removed from the recta of five fish from each of the three replicates in each treatment. Before the removal of faecal material, the fish were anaesthetized in a solution of 0.2 ml phenoxyethanol/L for approximately 1 minute. The fish were then removed from the anaesthetic solution, blotted dry on the underside and stripped of faeces by applying gentle pressure to the abdomen between the anal fin and the anus. On the first appearance of the green dye in the faeces, a further 10 fish from each replicate were examined. Total evacuation time was measured as the time taken for 75% of fish sampled to evacuate dyed faeces. Unexamined fish continued to be fed at their respective feeding frequencies throughout the experimental period. Water temperature was maintained at the same level (24.8 ± 0.3°C) used in the growth trial. Water quality parameters were measured daily and remained optimal throughout the experimental period with nitrite levels remaining below 0.1mg/L and unionised ammonia remaining below 0.02 mg/L. Photoperiod was controlled to 14L:10D cycle. Salinity was kept constant at 35‰, with the addition of distilled water when necessary.

Gut evacuation

Fish were divided into groups of ten and randomly allocated to 40 L aquaria then acclimated to a feeding frequency of 3 times daily for a period of two weeks. Marked food (described above) was substituted for the normal diet at one of the feeding intervals. At hourly intervals faecal matter was removed from the recta of 10 fish. The procedure for the collection of faecal matter was the same as that used in the gut passage time experiment. The faecal matter was collected in sterile glass vials and dried in a convection oven at 70°C. The fish were not fed once collection of faeces had started. Faeces were collected hourly for a ten hour period. Water quality and temperature were maintained at the same levels described for the gut passage time.

The dried faecal samples and a sample of the marked food were digested according to the standard micro-Kjeldhal digestion technique (Irish, 1997). Chromic oxide concentration was determined using a GBC Atomic Absorption spectrophotometer at an absorbance level 357.9μm using nitrous oxide acetylene (Irish, 1997). The chromic oxide concentration was determined from a standard curve constructed from the dilution of a standard chromium solution. Gut passage time was calculated from a modified version of the Maynard and Loosli (1956) equation for the calculation of apparent digestibility (AD):

$$\text{Total evacuation (\%)} = \frac{\text{\% marker in diet - \% marker in faeces}}{\text{\% marker in diet}}$$

Results

Effect of feeding frequency on growth

An increase in feeding frequency was found to have a significant effect on the food consumption, growth, condition and FCR of juvenile spotted grunter (Table 6.1). A feeding regime of three times daily was found to be the minimum frequency to provide the consumption of a maximum daily ration of 14.29% wet body weight per day (Fig 6.1). More frequent feeding, 4 and 5 times daily, resulted in no significant increase in the amount of food consumed (Fig 6.1). Feeding twice daily resulted in lower food consumption which reduced growth (Table 6.2). Food consumption at a once daily feeding regime was significantly lower than the maximum daily ration (Table 6.1). The effect of feeding frequency on food consumption was reflected in the growth performance of juvenile spotted grunter. Specific growth rate (SGR) was significantly improved with an increase in feeding frequency from once daily to three times daily (Fig 6.2). Feeding more often than three times daily did not result in a further improvement in growth. Food conversion ratios of fish fed once daily were significantly better than those at higher feeding frequencies (Table 6.1 & 6.2). The condition factor of fish at frequencies of 3-5 times daily were all similar and, although higher than those fed twice daily, were not significantly different. Fish fed once daily showed significantly lower condition factors than those at higher feeding frequencies (Table 6.2).

Table 6.1: Summary of one-way ANOVA tests of the effect of feeding frequency on the growth performance of juvenile spotted grunter, *Pomadasys commersonnii*.

FACTOR	F-RATIO	D.F.	SIG. LEVEL
Feeding Frequencies: 1, 2, 3, 4 & 5x daily			
Condition factor	11.238	4	0.0000
Food consumption	4.606	4	0.0021
Food conversion ratio	20.389	4	0.0000
Specific growth rate	6.076	4	0.0001

Table 6.2: Tukey's Multiple Range Analyses' of the effect of feeding frequency on the condition factor, food consumption, food conversion, survival, growth and protein efficiency ratio of juvenile spotted grunter, *Pomadasys commersonnii*. Superscripts (a,b & c) indicate homogenous groupings of means. Feeding frequency treatments which share a common superscript are not significantly different from one another at $P<0.05$.

Growth parameter	**Feeding Frequency**	**Tukey HSD Average**
Condition factor	1	0.84465^{a}
	2	0.85678^{ab}
	3	0.86474^{b}
	4	0.87304^{b}
	5	0.87319^{b}
Food consumption	1	8.22791^{a}
	2	10.52782^{ab}
	3	14.2918^{b}
	4	14.7941^{b}
	5	14.8634^{b}
Food conversion ratio (FCR)	1	2.25749^{a}
	2	2.95462^{b}
	3	3.78831^{c}
	4	3.85462^{c}
	5	3.98437^{c}
Specific growth rate (SGR)	1	1.60418^{a}
	2	1.95132^{ab}
	3	2.51528^{bc}
	4	2.51516^{bc}
	5	2.51894^{bc}

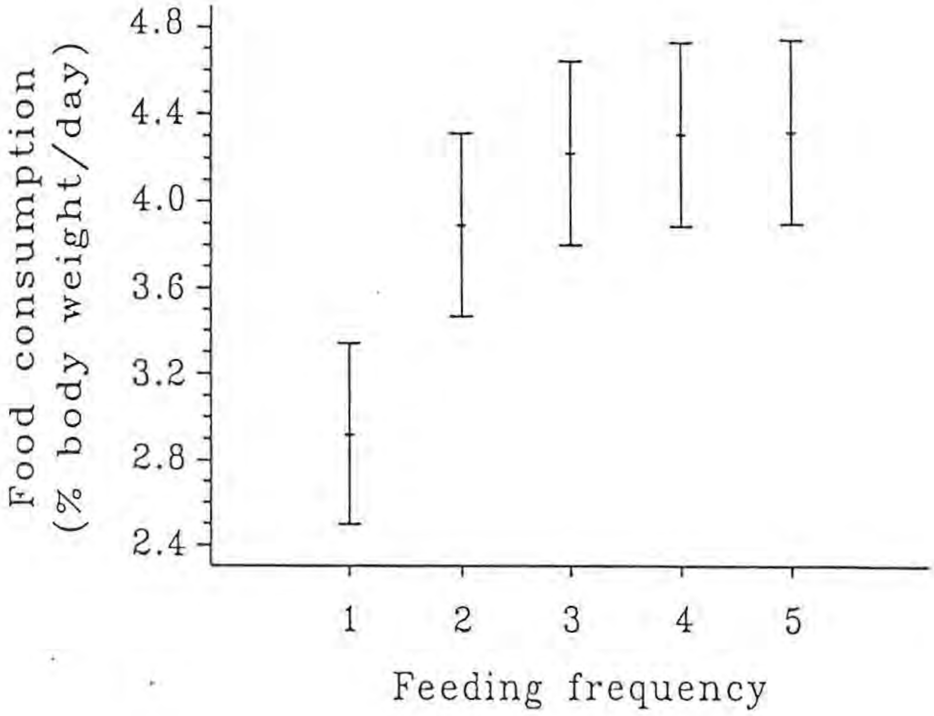

Figure 6.1: Daily food consumption of juvenile *P. commersonnii* fed at frequencies of 1, 2, 3, 4

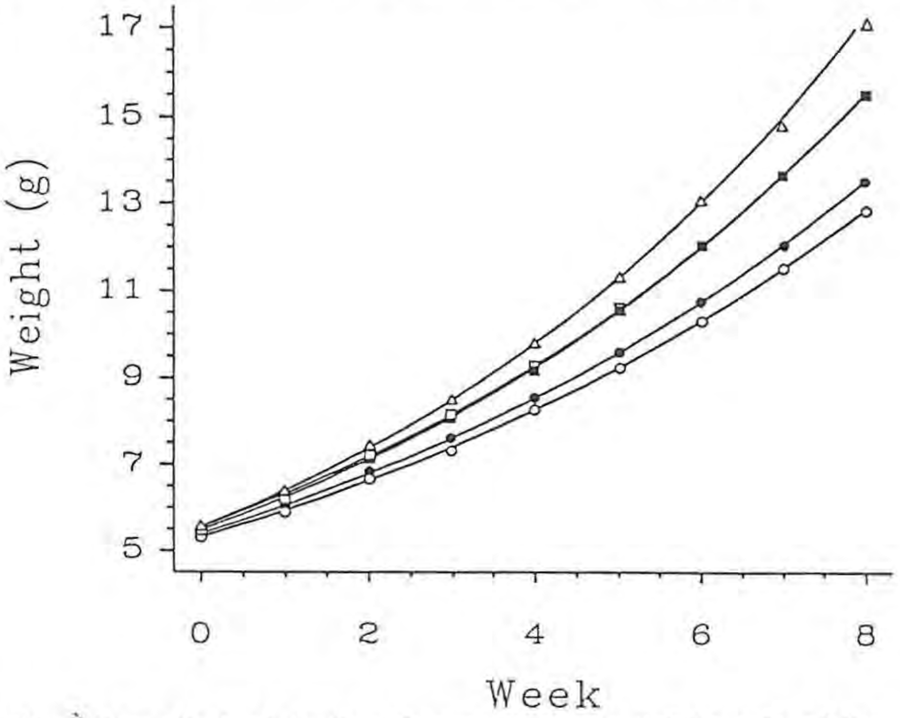

Figure 6.2: The effect of feeding frequency on the weight gain of juvenile spotted grunter, *Pomadasys commersonnii*. Each datum for 1 (○), 2 (●), 3 (□), 4 (■) and 5 times daily (▲) is the mean weight (g).

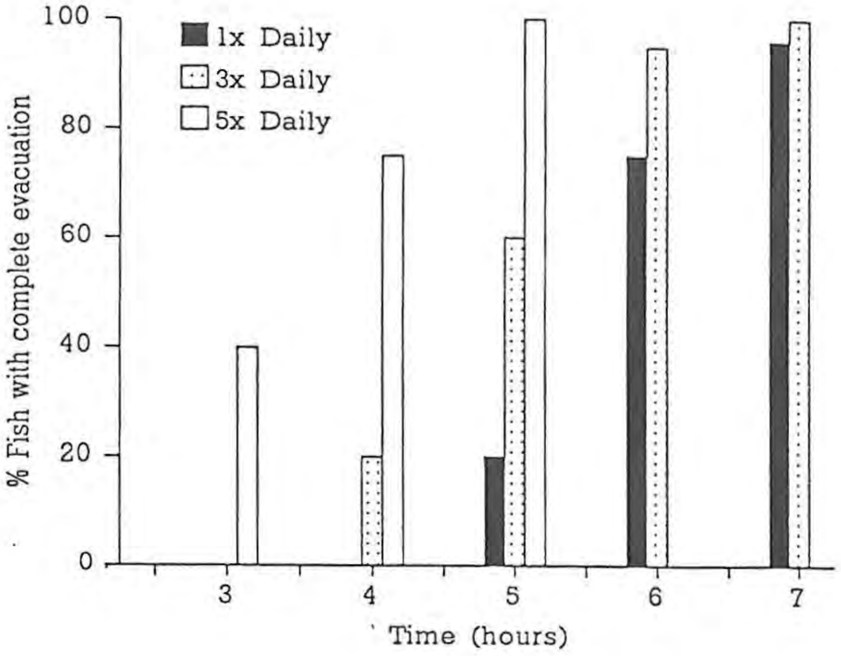

Figure 6.3: Gut passage time of fish acclimated to feeding frequencies of 1, 3 and 5x daily.

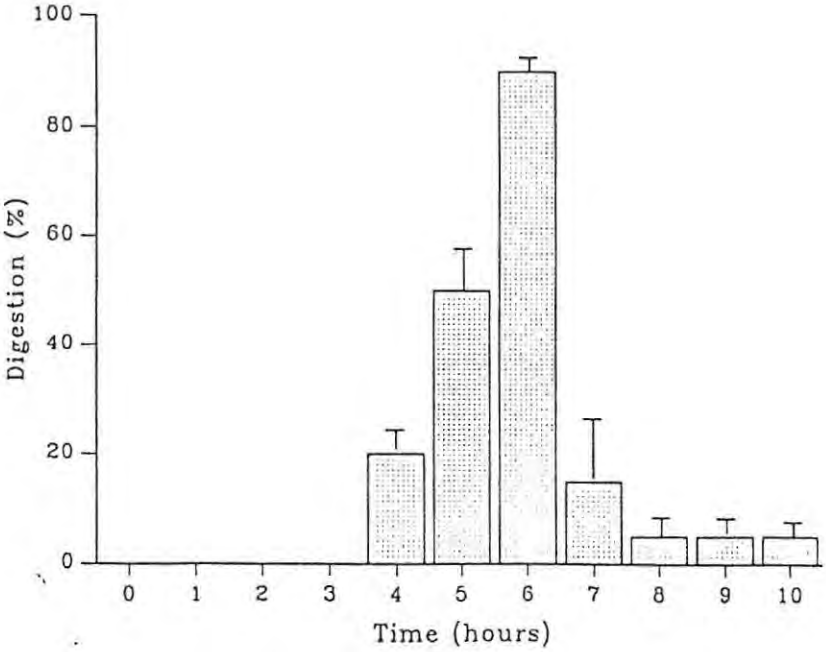

Figure 6.4: Gut evacuation rate of juvenile spotted grunter.

Gut passage time

The gut passage time of juvenile spotted grunter was found to decrease significantly with increasing feeding frequency. The gut passage time for at least 75% of fish fed five times daily was 4 hours. For fish fed 3 times and once daily the gut passage time was significantly longer at 6 hours (Fig 6.3).

Gut evacuation

Figure 6.4 illustrates the relationship between gut evacuation and time. The presence of the marker was only measurable 4 hours after feeding. At this time rate of evacuation was 20% of maximum. Five hours after feeding, rate of evacuation had increased to 50% of maximum. Maximum gut evacuation of 90% was observed after 6 hours. At 7 hours after feeding, gut evacuation decreased to 5% of maximum. This level remained constant through up to 10 hours after feeding.

Discussion

The daily ration consumed by juvenile *P. commersonnii* was increased by increasing the feeding frequency from one to three meals daily. At higher feeding frequencies no difference in the total daily intake was observed. This is consistent with results of feeding frequency studies on other species of marine fish (Ishiwata, 1969 a,b; Chua & Teng, 1978). Maximum daily intake of juvenile spotted grunter is therefore estimated to be approximately 14% of wet body weight/day. Tyler and Dunn (1976), however, found that food intake by winter flounder (*Pseudopleuronectes americanus*) was linnear and increased directly with meal frequency. Presumably total food intake of flounder would exhibit an asymptotic pattern, observed in the present and similar studies, with higher feeding frequencies.

An increase in feeding frequency only had a significant effect on the growth performance of juvenile spotted grunter when increased from once to three times daily. Lower feeding frequencies of once and twice daily showed decreased growth, lower condition factors and better food conversion. Feeding frequencies in excess of 3 times daily did not significantly improve growth performance. This is consistent with Grayton and Beamish's (1977) observation that until maximum food intake is facilitated, the effect of feeding frequency is a function of the total food consumed. The lowest frequency at which maximum intake is achieved corresponds to the optimum feeding frequency. For juvenile grunter this was achieved by feeding three times daily. This feeding regime supported the highest growth and best food conversion ratio thus also meets the criteria commonly used to define optimum feeding frequency (Chua & Teng, 1978; Kayano *et al.*, 1993).

Brett (1971), following his observations of appetite in sockeye salmon (*Oncorhynchus nerka*), found that food intake or appetite increased depending on the rate of digestion of a previous meal. Maximum food intake was attained only when 90% of the previous meal was evacuated from the stomach. As digestion proceeded at an exponential rate, gastric evacuation rate for 90% digestion was half of that for total digestion. On this basis he proposed that feeding at intervals equivalent to 90% digestion would optimise daily food intake and therefore growth of fish, especially if fed a restricted ration. Growth trials confirming this hypothesis were not performed. However, if this hypothesis is applied to food conversion and gastric evacuation in the estuary grouper (Chua & Teng, 1978), there is considerable loss in food conversion efficiency. The time for 90% evacuation of the stomach in this species is 27 hours and complete evacuation occurs 35 ± 1 hours after feeding. Amongst other feeding frequencies, Chua and Teng (1978) tested frequencies of once every 24 hours and once every 48 hours. In terms of stomach evacuation, these feeding frequencies were equivalent to feeding at 86% of gastric evacuation and 12 hours after complete evacuation of the stomach, respectively. The food conversion of fish fed once every two days was significantly higher than those fed once a day, in addition a higher growth rate (g/month) was observed in the lower feeding frequency. This finding emphasises the importance of allowing digestion to progress to its full extent before feeding again. However, it provides no insight into the effect of deprivation time. Although the effects of extreme deprivation have been documented, they have little relevance to normal hatchery feeding practices.

In this study feeding frequencies of 5, 4, 3, 2 and 1x daily corresponded to the provision of a meal once every 3, 4, 6, 12 and 24 hours, respectively. As the passage of food through the gut was not altered whether the fish were fed once or three times daily when fed the standard semi-moist pelleted diet, it is concluded that the gut passage time at 24.8 ± 0.3°C was 6 hours with this diet. Feeding frequencies of 5, 3 and once daily therefore coincided with a meal at every 66.7, 100 and 200% of gut passage time, respectively. As a three times daily feeding regime proved optimal for both growth and food conversion, it is clear that digestion must progress to its full extent to maximise growth performance. Feeding five times daily resulted in a 33% more rapid evacuation time and a slightly increased food conversion ratio. This supports previous observations that poor food conversion ratios at high feeding frequencies may be attributed to incomplete digestion due to decreased gut passage time (Dawes, 1930; Hickling, 1962; Chua & Teng, 1978). On the other hand, increasing deprivation to 200% of evacuation time (one meal daily) resulted in a significantly reduced growth rate. However, as feeding once daily also resulted in a concomitant reduction in total daily food intake, the observed decline in growth cannot be attributed to deprivation time *per se*. It appears then, that to test the effect of deprivation it is necessary to feed a restricted ration at different feeding frequencies.

The correlation between the optimum feeding frequency of three times daily and total gut evacuation time in juvenile *P. commersonnii* suggests that examination of food passage time may be used to predict optimum feeding frequency. However, further examination of the relationship between these variables in other species is required before the predictive potential of this method can be assessed.

Chapter 7

The relationship between growth, ration and feeding frequency

Introduction

The relationship between metabolism and ration is the fundamental basis of fish growth (Brett, 1979). Food intake acts as a potential limiting factor on fish metabolism (Fry, 1971; Brett, 1979). As with other limiting environmental factors, the effect of ration size involves independent and dependent states which become operational at a particular level. Growth rate increases with increasing ration from a minimum negative value at zero ration to a maximum at the point of maximum ration (Elliot, 1975a, Brett, 1979). Above maximum ration, growth rate reaches a plateau where, theoretically, it is independent of any increase in ration size. Maximum growth is, therefore, dependent on the consumption of the maximum ration.

In the development of the theoretical protocol for the sequential determination of the optimum environmental requirements of a species (Chapter 1), limiting factors were eliminated from the protocol on the assumption that their effects are negligible if maintained at appropriate levels. This implies that the maximum ration must be consumed by the fish under the experimental conditions in order for food intake to have no limiting effect. However, research on the effect of feeding frequency on growth indicates that feeding regime is a factor which potentially restricts food intake (Grayton & Beamish, 1977; Chua & Teng, 1978, Jobling, 1983). In the investigation of the effect of feeding frequency on growth of juvenile spotted grunter it was revealed that feeding to satiation at frequencies lower than three times daily resulted in significantly lower food intake (Chapter 6). Consumption of maximum ration in juvenile spotted grunter is only possible if the fish are fed to satiation 3 or more times daily. Since a satiation feeding regime of 3 times daily was used in the experimental determination of the effects of temperature, photoperiod, light factor in the experimental procedure. However, it has been suggested that *ad libitum* access to food is virtually a prerequisite for eliminating potential biases arising from the feeding regime when testing the effects of non-food related parameters (Jobling, 1983). Although there are means of achieving this, such as demand feeding, providing fish with *ad libitum* access to food is not always practical or possible. Juvenile spotted grunter fall into this category as they will only accept a semi-moist diet, which makes cold storage a necessity. In such cases, as in this study, it is necessary to compromise and adopt a feeding regime whereby the fish are fed to satiation at a set frequency. However, this methodology has been questioned as it is unknown whether the growth rates achieved on a set satiation feeding regime are always comparable to those with *ad libitum* access to food (Jobling, 1983).

Numerous feeding frequency experiments have shown that the effect of feeding frequency is a function of ration size, at least until maximum daily intake is achieved (Grayton & Beamish, 1977; Chua & Teng, 1978; Chapter 6). The optimum feeding frequency normally corresponds to the lowest frequency at which maximum daily intake is attained. Increasing feeding frequency above the optimum does not affect growth although food conversion declines as a result of incomplete digestion (Dawes, 1930; Hicking, 1962; Chua & Teng, 1982). Since maximum daily intake appears to have a finite effect on growth (Elliot, 1975a; Wurtsbaugh & Davis, 1977; Chua & Teng, 1982), any difference between growth of fish on *ad libitum* feeding and those fed to satiation would most likely be caused by the time of deprivation.

The relationship between growth and ration is determined by analysing either growth rate in relation to ration size or food conversion efficiency in relation to ration size. The data is derived from growth trials in which fish are fed a number of prescribed rations, expressed as percent of wet body weight, at the optimum feeding frequency. Weight of food fed is adjusted to maintain the prescribed ration after each weighing and measuring of the fish. The maximum ration size is determined as the point where growth no longer increases in relation to an increase in ration. Feeding at the optimum feeding frequency is a prerequisite to the accurate determination of maximum ration size as maximum food intake must be possible in the first place. Since optimum feeding frequency is essentially the lowest frequency at which maximum food consumption is attained, an estimate of the maximum is ensured (Grayton & Beamish, 1977; Jobling, 1983).

Analyses of the relationships between growth and ration can also be used to determine the optimum ration. The optimum ration is important from an economic point of view as it is the ration which provides for the highest growth rate for the least intake of food (Brett *et al.*, 1969; Elliot, 1975a). From an economic perspective, a correct food ration must fulfil a number of criteria. It must promote assimilation of dietary protein to muscle rather than lipid storage, support a high survival rate and maintain a high food conversion efficiency, without limiting growth. While maximum growth occurs at the limit of voluntary food intake (satiation), maximum food conversion efficiency occurs at some level below satiation (Andrews & Stickney, 1972; Brett, 1979; Chua & Teng, 1982). The optimum ration size is determined geometrically by drawing a tangent to the growth-ration curve from the origin, on a plot of weight increase (g/fish per day) against ration size (% wet weight of fish)(Brett *et al.*, 1969; Elliot, 1975; Chua & Teng, 1982). Alternatively, determination of the optimum ration can be achieved by analysing the relationship between food conversion ratio and ration size (Chua & Teng, 1982). Size of food ration affects food conversion ratio in fish (Pandian, 1967; Chua & Teng, 1982). In most studies gross food conversion efficiency, the inverse of food conversion ratio, is found to increase with an increase in ration size, from near maintenance levels to a maximum at an intermediate level, whereafter it remains largely unchanged or decreases if the ration is further increased (Elliot, 1975b; Wurtsbaugh & Davis, 1977 a,b; Chua & Teng, 1982).

This investigation was aimed at determining the relationship between growth and ration size, and investigated the effect of short term food deprivation on the growth of juvenile spotted grunter.

Materials and methods

Ration Size

The investigation into the effect of ration size on growth was conducted in 40 L glass aquaria linked to the experimental recirculating system (Chapter 2). Fifteen 40 L aquaria were each randomly stocked with 8 individually marked fish. The fish were marked according the protocol described in Chapter 2. Initial length and weight of the fish were 50.2 ± 2.9mm and 4.23 ± 0.75g. The aquaria were divided into five triplicate groups and the fish were fed three times daily with the standard semi-moist pelleted diet at five different ration levels. Calculation of the experimental ration was based on maximum daily food intake estimated from the feeding frequency experiment (Chapter 6) and corresponded to 3, 5, 7, 10.5 and 15% wet body weight/day. Ration size was calculated according to fish biomass and were adjusted weekly when the standard length (mm) and weight (g) of the fish were measured. Water quality parameters were monitored daily and these remained optimal throughout the experiment with nitrite levels remaining below 0.1 mg/L and unionised ammonia remaining below 0.02 mg/L. Water temperature was maintained at optimum temperature for growth of 24.5 ± 0.5°C for the species (Deacon & Hecht, 1996). Photoperiod was controlled to 14L:10D cycle. Salinity was kept constant at 35‰, with the addition of distilled water when necessary. The experiment was run for eight weeks.

Feeding frequency with a restricted ration

Twelve 40 L aquaria were each randomly stocked with 8 individually marked fish. Initial standard length (SL) and weight of the fish were 55 ± 4.2mm and 3.71 ± 0.92g, respectively. Feeding frequencies of 2, 3, 4 and 5 times daily were allocated randomly amongst the tanks with each frequency represented in triplicate. The fish were fed the standard semi-moist pelleted diet at maximum daily intake of 10.5% wet body weight/day at the different feeding frequencies. These were equivalent to deprivation times of 12, 6, 4 & 3 hours, respectively. The ration size for each tank were calculated according to fish biomass and were adjusted weekly when the standard length (mm) and weight (g) of the fish were measured. Water quality parameters were maintained at optimum levels throughout the experiment as described above. The experiment was conducted over a period of 8 weeks.

Results

Ration size

Juvenile spotted grunter exhibited a typical growth response to increasing ration size. Rapid and significant increases in growth rate were observed with increased daily intake up to 7% wet body weight/day (Fig 7.1, Table 7.1). Further increases in ration size from 7-15% wet body weight/day resulted in only marginal improvements in growth which were not significantly ($P<0.05$) different (Table 7.1). As the relationship between specific growth rate and ration size is curvilinear (Fig 7.2), it was possible to determine the maintenance, optimum and maximum ration from the growth/ration curve. Maintenance ration was determined as the ration which maintains fish with no change in weight, while maximum ration is the lowest ration which results in maximum growth rate. The optimum ration was determined by drawing a tangent to the growth curve, as described by Brett *et al.* (1969), and Chua and Teng (1982). Therefore the theoretical maintenance, optimum and maximum food rations of juvenile spotted grunter are 0.51, 3.6 and 10.7%, respectively.

Food conversion ratio decreased with decreasing ration size from 15-5% wet body weight/day, indicating more effective use of food as daily intake decreased. Further reduction of the ration size below 5% wet body weight/day did not significantly alter the food conversion ratio (Table 7.2, Fig 7.3), although a slight increase was observed from 5 to 3% wet body weight/day. The ration providing the optimum food conversion, therefore, lies between 3 and 5% wet body weight/day. This correlates well with the optimum ration determined from the growth-ration relationship (Fig 7.3).

Condition factor of the fish increased with increasing daily intake. However, the only significant improvement was observed when the ration was increased from 3 to 5% wet body weight/day (Table 7.2). No further improvement in condition factor was observed above 7% wet body weight/day. Daily food intake had no significant effect on survival rate of fish at any of the rations tested.

Table 7.1: Summary of one-way ANOVA tests of the effect of ration size and feeding frequency with a restricted ration on the growth performance of juvenile spotted grunter, *Pomadasys commersonnii*.

FACTOR	F-RATIO	D.F.	SIG. LEVEL
Ration size: 3, 5, 7, 10.5 & 15% wet body weight/day			
Condition factor	3.479	4	0.0078
Food conversion ratio	5.786	4	0.0003
Specific growth rate	11.288	4	0.0000
Feeding Frequency/Restricted Ration: 2, 3, 4 & 5x daily			
Condition factor	0.749	3	0.4126
Food conversion ratio	0.335	3	0.8004
Specific growth rate	0.808	3	0.4895

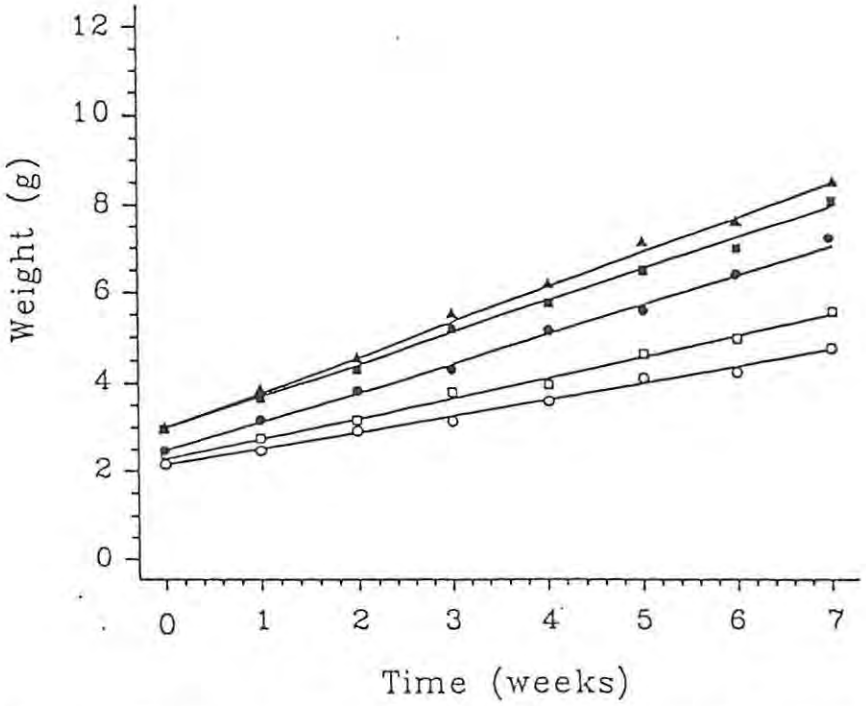

Figure 7.1: The effect of ration size on the weight gain of juvenile spotted grunter, *Pomadasys commersonnii*. Each datum for 3 (○), 5 (□), 7 (■), 10.5 (●) and 15 %wet body weight/ day (▲) is the mean of *ln* weight (g).

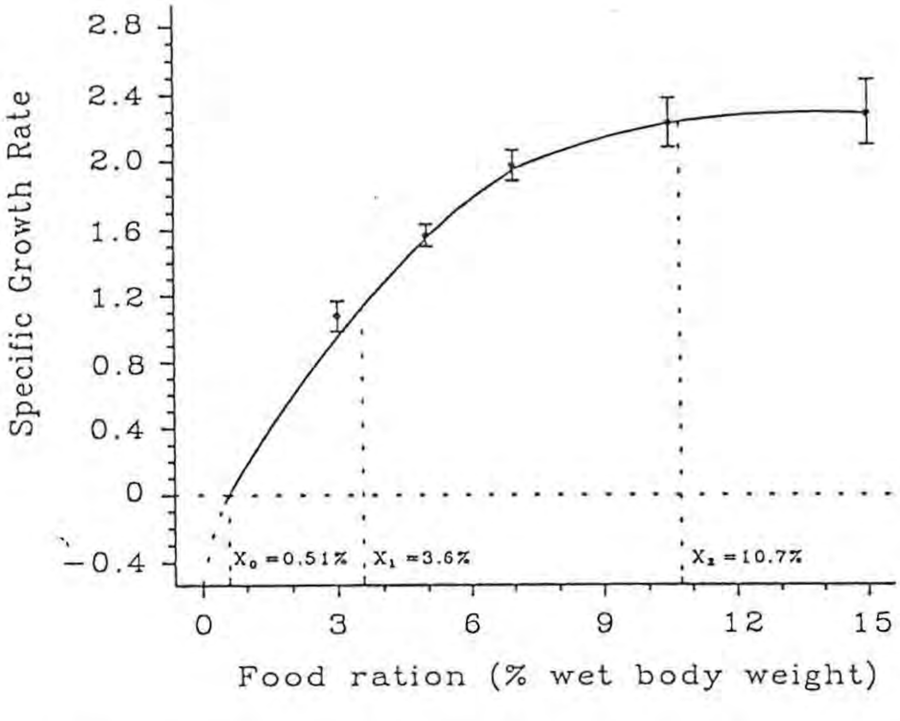

Figure 7.2: The relationship between growth and ration relationship for juvenile spotted grunter, *Pomadasys commersonnii*. X_0 = maintenance ration, X_1 = optimum ration and X_2 = maximum ration. Vertical bars represent standard deviations.

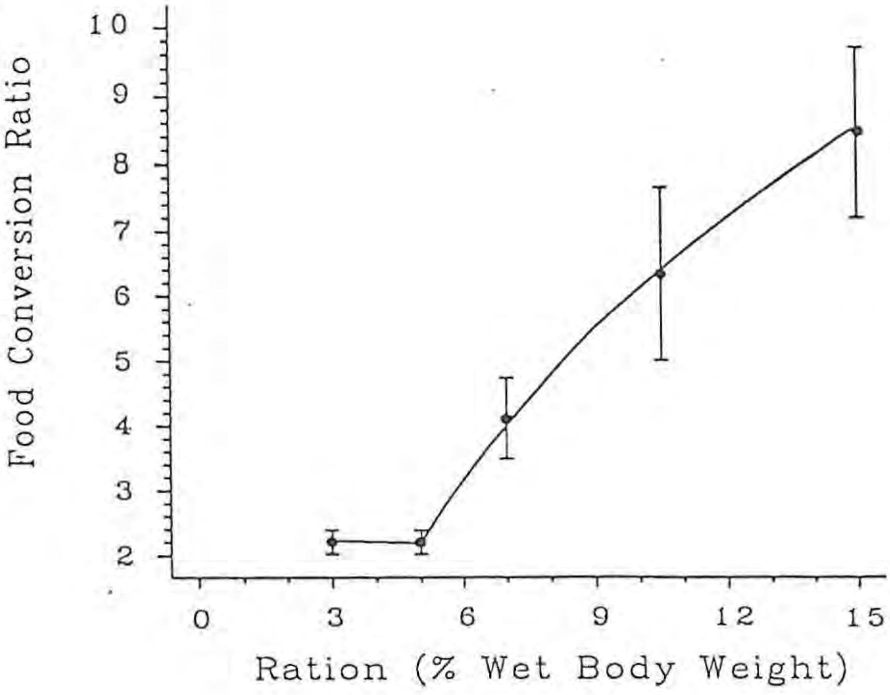

Figure 7.3: Effect of ration size on food conversion ratio of juvenile spotted grunter, *Pomadasys commersonnii*. Vertical bars represent standard deviations.

Feeding frequency with a restricted ration

The growth rate of juvenile *P. commersonnii* fed a restricted ration of 10.5% wet body weight/day was found to be independent of feeding frequency. Growth expressed as weight gain over time and specific growth rate were found to be not significantly different whether fish were fed 2, 3, 4 or 5 times daily (Table 7.1). Condition factor also did not differ significantly between the respective treatments (Table 7.1). Food conversion ratio was also found to be not significantly different whether the fish were fed 2, 3, 4 or 5x daily (Table 7.1). However, the food conversion of fish fed 3 times daily was approximately 1% lower than that of any of the other treatments.

Table 7.2: Tukey's Multiple Range Analyses' of the effect of food ration on the condition factor, food conversion and growth of juvenile spotted grunter, *Pomadasys commersonnii*. Superscripts (a & b) indicate a homogenous grouping of means. Rations that share a common superscript are not significantly different from one another at $P<0.05$.

Growth parameter	**Ration** **% wet W/day**	**Tukey HSD** **Average**
Condition factor	3	0.76910^{a}
	5	0.79067^{a}
	7	0.82609^{b}
	10.5	0.83538^{b}
	15	0.83593^{b}
Food conversion ratio (FCR)	3	2.27260^{a}
	5	2.26644^{a}
	7	4.16550^{ab}
	10.5	6.32944^{b}
	15	9.24716^{b}
Specific growth rate (SGR)	3	1.11206^{a}
	5	1.56598^{ab}
	7	2.00030^{ab}
	10.5	2.34746^{b}
	15	2.36451^{b}

Discussion

Before an experiment could be conducted to examine the effect of deprivation it was necessary to first obtain an accurate estimate of the maximum ration. The most reliable method of accomplishing this was to establish the relationship between growth and ration for this species (Elliot & Persson, 1978). Juvenile grunter exhibited a curvilinear growth/ration relationship similar that observed in salmonids (Brett, 1979; Elliot, 1982) and serranids, such as *Dicentrarchus labrax* (Hidalgo *et al.*, 1987) and *Epinephelus salmoides* (Chua & Teng, 1982). Linear functions have been found to apply to some species, such as *Hippoglossus hippoglossus* (Tuene & Noŕtvedt, 1995) and *H. stenolepis* (Paul *et al.* 1994). Maintenance, optimum and maximum rations were determined geometrically from the growth/ration curve (Brett *et al.* 1969; Elliot, 1975a; Chua & Teng, 1982), and were estimated at 0.51, 3.6 and 10.7% wet body weight per day, respectively. Increasing the ration from 10.5 to 15% wet body weight/day did not improve growth or condition of juvenile grunter, however food conversion ratio was increased by ± 30%. Since optimum food ration is defined as that ration which provides the best growth for the least intake of food (Brett *et al.*, 1969; Elliot, 1975a), a correspondingly low food conversion ratio in the 3-5% wet weight/day provides confirmation of the optimum ration. Although there was a slight decrease in food conversion between 3 and 5% wet body weight/day, the lowest FCR was not well defined. The relationship between food conversion ratio and ration often describes a parabolic curve with the peak defining the optimum (Chua & Teng, 1982; Hidalgo *et al.*, 1987; Tsevis *et al.*, 1992). This was not possible with our data as the lowest ration was not low enough to give a parabolic relationship.

Juvenile grunter which were fed a ration controlled at maximum daily intake, showed almost no variation in growth whether they were fed 3, 4 or 5 times daily. However, a slight, although not significant, decline in growth was observed when they were fed twice daily (12 hours deprivation). In the study of the effect of feeding frequency on the growth of juvenile spotted grunter (Chapter 6), it was found that fish of this size were unable to achieve maximum daily intake when fed only twice daily. Therefore, the decline in growth of fish fed twice daily observed in this study is probably due to the same factor rather than the result of deprivation. The increase in FCR of fish fed twice daily supports this explanation. Nonetheless, the similarity in growth rate of fish fed 3 times daily or more, indicates that short term deprivation of 3 - 12 hours has no negative effects on growth of fish provided they can achieve a maximum daily intake. This suggests that feeding to satiation at the optimum feeding frequency should be as effective as *ad libitum* access to food when attempting to eliminate any biases stemming from feeding regime. Comparison between growth rate of juvenile summer flounder (*Paralichthys dentatus*) fed maximum daily intake and those fed *ad libitum* showed no differences between the two feeding regimes (Malloy & Targett, 1994). Furthermore, the slight decrease in FCR of juvenile grunter fed 3 times daily suggests that more reliable estimates of food utilisation would be obtained if fish were fed to satiation at the optimum feeding frequency in contrast to *ad libitum* feeding. In the previous chapter (Chapter 6) it was found that feeding more often 3 times daily results in a significant reduction in food conversion efficiency due to an increase in the gut passage time. At this feeding frequency, food is more than 90% digested. Since fish rapidly regain their appetite after 75% digestion (Brett, 1971; Gwyther & Grove, 1981; Grove *et al*, 1985), feeding on an *ad libitum* basis could result in inflated estimates of food conversion thus reducing the reliability of this parameter for comparison between experimental variables.

This research has shown that short term food deprivation has no effect on the growth of fish provided that maximum daily intake can be attained. Therefore it is concluded that feeding to satiation at the optimum feeding frequency eliminates the potential biases resulting from the feeding regime in experiments testing the effect of non-food related factors (Chapters 3-5). Consequently, the elimination of limiting factors from the theoretical protocol developed for the determination of optimum environmental requirements of a species (Chapter 1) is justified.

In Chapter 6 it was found that the optimum feeding frequency for juvenile spotted grunter corresponded to feeding at an interval that was equivalent to one meal per total gut passage time.

From this it was hypothesised that optimum feeding frequency may be directly related to the rate of digestion and that it should correspond to feeding at an interval that combines maximum digestion with minimum deprivation time. Furthermore it was found that the optimum feeding interval corresponded to >90% digestion of the previous meal, which implied that digestion must progress to its fullest extent before the next meal. However, the results on the effect of deprivation on growth of juvenile grunter do not support this hypothesis as the decrease in food conversion at the optimum feeding frequency was not significantly different from higher feeding frequencies. Therefore it can be concluded that the effect of feeding frequency on fish growth is purely a function of the food intake.

Chapter 8

The comparative effects of environmental factors on growth performance

Introduction

The sequential determination of the effect of environmental factors on growth of juvenile spotted grunter was conducted according to a theoretical ranking of Fry's (1971) functional categories (Chapter 1). The key environmental factors considered to influence the growth of juvenile spotted grunter were designated as controlling, masking, directive and limiting factors according to their respective effect on metabolism (Fry, 1971, Brett, 1979). These factors were identified from natural history studies on spotted grunter as well as ecological studies of southern African east coast estuaries (Wallace, 1975b; Whitfield *et al.*, 1981; Wooldridge & Bailey, 1982; Beckley, 1984; Cyrus, 1988; Whitfield, 1990; Ter Morshuizen *et al.*, in press). In this chapter the individual models of each of the respective environmental factors are pooled together into a multiple regression analyses for comparison of their relative effects on growth. The statistical ranking provides a basis upon which a protocol can be developed from the experimental data.

In Chapter 1 it was hypothesised that the effects of controlling factors should be investigated before all others as these control the molecular activation of metabolites and enzyme systems and, therefore, the rate of biochemical reactions. The upper limit of metabolic processes are ultimately defined by the point where the biochemical reactions are optimized (Brett, 1971; Crawshaw, 1979; Jobling, 1981). Temperature was determined to be the major controlling factor influencing the growth of juvenile spotted grunter under natural conditions (Wooldridge & Bailey, 1982; Ter Morshuizen *et al.*, in press) and was, therefore, investigated first in the experimental sequence.

Metabolism is a combination of physiological and biochemical processes. Therefore, the energy available for catabolism is a function of the difference between the external environment and the internal requirements (Woo & Fung, 1981; Morgan & Iwama, 1991). Since masking factors compensate for the differences between the internal and external environments, they modify the level of optimum metabolism. It was concluded that before research could be conducted into the effects of directive factors, the external environmental conditions should provide for the attainment of optimum metabolism. As juvenile spotted grunter are exposed to fluctuations in salinity in their natural environment (Whitfield *et al.*, 1981; Ter Morshuizen, *et al.*, in press), salinity was identified as a potential masking factor.

Directive factors synchronize physiological and metabolic processes (Schwassmann, 1971) and therefore complement the effect of controlling factors in the attainment of optimum metabolism. In addition, directive factors also control activity, such as food intake, through cueing of behaviour (Reynolds & Casterlin, 1976). Consequently, it was hypothesised that research on the effect of masking factors should be followed by the effect of directive factors on growth performance. Light intensity and photoperiod were hypothesised to be the principal directive factors influencing the growth of juvenile spotted grunter. Under natural conditions this species is exposed to varying levels of turbidity (Cyrus, 1988; Ter Morshuizen *et al.*, in press) which results in changes in light intensity (Alabaster & Lloyd, 1980). There is also considerable variation between the summer and winter photoperiods in the southerly distribution of juvenile spotted grunter (Beckley, 1984).

Investigations into the effects of limiting factors were last in the experimental sequence. These were included to confirm the assumption that optimum growth was not restricted under the experimental conditions used for the determination of the effects of environmental factors. As a consequence of the fundamental relationship between food intake and metabolism (Fry, 1971; Brett, 1979), food consumption was considered to be a potential limiting factor on the growth of juvenile spotted grunter under experimental conditions. Since food intake is a function of the feeding regime (Grayton & Beamish, 1977; Chua & Teng, 1978) and ration size (Brett *et al.*, 1969; Elliot, 1975a; Chua & Teng, 1982), separate experiments were conducted to determine the independent effects of the respective factors on the growth performance of juvenile spotted grunter and to establish the relationship between them.

The sequence of experimentation used in the present study, namely:

temperature	→ **photoperiod/light intensity** →	**ration size** →	**salinity**
(controlling factor)	**(directive factors)**	**(limiting factor)**	**(masking factor)**

may seem contrary to the theoretical protocol. However, the above experimental sequence ignores the previous studies on temperature preference (Deacon & Hecht, 1995), and the effect of salinity on the growth of juvenile grunter (Bussiahn, 1992). If these studies are included in the sequence of experimental determination of the environmental requirements of juvenile spotted grunter, the protocol translates to the following experimental sequence:

temperature → salinity → photoperiod → light intensity → feeding frequency → ration size.

This experimental sequence is in accordance with the theoretical protocol.

Generally, research on the environmental requirements of fish has been conducted on a strictly goal orientated basis with independent studies of the effects of one or two environmental factors which are considered important for the culture of a species. Investigations into further environmental requirements of the species are often conducted by different researchers under different experimental conditions at a later stage. Consequently, our knowledge of the influence of environmental factors on the growth of fish is a synthesis of the information on their independent effects on a multitude of species of different ages and under a variety of experimental conditions (Fry, 1971; Brett, 1979). Although a general pattern in the responses of fish to certain environmental factors, such as food intake and temperature, emerges from interpretation of this information, it still provides no clue as to the relative effect of environmental factors on the growth of a fish. The development of a protocol for the sequential determination of the optimum environmental requirements is, therefore, based mainly on assumptions. Many of these are unproven.

In this study, the evaluation of the effects of individual environmental factors on the growth of spotted grunter were conducted under similar experimental conditions. Although experiments were not undertaken simultaneously, the experimental procedures used to determine the effects of the individual factors were similar. Experiments were designed so that only one factor was varied during each experimental period. Otherwise, all factors remained at a constant level or at the pre-determined optimum. For example, after the effect of temperature on growth was determined in the first experiment all subsequent experiments were conducted at the optimum temperature for growth at 24.5°C. A salinity of 35‰ was used in the temperature experiment. In the following experiment on the effect of photoperiod, temperature was maintained at 24.5°C and salinity was kept constant at 35‰. Coincidently, the salinity, light intensity and photoperiod levels chosen for the initial experiment on temperature were discovered, in subsequent experiments, to be the optima for juvenile spotted grunter. Generally the experiments were conducted in the same experimental recirculating system and 40 L experimental aquaria (Chapter 2). In experiments where common recirculated water could not be used for all treatments, such as the salinity experiment (Chapter 5), the experimental conditions closely approximated that of the standard system. Water quality criteria such as pH, ammonia, nitrite and dissolved oxygen were kept constant at optimum levels during all experiments.

Statistical methods require simultaneous investigation of experimental variables for any multifactorial analyses. However, to conduct experiments of this nature when determining the environmental requirements is logistically almost impossible. An experiment of this scale is not feasible under laboratory conditions considering the numbers of experimental aquaria and test animals required for replicate treatments of each experimental variable for all the environmental factors which could influence growth. This study of the environmental requirements of juvenile spotted grunter was unique in that evaluation of the effects of individual environmental factors on the growth of juvenile spotted grunter were essentially conducted under the same experimental conditions. Therefore, although not strictly correct, this study nevertheless provides an opportunity to statistically compare and model the independent effects of environmental factors on the growth of fish on an equal basis.

The aim of this investigation was to rank the independent environmental factors according to their respective effects on juvenile spotted grunter. Then, using this information, to develop a protocol for the sequential determination of the optimum environmental requirements based on the experimental data.

Methods

Experimental data

In the growth trials to determine the effects of the individual environmental factors, length and weight data of growth were recorded weekly for each fish in each treatment. For the statistical analysis of the effects of the respective treatments within experiments all growth data were expressed as Specific Growth Rates (SGR). Specific Growth Rates were compared using a one-way ANOVA and differences among means were tested by applying Tukey's Multiple Range Analysis. Growth data were tested for differences within the replicates of experimental variables, before the respective variables were compared with each other. Furthermore, the data were also tested for differences in weekly growth rate to ensure that growth was constant through out the experimental period. Equality of variances among individual fish growth rates and replicates in experimental variables were confirmed with a Barlett's Test (Zar, 1984).

Comparison of experimental data

The specific growth rates of individual fish in each experiment were pooled and mean weekly growth rates were calculated for each replicate in the respective experimental variables. The mean weekly growth rates of all the variables in each of the experiments were then pooled. A matrix of dummy variables was constructed to facilitate the multiple regression analyses where Specific Growth Rate was the dependent variable and time, environmental factors and dummy variables were the independent variables. Comparison of experimental data was undertaken with a series of multiple regression analyses. The multiple regression analyses of the growth data was divided into phases. In the initial multiple regression analyses, the growth data of all the variables which did not contribute significantly to growth in the individual experiments were compared to each other. For example, all the ration size variables above maximum daily intake were compared to the salinity variables which did not influence growth i.e 12, 25 and 35‰ (Table 8.1). This was followed by a multiple regression analyses of all the variables which did have an effect on growth in the individual experiments. The experiment (e.g. all the ration size treatments) which had the highest significance level were then removed from the analyses and the regression was recalculated. This process of elimination was repeated until the contributions of the respective experimental variables did not differ significantly. In each regression analyses only the variables of one experiment were removed from calculation at any one time. In the multiple regressions where there was more than one experiment with variables with a highly significant P-value, a separate multiple regression analyses was calculated to establish which experimental variables had the dominant effect. The experiment with the most number of significant variables in the separate regression was removed from the next analyses.

Results and Discussion

Before comparing the effects of the various treatments within experiments, growth data was rigorously tested to ensure that the growth of fish within the replicates of the same treatments were not significantly different from one another. Homogeneity amongst the replicates was tested to confirm that there were no experimental artifacts influencing the growth of the fish in any one replicate. Weekly growth rates in each replicate were also compared to ensure that growth remained constant throughout the experimental period. Performing these tests provided a valid basis for pooling replicate treatments for comparison with the other experimental variables within the same experiment.

Rigorous testing of the experimental data was equally important for this investigation as the mean weekly growth of the individual fish in each replicate treatment was used for comparison between environmental factors. Mean weekly growth rates were calculated from the pooled individual growth rates of all fish within the same replicate. This compromise in statistical resolution was necessary due to the limitations of the statistical software used to perform the multiple regression analyses.

In the introduction to this study it was recognised that interpretation of the effect of an isolated environmental factor may lead to over-simplification, as interaction between factors is ignored. This can lead to the misinterpretation of results as certain dominant factors mask the effects of others (Fry, 1971). In the first multiple regression it was found that there were no significant differences in growth between environmental factors provided that they were at the respective optimum levels (Table 8.1). However, if limited (i.e. in the second multiple regression) ration size overwhelmed the effects of all other environmental factors. The lower ration sizes of 3 and 5% wet weight/day and the lowest feeding frequency treatment of 1x daily had a highly significant effect on the growth of juvenile spotted grunter (Table 8.1). Since food intake was restricted in these treatments, it can be concluded that limiting factors had the greatest impact on the growth of juvenile spotted grunter.

The dominant influence of these factors resulted in the reduction of the significance level of temperature. In the first regression, the P-value for temperature was 0.0367 (Table 8.1). Removal of the ration size and feeding frequency treatments (regression analyses 1 and 2) resulted in a decrease in the P-value to 0.0000 in the third multiple regression (Table 8.1). Therefore, although temperature appeared to have little effect on growth in the first regression it was found to have a highly significant effect when food intake was not restricted. Therefore, controlling factors are ranked after limiting factors in terms of their importance on the growth of juvenile spotted grunter.

The dominant effect of temperature over other environmental factors became apparent when all temperature treatments were removed in the fourth multiple regression analyses. In the fourth regression the 5‰ salinity treatment had a P-value of 0.0321, while in the previous regression the P-value was 0.0500 (Table 8.1). The increase in significance level of the 5‰ salinity in the fourth regression suggests that masking factors are subordinate to the effect of temperature. The removal of the salinity treatments from the fifth multiple regression resulted in an increase of the significance of the lowest light intensity treatment. The P-value of the lowest light intensity was decreased from 0.0632 to 0.0267 by the removal of the salinity treatments. As photoperiod and food deprivation were eliminated in the first multiple regression analyses the effects of light intensity were greater than those of photoperiod or food deprivation. The removal of the light intensity treatments had little effect on the significance levels of either the photoperiod or food deprivation treatments. The respective treatments were not significantly different even when the light intensity treatments were removed (Table 8.1). Therefore, a ranking of remaining experimental treatments could not be undertaken. However, the elimination of the light intensity treatments before food deprivation suggest that investigation into directive factors should precede examination of the effects of limiting factors on the growth of fish.

Through the process of elimination the experimental factors were removed in the following order:

ration size → feeding frequency → temperature → salinity → light intensity → photoperiod and food deprivation

If the experimental factors are classified into functional categories according to their metabolic effect it is possible to develop a sequential protocol based on the statistical ranking of the experimental data. The experimental treatments are classified as follows:

ration size → feeding frequency → temperature → salinity → light intensity → photoperiod and food deprivation
(limiting) (limiting) (controlling) (masking) (directive) (directive and limiting) .

Therefore, the statistical ranking of the independent effect of environmental factors results in the following protocol:

limiting factors →.controlling factors → masking factors → directive factors → limiting factors.

Table 8.1: The statistical ranking of the independent variables by the serial multiple regression analyses (1 - 6). The relative contribution of individual independent variables are tabulated as P-values.

Independent variables	**Multiple Regression Analyses**					
	1	**2**	**3**	**4**	**5**	**6**
Ration size (3%)		0.0000				
Ration size (5%)		0.0000				
Ration size (7%)		0.0049				
Ration size (10.5%)	0.7789					
Ration size (15%)	0.7896					
Feeding frequency (1x)		0.0197	0.0015			
Feeding frequency (2x)		0.0892	0.0089			
Feeding frequency (3x)	0.6785					
Feeding frequency (4x)	0.7034					
Feeding frequency (5x)	0.6932					
Temperature (20.5°C)		0.0367	0.0049	0.0000		
Temperature (22.5°C)		0.0497	0.0213	0.0114		
Temperature (24.5°C)		0.0967	0.0745	0.0678		
Temperature (26.5°C)						
Temperature (28.5°C)						
Salinity (5‰)		0.0919	0.0715	0.0500	0.0321	
Salinity (12‰)	0.2648					
Salinity (25‰)	0.35894					
Salinity (35‰)	0.4002					
Light intensity (low)		0.0937	0.0841	0.0716	0.0632	0.0267
Light intensity (medium)	0.3765					
Light intensity (high)	0.3990					
Photoperiod (8L:16D)	0.7539					
Photoperiod (12L:12D)	0.7819					
Photoperiod (16L:8D)	0.7899					
Food deprivation 2	0.6960					
Food deprivation 3	0.8245					
Food deprivation 4	0.7931					
Food deprivation 5	0.7733					

Chapter 9

Discussion

In the introduction to this study individual environmental factors were classified into five functional categories according to their respective influences on metabolic processes, namely controlling, limiting, masking, directive and lethal factors (Chapter 1). The effects of these factors were contextualised and a theoretical sequential protocol for the experimental investigation of the optimum environmental conditions of a species was developed (hereon referred to as the theoretical protocol). The sequence was as follows:

controlling factors → masking factors → directive factors → limiting factors.

This theoretical protocol was used as the basis for investigating the effect of individual environmental factors on growth of juvenile spotted grunter. The sequence of experimentation used in the present study, namely:

temperature	→	**photoperiod/light intensity**	→	**ration size**	→	**salinity**
(controlling factor)		(directive factors)		(limiting factor)		(masking factor)

may seem contrary to the theoretical protocol. However, this experimental sequence ignores the previous studies of temperature preference (Deacon & Hecht, 1995), and the effect of salinity on the growth of juvenile grunter (Bussiahn, 1992). If these studies are included in the sequence of experimental determination of the environmental requirements of juvenile spotted grunter, the protocol translates to the following experimental sequence:

temperature → salinity → photoperiod → light intensity → feeding frequency → ration size

This experimental sequence is in accordance with the theoretical protocol.

In Chapter 8, the individual environmental factors (temperature, salinity, etc) were independently ranked according to their respective effect on growth performance of juvenile grunter. Interpretation of this ranking results in the following protocol for the sequential determination of the optimum environmental requirements of a species (hereon referred to as the experimental protocol):

Limiting factors → controlling factors → masking factors → directive factors → Limiting factors.

The theoretical and experimental protocols are fundamentally different as effects of the lethal factors are either investigated first or last according to which sequence is used. The growth of fish in response to limiting factors involves dependent and independent states which become operational at different levels. Therefore, depending on the sequence used, the estimation of maximum growth may or may not be influenced by the effect of limiting factors. Since the experimental determination of the optimum environmental requirements of juvenile spotted grunter was conducted according to the theoretical protocol (i.e. limiting factors last), it could be hypothesised that the whole experimental determination was influenced by the effect of one or more limiting factors. For example, if food intake was unintentionally restricted by an inappropriate feeding regime, there may have been a concomitant decrease in the optimum temperature for growth. This has been observed in juvenile sockeye salmon (*Oncorhynchus nerka*) where the optimum temperature for growth decreases from 15°C on maximum ration to 5°C when ration is restricted to 1.5% weight/day (Brett *et al.*, 1969). It could, therefore, be argued that the growth performance of juvenile grunter was only optimised to the point where any one environmental factor became limiting.

In the introduction to this study (Chapter 1), it was rationalised that the optimization of limiting factors was not necessary in a protocol for determining the optimum environmental requirements of a species as the appropriate levels of these factors correspond to the maximum or minimum concentration of the respective factors. Therefore, in the experimental application of the theoretical protocol to determine the maximum growth of juvenile spotted grunter, it was assumed that effects of limiting factors did not affect growth. This assumption was tested in the investigations on the effect of food intake on growth performance (Chapter 6 & 7).

As the relationship between food intake and metabolism is the fundamental basis of fish growth (Fry, 1971; Elliot, 1975a; Brett, 1979), it is vital that food availability does not act as a growth limiting factor when testing the effect of non-food related parameters (Jobling, 1983). This implies that *ad libitum* access to food is virtually a prerequisite for eliminating potential biases arising from the feeding regime (Jobling, 1983). Therefore, the assumption in the experimental determination of the effects of environmental factors on juvenile grunter is that they were able maximise food intake. However, the provision of *ad libitum* access to food during experiments to determine the effects of non-food related parameters was not possible for juvenile spotted grunter. Consequently, it was necessary to compromise and adopt a satiation feeding regime of 3 times per day during the experiments. Numerous feeding frequency experiments have shown that the effect of feeding frequency is a function of ration size (Grayton & Beamish, 1977; Chua & Teng, 1978; Chapter 6). Food intake was, therefore, potentially limiting under the experimental conditions used to determine the effects of non-food related environmental factors. In the experimental determination of the effect of feeding frequency on growth it was found that juvenile spotted grunter consumed 14% wet body weight/day when fed 3 times daily (Chapter 6). The theoretical maximum ration, determined from the relationship between ration size and growth for this species, was estimated to be 10.7% wet body weight/day (Chapter 7). Therefore it can be concluded that food intake was not restricted by the feeding regime under the experimental conditions. Furthermore, the food consumption of juvenile spotted grunter fed to satiation 3 times daily was ± 4% (wet body weight/day) higher than the theoretical maximum daily ration.

Even though the juvenile spotted grunter were capable of consuming the maximum daily ration under the experimental conditions used for the determination of the optimum environmental requirements, this methodology is still questionable (Jobling, 1983). A satiation feeding regime still represents a compromise as it is unknown whether the growth rates achieved on a fixed satiation feeding regime are not always comparable to those with *ad libitum* access to food (Jobling, 1983). In Chapter 7 it was proposed that one of the differences between the two regimes is the interval between meals. Therefore, an experiment was conducted to investigate the effect of deprivation time on the growth of juvenile spotted grunter. The results revealed that there was no difference in growth performance between fish fed a maximum ration 2, 3, 4 or 5 times daily. It was concluded that deprivation time had no effect on growth performance implying that there is no difference between a satiation feeding regime and *ad libitum* feeding. This conclusion is consistent with the findings on juvenile summer flounder (*Paralichthys dentatus*), where the growth rate of fish fed at maximum daily ration and those fed *ad libitum* were not significantly different (Malloy & Targett, 1994).

In Chapter 6 it was found that the optimum feeding frequency for juvenile spotted grunter corresponded to feeding at an interval that was equivalent to one meal per total gut passage time. From this it was hypothesised that optimum feeding frequency may be directly related to the rate of digestion and that it should correspond to feeding at an interval that combines maximum digestion with minimum deprivation time. The optimum feeding interval corresponded to a level at which at least 90% of the previous meal had been digested. This implied that digestion must progress to its fullest extent before the next meal. The results of the effect of food deprivation on growth of juvenile grunter do not support this hypothesis as the decrease in food conversion at the optimum feeding frequency was not significantly different from higher feeding frequencies. However, the relationship between gut passage time and the minimum feeding frequency for maximum food intake provides a method of ensuring maximum food intake during the experimental determination of the optimum environmental requirements.

The statistical ranking of environmental factors for the development of the experimental protocol was based on their individual effects on the growth performance of juvenile spotted grunter

(Chapter 8). Therefore, the ranking also included experimental variables where food intake was restricted to below maximum daily intake. For example, food intake was unintentionally restricted to 8.2% of body weight/day when fish were fed once daily in the feeding frequency experiment. In the experiment to determine the relationship between growth and ration, food intake was restricted to 3, 5 and 7% wet body weight/day. Since food was not a limiting factor in the experimental determination of the effects of non-food related environmental factors, all treatments in which food intake was limited should be eliminated from the ranking. Therefore, the ranking of the environmental effects of feeding frequency and ration size should only be based on the treatments where food intake was not restricted to below maximum ration. In effect this was achieved when the experimental parameters used for the experiment to determine the effect of food deprivation were included in the statistical analyses. When the experimental factors where food intake was limited, such as feeding frequency and ration size, were removed from the statistical analysis, deprivation variables had the lowest ranking. Therefore, according to the new ranking the position of limiting factors in the experimental protocol was altered from first to last. Therefore, the modified experimental sequence would be as follows:

controlling factors → masking factors → directive factors → limiting factors.

Using food intake as an example, the position of limiting factors was altered from the most to least important factor in the experimental protocol sequence, depending on whether it is restricted or not. However, the statistical analyses excluded the possible effects of all the limiting factors, such as dissolved oxygen and toxic metabolites, which were not examined in this study. It is conceivable that any one of these factors could have the same effect as a restricted food intake. Nonetheless, the low ranking of food intake in the modified experimental protocol provides evidence in support of the assumption that the effect of limiting factors on growth are negligible provided that they are maintained at appropriate levels.

Although the effects of other limiting factors, such as dissolved oxygen and toxic metabolites, were not tested, they were not ignored. In the sequential determination of the optimum environmental requirements of juvenile spotted grunter, dissolved oxygen was maintained at 100% of saturation and ammonia and nitrite concentrations remained below 0.02 mg/L and 0.1 mg/L, respectively. These concentrations were based on the effect of the respective factors on growth of other juvenile fish. In juvenile striped bass (*Morone saxatilus*) dissolved oxygen levels between 53% and 60% restrict growth, however between 60 and 100% saturation growth is maximised (Chiba, 1988). A similar growth response has been observed in *Micropterus salmoides* (Stewart *et al.*, 1967), *Oncorhynchus kisutch* (Herrmann *et al.*, 1962) and *Cyprinus carpio* (Chiba, 1966), although the lower critical oxygen concentrations range from 50 to 60% saturation depending on the species. As dissolved oxygen levels were maintained above the levels considered critical for other juvenile fish, it is concluded that this factor did not limit the growth of juvenile spotted grunter under the experimental conditions used in this study.

The growth of fish in response to toxic metabolites is similar to that of oxygen, although in reverse. At low concentrations toxic metabolites have no effect on growth, but from a critical concentration their effect increases as the concentration of the metabolite increases. The critical ammonia concentration which affects growth varies according to species (Handy & Poxton, 1993). The chronic effects of ammonia on marine fish have only been measured in juvenile Dover sole (*Solea solea*) and Turbot (*Scophthalmus maximus*)(Alderson, 1979). Ammonia concentrations in excess of 0.1 mg/L were found to have a negative effect on growth of these species (Alderson, 1979). Juvenile marine fish are generally more tolerant of high nitrite concentrations (Handy & Poxton, 1993). Acute toxicity in juvenile marine fish varies from 980 mg/L (24h L.C. 50) in larval *Cynoscion nebulosus* (Daniels *et al.*, 1978) to 154 mg/L in juvenile *Dicentrarchus labrax* (Saroglia *et al.*, 1981). The chronic effects of nitrite on growth have not been investigated (Handy & Poxton, 1993), however, the minimum concentration for the formation of methaemoglobin is 12.9 mg/L in *Dicentrarchus labrax* (Saroglia *et al.*, 1981). Nitrite in the blood oxidises the ferrous (Fe^{2+}) ion in the haemoglobin to the ferric (Fe^{3+}) state

(Handy & Poxton, 1993). The resulting product, methaemoglobin, irreversibly combines with oxygen thus impairing oxygen transport (Handy & Poxton, 1993). The formation of methaemoglobin would, therefore, presumably affect growth (Handy & Poxton, 1993). Since ammonia and nitrite concentrations were well below levels considered critical for other juvenile marine fish, it is concluded that the respective factors had no limiting effect on the growth of juvenile spotted grunter under the experimental conditions used in this study.

In the theoretical protocol, it was hypothesized that investigation of controlling factors should precede all others in the sequential determination of the optimum environmental conditions of a species. Since temperature is considered to be the principal factor controlling the rate of metabolic processes (Fry, 1971; Brett, 1979), it was the first environmental parameter to be investigated in this study. Temperature was ranked as having the greatest effect on the growth of juvenile spotted grunter in the statistical ranking of the effects of the individual environmental factors (Chapter 8). Therefore, the hypothesis that controlling factors should be investigated first in the sequential investigation of optimum environmental conditions was accepted.

In the investigation of the thermal requirements of juvenile spotted grunter it was revealed that the optimum temperature for growth of 24.5°C fell within the zone of final preference of 24-25°C for the species (Deacon & Hecht, 1995). This result provides further confirmation of the correlation between the final thermal preferendum and the optimum temperature for growth (Jobling, 1981; Britz & Hecht, 1987). Temperature preference studies therefore provide a rapid and valid method of determining the optimum temperature for growth (Jobling, 1981; Deacon & Hecht, 1996). Investigations were also conducted to evaluate the CTMax method for determining the final preferendum (Tsuchida, 1995). However, it was concluded that the CTMax studies could not substitute temperature preference studies in the determination of the final preferendum as the CTMax methodology relies on an independent estimate of the final preferendum for further data analyses. Substitution of the appropriate CTMax or temperature preference data into Tsuchida's (1995) relationship between CTMax and final preferendum, revealed that the relationship could provide confirmation of the final preferendum in several fish species (Chapter 3). Therefore, the CTMax method provides a corollary test of the final preferendum on purely physiological criteria (Becker & Genoway, 1979).

The importance of accurate assessment of optimum temperature in the determining the effects of environmental factors is well illustrated by comparing previous studies into the effect of salinity on growth of juvenile spotted grunter to those of the present study (Bussiahn, 1992; Chapter 5). The first experiment tested the effect of three salinities, 5, 15 and 35‰, on the growth of juvenile spotted grunter at a temperature of 17°C (Bussiahn, 1992). From this experiment (*op cit*) it was concluded that salinity had no effect on growth. However, in the present study it was found that salinity had a significant effect on the growth of juvenile spotted grunter when tested at the optimum temperature (Chapter 5). Growth performance of juvenile spotted grunter was found to decrease when the fish were exposed to a reduced salinity of 5‰ at a temperature of 24.5°C. The differences between the two studies can be interpreted in two ways. Firstly, it could be argued that in Bussiahn's (1992) study low salinity had a mitigating effect on metabolism and, therefore, masked the effect of sub-optimum temperature on growth. Alternatively it could be hypothesised that the effect of salinity on growth is subordinate to that of temperature due to the controlling effect of temperature on metabolism. The overriding effect of temperature on growth, therefore, masks any effect that salinity might have.

Masking factors are similar to limiting factors in that they have a moderating effect on metabolism which results in a lower measure of maximum growth. Consequently it was hypothesised that the effect of masking factors should be investigated before assessing the effects of directive factors. By implication, masking factors are, therefore, investigated after controlling factors in the theoretical protocol. The above example, however, illustrates the paradox between controlling and masking factors. According to the theoretical protocol the effects of masking factors on growth should be subordinate to those of controlling factors. This deduction was correct. In the statistical ranking of the independent effects of environmental factors on the growth of juvenile spotted grunter, temperature was ranked higher than salinity. Observations on the effect of salinity on thermoregulatory behaviour also support this deduction. Deacon and Hecht (1995) found that the final thermal preferendum of juvenile spotted grunter was not influenced by a variation in salinity which indicates that temperature is more important for controlling metabolism than salinity. Therefore, the optimum salinity of 12-35‰ determined at the optimum temperature in the present study is an accurate reflection of the salinity requirements of juvenile spotted grunter (Chapter 5).

The optimum salinity range of 12-35‰ of juvenile spotted grunter supports the hypothesis that the optimum salinity of a species usually corresponds with the salinity levels in the environment in which the species is commonly found (Morgan & Iwama, 1991). In the natural habitat juvenile spotted grunter are exposed to salinity changes that regularly fluctuate between 0-35‰ (Ter Morshuizen *et al.*, in press). Under extreme conditions, for example in closed estuaries, spotted grunter survive in salinities of up to 70‰ (Whitfield *et al.*, 1981). Therefore, the lack of a clearly defined optimum salinity for growth in juvenile spotted grunter may indicate adaptation to an unstable natural environment. In contrast, stenohaline freshwater species such as grass carp (*Ctenopharyngodon idella*) and freshwater catfish (*Mystus vittatus*) exhibit a narrow range of optimum salinity (0-2‰) for growth (Arunachalam & Reddy, 1979; Kilambi, 1980). This suggests that salinity may not be an important factor in the investigation of the environmental requirements of stenohaline marine species.

Optimum metabolism requires the synchronization of metabolic and physiological processes (Brett, 1979). Light intensity and photoperiod have been implicated as the key directive environmental factors synchronizing the activity and behaviour of fish (Fry, 1971; Schwassmann, 1971; Reynolds & Casterlin, 1976). In this study photoperiod was investigated before light intensity. This was based on the assumption that the dominant factor influencing the daily activity pattern is the periodicity of light (Schwassmann, 1971), while the locomotor activity within this pattern is determined by the ambient light intensity (Reynolds & Casterlin, 1976). Therefore, it was hypothesised that the period of activity would have to be determined before light intensity could be optimised. Consequently, the aim of the photoperiod experiment was to determine whether spotted grunter are diurnal or nocturnal during the early juvenile phase. Some marine fish, such as *Gilbertia sigalutes*, are diurnal during the larval phase but become nocturnal as they develop into juveniles (Marlivae, 1981). Generally diurnal fish grow better under longer periods of light (Gross *et al.*, 1965; Hu *et al.*, 1976), while nocturnal fish exhibit better growth under longer or even continual periods of darkness (Britz & Pienaar, 1992). Once the photoperiod response of the species has been determined, investigation into the appropriate light intensity can be undertaken.

In the ranking of the independent effect of environmental factors on growth, light intensity was ranked higher than photoperiod (Chapter 8). This implies that in the experimental sequence light intensity should be investigated before photoperiod. This conclusion is supported if the independent effects of light intensity and photoperiod on juvenile spotted grunter are contextualised. In the light intensity experiment, the optimum light intensity for growth, food conversion, condition factor and survival of juvenile spotted grunter under a photoperiod of 12L:12D was 1.94×10^{-3} μE.sec^{-1}. cm^{2} (Chapter 4). By chance, this light intensity was used in the photoperiod experiment. Theoretically, therefore, growth should have been maximised at a photoperiod of 16L:8D. However, although maximum growth did occur at 16L:8D, it was not significantly higher than that observed at 12L:12D (Chapter 4). On the other hand, fish under the 16L:8D photoperiod exhibited a lower condition factor and food conversion ratio than those at 12L:12D. It was concluded that modified photoperiods do not increase growth in juvenile spotted grunter. Kiyono & Hirano (1981) observed that even when juvenile black porgy (*Mylio macrocephalus*) were fed *ad libitum*, growth did not increase when photoperiod was modified from 12L:12D to 18L:6D. A similar lack of response to increased photoperiod has also been observed by Fuchs (1978) in juvenile sole (*Solea solea*). It can be concluded that the investigation of the effect of photoperiod is not as important as previously thought in the determination of the optimum environmental requirements of juvenile marine fish.

The investigation of the effect of light intensity on the growth of juvenile spotted grunter was pre-empted by a light intensity preference study (Burton, 1995). Radenko and Alimov (1992) found that the preferred light intensity of larval carp corresponded well with the optimum light intensity for growth. It was, therefore, hypothesised that light intensity preference may represent a method of determining the light intensity requirements of juvenile fish (Burton, 1995). In this study (*op cit.*) juvenile spotted grunter consistently selected the darkest region of the gradient. This behaviour was observed even when the darkest region of the gradient differed by <1 lux (Burton, 1995). However, in the growth trial investigating the effect of light intensity on juvenile spotted grunter, the lowest light intensity of 1.94×10^{-3} μE.sec^{-1}. cm^{2} (<5 lux) resulted in poor growth, condition factor and survival (Chapter 6). It was concluded that behavioural studies are unreliable indicators of preferred light intensity.

In Chapter 6 it was observed that the quality, or wavelength spectrum, of light may be an important factor in the investigation of the effect of light intensity on the growth of a species. This is particularly important when experiments are conducted under artificial light. The artificial light used in the investigation of the environmental requirements of juvenile spotted grunter provided a wavelength spectrum which had spectral peaks in the range of 540 and 620nm. This spectrum encompasses most of the wavelength range to which fish visual pigments have been found to be most sensitive, however the shorter wavelengths were not provided by this light source. In fish such as perch (*Perca fluviatilis*) and the black porgy (*Acanthopagrus schlegeli*), which commonly inhabit poor photic conditions additional peak spectral absorbencies also occur in the 550-680nm ranges (Cameron, 1982; Huang, 1989). Since juvenile spotted grunter in the size range used in this experiment are found in highly turbid water it was assumed that they also possess retinal pigments required for sight under poor photic conditions. This hypothesis was, however, not tested. Most fish retinal pigments have a peak spectral absorbance of 500nm (Hobson *et al.*, 1981; Huang, 1989; Loew *et al.*, 1993). Larval fish retinae have maximum absorbance at a lower wavelength 400nm (Loew *et al.*, 1993). This suggests that the influence of wavelength spectra on fish growth should be investigated in the experimental determination of optimum environmental requirements.

Although the sequence of experimental investigation into the effects of photoperiod and light intensity were incorrect according to the statistical ranking, the overall effect of directive factors on growth was less than that of the masking factors. Sequencing of directive factors after masking factors is, therefore, a valid assumption. The effects of light intensity and photoperiod on the growth of juvenile grunter were ranked above the effect of food deprivation. Therefore limiting factors are ranked last in the experimental protocol, provided food intake is not restricted. The implications of this finding have been explained in previous discussions.

The experimental protocol, therefore, supports the theoretical protocol for sequentially determining the environmental requirements of juvenile marine fish. Consequently, it can be concluded that the determination of the optimum environmental requirements of a candidate mariculture species should be conducted according to the following protocol:

controlling factors → masking factors → directive factors → limiting factors.

The investigation into the effects of limiting factors have been included in the protocol as the determination of the optimum levels of controlling, masking and directive provide a platform upon which research into the effects of limiting factors can be conducted. Food is the most important cost factor in finfish culture and under commercial farming conditions food intake is intentionally restricted. From an economic perspective, a correct food ration must fulfill a number of criteria. It must promote assimilation of dietary protein to muscle rather than lipid storage, support a high survival rate and maintain a high food conversion efficiency, without limiting growth. Maximum growth occurs at the limit of voluntary food intake (satiation), while maximum food conversion efficiency occurs at some level below satiation (Andrews & Stickney, 1972; Brett el al., 1969; Chua & Teng, 1982). The optimum ration size is a compromise between these factors and is defined as the ration providing the highest growth rate for the lowest intake of food (Brett *et al.*, 1969; Elliot, 1975a). For *Epinephelus tauvina* and *Seriola quinqueradiata* the optimum ration is approximately 65% of maximum intake (Chua & Teng, 1982). In juvenile spotted grunter the optimum ration size is much lower, at 33.6% of maximum daily intake. The determination of the optimum ration size under optimum environmental conditions also provides a basis upon which other factors which limit growth, such as stocking density (Brett, 1979), can be investigated.

In Chapter 1 it was proposed that this study would attempt to determine whether the optimum environmental requirements of a candidate species should be determined in a particular sequence, and, if so, in what sequence? Based on the results of the independent experiments and interpretation of the findings it is clear that the optimum environmental requirements should indeed be determined in a particular sequence. If not, then the experimental protocol would not have corroborated the theoretical protocol. Therefore, using the protocol as a basis, a preliminary experimental sequence for determining optimum environmental requirements of a species can be developed. In the development of the experimental, the protocol was interpreted in conjunction with the experimental methods used to determine the environmental requirements of juvenile spotted grunter.

Figure 9.1 represents a preliminary protocol for determining the environmental requirements of juvenile marine fish. Temperature was ranked as the primary controlling factor affecting the growth performance of juvenile spotted grunter and, therefore, has priority over all other factors. A combination of temperature preference and CTMax studies together constitute the most rapid and reliable means of determining the optimum temperature for growth. The strong correlation between the zone of final preference and optimum temperature for growth (Jobling, 1981), was further supported by the present study. CTMax studies have been included in the protocol as they measure the response to temperature on a purely physiological basis and therefore provide an independent method of validating the final preferendum (Bekker & Genoway, 1979; Tsuchida, 1995) (Chapter 3). The non-growth based evaluation of optimum temperature provided by CTMax and temperature studies is vital as it is independent of the effect of food.

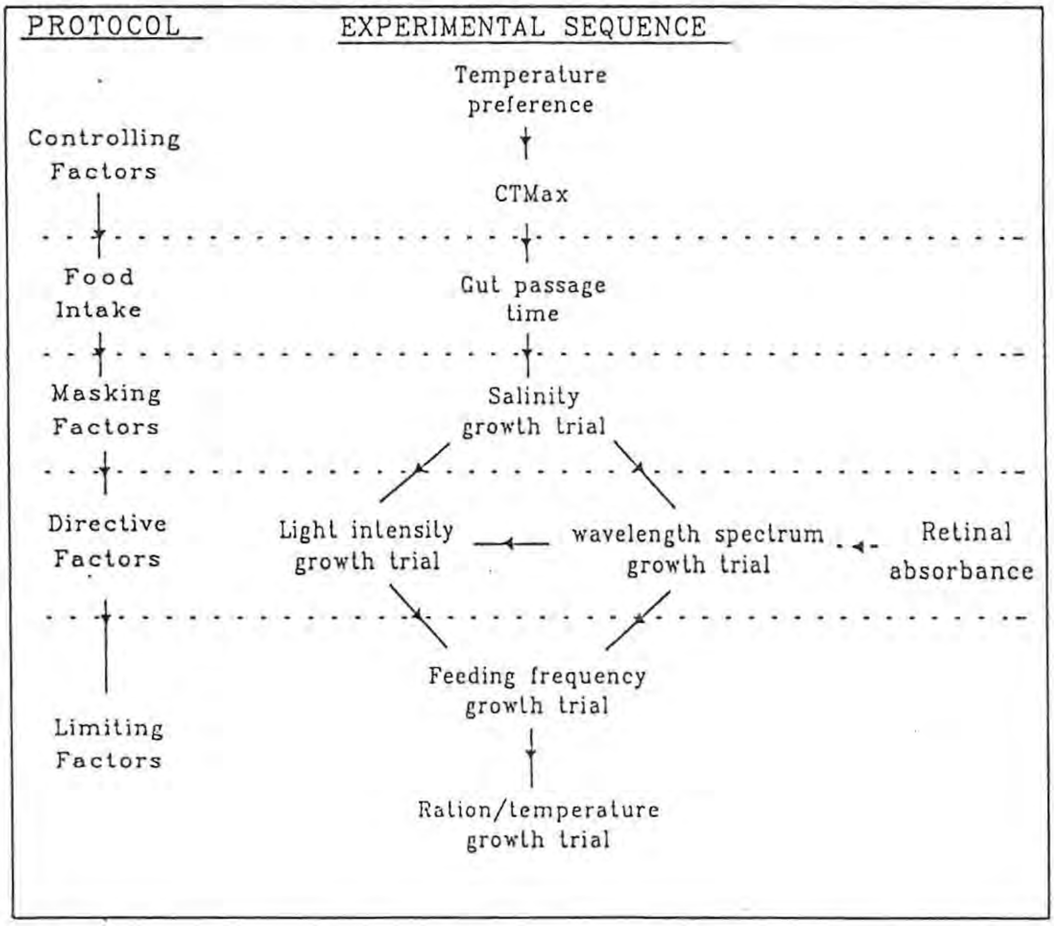

Figure 9.1: The preliminary protocol and sequence of experimentation for the determination of the optimum environmental requirements of juvenile marine fish.

In this respect, growth, unlike other activities, such as temperature preference, is inseparably coupled with food intake. Food intake is, therefore, a potential limiting factor (Fry, 1971; Brett, 1979). The relationship between gut passage time and optimum feeding frequency established in the experiment on the effect of feeding frequency on growth (Chapter 6), however, provides a possible method of ensuring that food is not limiting. Feeding to satiation at an interval that is equivalent to gut passage time ensures that maximum daily intake is achieved (Chapter 6 & 7). This feeding regime, for practical and theoretical reasons, is superior to the only alternative of feeding *ad libitum* (Chapter 7). Therefore, the next step in the protocol would be an investigation into gut passage time conducted at the preferred (optimum) temperature (Fig 9.1). In the protocol outlined in Figure 9.1, food intake has not been classified according to a functional category in the first instance. This is because the study of gut passage time is not designed to optimize food intake, but rather to ensure that it is not restricted. Provided food intake and other limiting factors, such as dissolved oxygen and toxic metabolites, are at appropriate levels they are assumed to have no effect on growth in subsequent growth trials. Investigations into limiting factors are undertaken last in the protocol. In these experiments they are intentionally restricted.

Determination of the optimum temperature and feeding regime for maximum daily food intake serves as a basis upon which all growth trials can be conducted. The effects of masking factors may act as confounding variables resulting in biased estimates of optimum levels of other factors. In juvenile spotted grunter, salinity proved to be the second most important factor regulating survival, growth and food conversion (Chapter 8). Since the majority of potential mariculture species are euryhaline, an investigation into the effects of this factor would be the consecutive step in the protocol (Fig 9.1). However, this may not be relevant to stenohaline marine species.

Investigation into the effect of masking factors is followed by an examination of directive factors. Light has been identified as the principal directive factor controlling the endogenous cycles of metabolism in fish and other organisms (Fry, 1971; Schwassmann, 1971; Reynolds & Casterlin, 1976). In juvenile spotted grunter, alteration of the photoperiod component of light had no beneficial effects on growth performance even when conducted at the optimum light intensity (Chapter 4). From this it was concluded that determining the effect of photoperiod is unnecessary. Therefore it has been omitted from the protocol (Fig 8.1). Light intensity and wavelength spectrum may have a behavioural effect on activity and, therefore, indirectly growth (Chapter 4). Light intensity had a significant effect on growth performance in juvenile grunter, however, this did not correspond to the preferred light intensity (Chapter 4). Behavioural studies of light intensity do not appear to be reliable indicators of the light requirements of all species and as a consequence are excluded from the protocol (Fig 9.1). Although the quality (i.e wavelength spectrum) of light has been found to influence behaviour its effect on growth in fish is not well documented (Loew *et al.*, 1993). Absorbance of retinal pigments may provide some clue into wavelength spectrum requirements (Chapter 4). Although this aspect was not investigated in the present study, the quality of light may represent an important abiotic factor affecting the growth of fish.

Under commercial conditions every effort is made to optimise utilisation. This implies that food intake must be limited (Andrews & Stickney, 1972; Chua & Teng, 1982). However, an accurate estimate of maximum ration is inherent to modelling of the relationship between growth and ration. This can only be achieved if the feeding regime permits maximum daily food intake. Since frequency of feeding controls daily food intake (Chapter 6), it must precede any investigation of the relationship between growth and ration (Fig 8.1). However, analyses of food consumption at the various feeding frequencies indicated that an accurate estimate of maximum ration cannot be determined from a feeding frequency experiment *per se*. Maximum ration was consistently overestimated by ± 4% at the higher feeding frequencies. In the present study the relationship between growth and ration in juvenile spotted grunter was determined at the optimum temperature for growth. Normally the relationship would be conducted to determine the optimum ration for commercial application (Brett *et al.*, 1969; Elliot, 1975a; Chua & Teng, 1982). Since the optimum ration has been found to vary according to temperature (Elliot, 1975; Brett, 1979), the relationship between growth and temperature should be determined over a range of temperatures (Fig 8.1). Maintenance of water temperatures at optimal levels is seldom practical or possible under hatchery conditions.

In the introduction to this study it was stated that the estimation of growth performance under a random combination of environmental conditions is a highly biased evaluation of the mariculture potential of a species. Maximum growth is the only measure of fish growth where the compromising effects of environmental factors on growth performance are negated. Since maximum growth only occurs under optimum environmental conditions, an investigation into the environmental requirements of a species is a prerequisite to evaluation of its mariculture potential. This study has shown that the sequence in which the effects of environmental factors are determined is important to the accurate assessment of the optimum environmental conditions. Therefore, the environmental factors influencing growth need to be identified and sequenced accordingly. From the interpretation of the independent effects of the environmental factors on the growth of juvenile spotted grunter and the sequence of experimentation it was possible to develop a preliminary protocol for the sequential determination of the optimum environmental requirements of a species. This was as follows:

controlling factors → Food → masking factors → directive factors → limiting factors.

At this point it must be emphasised that the environmental requirements of fish are species specific. Other specific environmental factors should, therefore, be considered within the framework of the protocol when investigating different species. For example turbidity may be a very important factor to a clear water predator. In this example, turbidity would restrict food intake and, therefore, would be classified as a limiting factor. Investigation of the growth limiting effect of this environmental factor would be undertaken at the end of the protocol (Fig 9.1). However, clear water would be assumed to be a critical requirement in the examination of the relevant controlling, masking and directive factors.

Table 9.1 is a summary of the optimum environmental conditions for growth of juvenile spotted grunter as determined according to the protocol. Under these conditions juvenile spotted grunter (SL= 50.2 ± 2.9mm, weight = 4.23 ± 0.75g) achieved a maximum specific growth rate of 2.36 ± 0.3 % body weight/day and a minimum food conversion ratio of 2.26 ± 0.4 at the optimum ration size of 3.6% wet body weight/day. Undoubtedly there are other specific factors, such as the nutritional requirements, which may affect the growth of juvenile spotted grunter. Optimisation of the key environmental factors provides the fundamental basis upon on which further research into these aspects can be conducted.

Table 9.1: The suite of environmental conditions required for optimum growth of juvenile spotted grunter, *Pomadasys commersonnii*, under culture conditions.

Environmental Factor	**Optimum Level**
Temperature	24 - 28°C
Salinity	12 - 35‰
Photoperiod	natural
Light intensity	$1.95\times10^{-3}\mu E.sec^{-1}$
Feeding frequency	3x daily
Ration size	3.6% wet body weight/day

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Appendix 1: A list of the marine fish species currently cultured on a commercial basis or are under consideration for aquaculture.

Family & Species	Euryhaline	Estuarine	Source
Chanidae			
Chanos chanos	y	y	Ferraris *et al.*, 1978
Gadidae			
Gadus morhua	y	n	Lambert *et al.*, 1994
Anarchichas minor	n	n	Tilseth, 1993
Anarchichas lupus	n	n	Tilseth, 1993
Serranidae			
Cromileptes altivelis			Liao, 1993
Dicentrarchus labrax	y	y	Buchet & Hussenot, 1993
Epinephelus akaara	y	y	Woo & Wu, 1982
Epinephelus malabaricus *(Epinephelus salmoides)*	y	y	Chua & Teng, 1982
Epinephelus striatus	y	y	Tucker, 1994
Plectropomus leopardus	n	n	Tucker, 1994
Haemulidae			
Pomadasys commersonnii	y	y	Deacon & Hecht, 1995
Pomadasys hasta	y	y	Liao, 1993
Latrididae			
Latris lineata	y	y	Riker, 1995
Lutjanidae			
Lutjanus argentimaculatus	y	y	Liao, 1993
Ocyurus chyrsurus	y	y	Riley *et al.*, 1995
Sparidae			
Acanthopagrus berda	y	y	Samiel & Matthews, 1987
Acanthropagrus bifasciatus	y	y	Samiel & Matthews, 1987
Acanthropagrus cuvieri	y	y	Samiel & Matthews, 1987
Acanthropagrus latus	y	y	Samiel & Matthews, 1987
Acanthropagrus schlegli	y	y	Liao, 1987
Archosargus probatocephalus	y	n	Tucker, 1987
Argyrozona arygyrozona	n	n	Davis, 1996
Cheimerius nufar	n	n	Garrrat, 1991

Family and species	Euryhaline	Estuarine	Source
Sparidae (continued)			
Chrysoblephus laticeps	n	n	Davis, 1996
Chrysoblephus puniceus	n	n	Garrat, 1991
Chrysophrys auratus (Chrysophrys major, Pagrus auratus, Pagrus auratus)	y	n	Woo & Fung, 1981
Dentex dentex	n	n	Tibaldi *et al.*, 1996
Diplodus sargus	y	y	Divanach *et al.*, 1993
Lithognathus lithognathus	y	y	Harris *et al.*, 1991
Mylio macrocephalus	y	y	Woo & Wu, 1982
Puntazzo puntazzo	y	y	Divanch *et al.*, 1993
Sparus aurata	y	n	Tandler *et al.*, 1995
Sparus sarba	y	y	Woo & Kelly, 1995
Lethrinidae			
Lethrinus russelli	y	y	Liao, 1993
Dichistidae			
Dichistius capensis	n	n	van der Lingen, 1986
Kyphosidae			
Kyphosus lembus	y	y	Liao, 1993
Gerridae			
Gerres filamentosus	y	y	Liao, 1993
Sciaenidae			
Argyrosomas hololepidotus	y	y	Thorpe, 1991
Cynoscion nebulosus	y	y	Daniels *et al.*, 1978
Micropogonius furnieri	y	y	Isaac, 1988
Pogonius chromis	y	y	Jones & Strawn, 1986
Sciaenops ocellatus	y	y	Craig *et al.*, 1995
Carangidae			
Pseudocaranx dentex	y	y	Arakawa *et al.*, 1993
Trachinotus carolinus	n	n	McMaster, 1988
Trachinotus falcatus	n	n	McMaster, 1988
Trachinotus goodei	n	n	McMaster, 1988
Seriola dumerili	n	n	Crespo *et al.*, 1994
Seriola quinqueradiata	n	n	Shimeno *et al.*, 1985

Family and Species	Euryhaline	Estuarine	Source
Coryphaenidae			
Coryphaena hippurus	n	n	Szyper, 1991
Mugilidae			
Chelon labrosus	y	y	Cardona & Castello-Orvay, 1993
Mugil carema	y	y	Bennett & Netto, 1991
Mugil cephalus	y	y	Liao, 1993
Mugil liza	y	y	Bennet & Netto, 1991
Liza aurata	y	y	Shusmin, 1990
Liza parsia	y	y	Paulraj & Kiron, 1988
Polynemidae			
Polydactylus sexifilus	y	y	Szyper *et al.*, 1991
Polynemus plebeius	y	y	Liao, 1993
Siganidae			
Siganus canaliculatus	y	y	El Sayed *et al.*, 1995
Siganus guttatus	y	y	Duray & Kohno, 1988
Siganus randalli	y	y	Nelson & Wilkins, 1994
Pleuronectidae			
Hippoglossus hippoglossus	n	n	Tuene & Norveldt, 1995
Hippoglossus stenolepis	n	n	Liu *et al.*, 1993
Paralichthys dentatus	y	y	Bengston & Nardi, 1995
Paralichthys microps	y	y	Silva, 1994
Paralichthys olivaceous	y	y	Takizawa *et al.*, 1996
Pleuronectes americanus	n	n	Lee & Litvak, 1996
Pleuronectes platessa	n	n	Tilseth, 1993
Rhombosolea taparina	y	y	Hart & Purser, 1995
Solea senegalensis	y	y	Vazquez *et al.*, 1994
Solea solea	y	y	Buchet & Hussenot, 1993
Bothidae			
Scopthalmus maximus	n	n	Person-Le Ruyet, 1990
Tetradontidae			
Fugu rubripes	y	y	Liao, 1993

List of Publications

Deacon, N. & Hecht, T. 1995. Observations on the thermoregulatory behaviour of juvenile spotted grunter, Pomadasys commersonnii (Pisces: Haemulidae). *J. Applied Ichthyol.* 11: 100-110.

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