FOOD AND FEEDING PATTERNS OF THE
# SOUTHERN BROWN SHRIMP Penaeus subtilis
PÉREZ-FARFANTE, 1967 (CRUSTACEA, PENAEIDAE)

ALBERTO JORGE PINTO NUNES

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SOUTHERN BROWN SHRIMP *Penaeus subtilis* PÉREZ-FARFANTE, 1967 (CRUSTACEA, PENAEIDAE)

BY

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BIOLOGY

FACULTY OF SCIENCE

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

OCTOBER, 1999

ST. JOHN'S   NEWFOUNDLAND

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ABSTRACT

Penaeid shrimp farming constitutes the most important aquaculture sector in economic value. In recent years however, the activity has faced slower progress in production despite a rapid intensification of production methods. The description and quantification of the food and feeding patterns of cultured penaeids may improve resource-use efficiency and conserve inputs critical to the sustainability of the activity.

This thesis investigated the food and feeding patterns of *Penaeus subtilis* under laboratory-controlled and culture conditions. Video-recording of shrimp feeding behaviours was used to examine food handling efficiency and size selectivity. Shrimp feeding levels in response to food dispersal method were determined through quantitative analysis of stomach contents and stable carbon isotope mass spectrometry. The abundance of polychaetes was used to study the effects of shrimp predation, stocking density and supplemental feeding. Partial integration of data was carried out using STELLA® II.

Results indicated that *Penaeus subtilis* feed manipulation was inversely related to food particle size, with large pellets being less preferred than small ones. Within the feed size range examined, shrimp size had no significant effect on handling efficiency. Feed broadcasting was a more effective method in regards to shrimp food intake, resulting in a greater access and a higher consumption of food among the cultured shrimp population, a lower number of empty stomachs and a greater occurrence of feed in *P. subtilis* diet. Ingestion of food was a function of shrimp body weight. Feeding intensity increased

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progressively with shrimp size, but inversely in percentage terms. Foregut clearance rates peaked 3 h after food recovery, while the bulk of faeces was produced within 1 h. Polychaete abundance was affected by higher shrimp stocking densities. Artificial feeding promoted higher polychaete levels, although was not capable of alleviating shrimp grazing pressure at increased stocking densities.

Results indicated that crumbles and broken pellets may be more advantageous in the culture of *Penaeus subtilis*. Feeds should be broadcast evenly over the culture area and administered regularly at continually reduced amounts. Rations should vary in accordance to estimates of *P. subtilis* body weight and account for the initial polychaete abundance and shrimp stocking densities.

ACKNOWLEDGEMENTS

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This thesis contains material published in peer-reviewed journals, magazines and conference proceedings. Portions of Chapter 1 were published in World Aquaculture (1998, 29: 27-37). A preliminary and modified version of Chapter 2 was published in the Boletim do Instituto de Pesca, (1997, 24: 11-20). Chapters 2 and 3 as they stand, were published in the Marine and Freshwater Behaviour and Physiology (1998, 31: 193-213) and in the Journal of the World Aquaculture Society (1999, 30: 331-348), respectively. Chapters 3 and 4 were presented at the Aquaculture Brazil'98 Meeting (Recife, Brazil), with abstracts published in the conference proceedings. Chapter 4 has been accepted for publication in the journal of Aquaculture. Both Chapters 5 and 6 are currently under review in the journals of Aquaculture and Aquacultural Engineering, respectively.

To Catia

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LIST OF ABBREVIATIONS AND SYMBOLS

ξ	dimensionless fraction from Table 6.3 at time i
λ	dimensionless fraction generated by $Se/100$
ω	dimensionless value given by $Rg + 1$
ψ	polychaete individual dry weight given as 0.7×10^{-3} g
$\propto$	statistical level of significance
$\delta^{13}C_a$	$\delta^{13}C$ of artificial food
$\delta^{13}C_n$	$\delta^{13}C$ of natural food (represented by benthic polychaetes)
$\delta^{13}C_{sc}$	$\delta^{13}C$ of shrimp stomach contents
μm	micron
A	total area
AC	number of attempts (successful or unsuccessful) to capture feed particles
a.d.	anno domini
ANOVA	Analysis of Variance
AR	mean appetite revival rate
ARI	mean appetite revival rate index
Broad	feed dispersal treatment
BW	shrimp wet body weight
C	centre

G_2	juvenile shrimp of a specific body weight range
G_3	pre-adult shrimp of a lower body weight range
G_4	adult shrimp of a higher body weight range
GE	gastric evacuation rate
h	hour(s)
ha	hectare(s)
IER	ingestion efficiency ratio
Ip	average daily percentage increase in polychaete abundance
IR	mean ingestion rate
IRI	mean ingestion rate index
kg	kilogram(s)
L	left
L	litre(s)
LD	light and dark
M	management
MANOVA	Multiple Analysis of Variance
MCI	manipulation capacity index
mg	milligram(s)
min	minute(s)
MM	mean maximum meal
mm	millimetre(s)
MMI	mean maximum meal index

n	number
N	total number of individuals sampled from a specific shrimp size group sampled
N	neutral
N'	total number of enclosures
NFNS	control enclosures without feed supply and shrimp
no	number
N_p	number of enclosures with each specific polychaete family
Np	number of polychaetes preyed per shrimp BW
Npm	number of polychaetes grazed per m^2
NR	number of shrimp alive per total culture area
Nsm	changes in number of shrimp per m^2
P	degree of significance
p	total number of each specific polychaete family
P'	total number of polychaetes observed in all samples
Ps	total shrimp population size per culture area
P_1	feed crumbles
P_2	broken feed pellets
P_3	pellets
PB	polychaete biomass
Pc	dimensionless fraction of polychaete contribution to shrimp's diet
PC	number of feed particles conducted successfully to the pre-oral cavity

Pd	polychaete population per m^2 after shrimp predation
PD	polychaete density
pers. obs.	personal observation
PI	number of movements performed by the mouthparts which may have led to ingestion of food placed successfully in the pre-oral cavity
PL	shrimp post-larvae
*P*max	maximum output value
*P*min	minimum output value
PSW	total shrimp population stomach weight
PVC	polyvinyl chloride
r	coefficient of statistical correlation
R	feed ratio[1]
R	right
Rg	polychaete daily population factor
Ri	amount of food ingested by shrimp after food administration
Ro	relative occurrence of carbon from artificial food
s	second(s)
S	percentage shrimp survival
S	south
s.d.	standard deviation
s.e.	standard error
Se	change in the degree of gastric evacuation

SG	shrimp growth
SI	sensitivity index
SNF	enclosures with shrimp and without feed supply
SW	shrimp stomach weight
t.	time
T_{0-4}	time between first detection of food up to 4 min
T_{4-8}	interval between 4 to 8 min after first food detection
Tf	total amount of feed consumed
T_i	time (i) after initial food exposure
Tr	total amount of food administered to shrimp
t-test	Student *t*-test
W	watts
W	west
W_C	shrimp stomach food contents wet weight
W_{ES}	shrimp stomach wet weight, without food contents
W_S	shrimp stomach wet weight, including existing food contents

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CHAPTER 1

GENERAL INTRODUCTION

1.1 General Considerations

Aquaculture is the fastest growing food production system in the world, expanding at an estimated annual rate of 10% since 1984 (FAO, 1997). In 1997, the industry contributed with 27.6% of the global aquatic output, generating 36.0 million mt of more than 300 species of finfish, shellfish and aquatic plants valued at US$ 50.3 billion (FAO, 1999). Despite its low overall volume representation (only 2.6%), penaeid shrimp[1] is the most important cultured group in monetary terms, accounting alone in 1997 for 12.0% or US$ 6.1 billion of the total estimated value generated by the aquaculture sector (Figure 1.1).

Historically, cultivation of marine shrimp originated thousands of years ago in the Mediterranean region (Brown, 1983) and to the 15th century a.d. in Indonesia (Ling, 1977). Nowadays, the activity has modernised and is now established in over 50 countries

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[1]The name "shrimp" is used here to denote the families Penaeidae and larger Palaemonidae (Holthuis, 1980), which occur in marine, estuarine and fresh waters. Penaeidae taxonomic classification adopted in this work in accordance to Pérez-Farfante (1988) and Dall *et al.* (1990). A recent change in nomenclature has been proposed for the Penaeoidean shrimp (Pérez-Farfante and Kensley, 1997), but it has not yet been widely accepted.

1
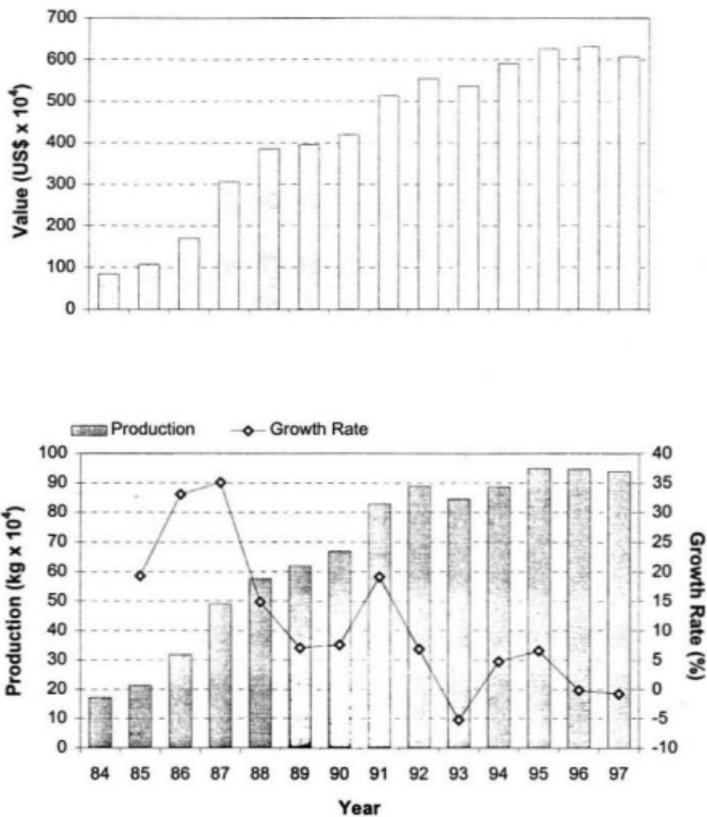
Figure 1.1: Annual estimated value (US\$ x 10^4), annual production (kg x 10^4) and annual compounded growth rate (%) of the penaeid shrimp farming industry since 1984. Data compiled from FAO (1999).

(Rosenberry, 1998) located in both tropical and sub-tropical areas around the globe. With its increasing demand and economic value (Figure 1.1), farmed penaeid shrimp production has grown 81.7% since 1984. The sector now constitutes almost half (47.3%) of the world penaeid landings, with over 86% of its total volume represented by only four species (*Penaeus monodon*, *P. vannamei*, *P. chinensis* and *P. merguiensis*).

In recent years however, the sustainability[2] of the activity has been questioned. The industry has shown relatively slower rates of development, with substantial production declines in some instances (Figure 1.1). Contradictorily, these reducing patterns have been associated with over-intensification of production driven primarily by market forces and competitive use of the resources (FAO, 1997, 1998). In marine shrimp farming, this rapid trend towards more intensive forms of husbandry has resulted in overloading the carrying capacity of the aquatic environment, creating self-pollution problems and disease outbreaks. These and other environmental-related difficulties have caused sudden losses and discontinuous progress in production and the industry now faces constant scrutiny for its ecological impacts. These constraints are now recognised as the major obstacles for further expansion of the activity (FAO, 1998).

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[2] Sustainable development is the management and conservation of the natural resource base and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development is environmentally non-degrading, technically appropriate, economically viable and socially acceptable (FAO, 1988).

1.2 Rationale and Research Objectives

At present, the bulk of marine shrimp production is still derived from extensive and semi-intensive culture systems, operating under low shrimp stocking densities and with either some or no external food provision. Under these conditions, natural productivity acts as a major food source, and although lower yields are achieved, these systems are considered more sustainable than intensive ones (Phillips, 1995; Tacon and De Silva, 1997; Nunes and Parsons, 1998a).

In less intensive production systems, environmental control, manipulation and management are intended primarily to relieve nutrient limitations of the ecosystem to the cultured animal, to achieve maximum yield with a minimum quantity of external input and ecological impact. Semi-intensive systems are characterised by a complex food web structure and network of relationships (Figure 1.2). The natural diet of the shrimp is supplemented with inputs of formulated food. Fertilisation promotes natural productivity and water quality is enhanced by increased water exchange rates, thereby allowing higher stocking densities and improved yields.

The functioning of the system involves chemical, biological and physical processes that interact with the pond biota in a continuous state of flux, and tends to be accompanied by physiological and behavioural responses from the cultured species. Water quality characteristics may change significantly over 24-h periods, mainly due to respiration and photosynthesis. Penaeid shrimp may alter food web structure through predation, while displaying ontogenetic variations in their feeding patterns. Some

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Figure 1.2: A conceptual model of nutrient flow in a tropical semi-intensive penaeid shrimp pond. Formulated food is used to supplement the shrimp's natural diet (Nunes and Parsons, 1998a).

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Penaeus spp. for example, may modify their diet during the rearing cycle, which often involves a shift from detritus source dependency to more animal-based sources at larger body sizes. In nature, these dietary alterations are apparently associated with the change in habitat as penaeids grow.

In this changing environment, feeding patterns of penaeids are structured on three elements: its own behavioural and physiological cues; the physical, chemical and biological components of the culture system; and, the elements that comprise a feed management regime (Figure 1.3). The way these structural elements change over time, are controlled, and their inter-relationships have yet to be described completely or quantified. As a consequence, only a portion of the organic matter and nutrients in pelleted feeds that enter the system is converted to shrimp flesh and removed from ponds at harvest. The remainder may either be consumed or recycled by the pond biotic community; flushed from the system with water exchange; or deposited in the pond sediment acting as a source of organic pollution. Little is also known about the factors that dictate the abundance and productivity of important shrimp prey species in aquaculture ponds, impairing attempts towards maximisation of natural food use.

It is now recognised that efforts to improve resource-use efficiency, including naturally occurring pond food sources, and to conserve critical inputs, such as formulated foods, will become increasingly important in aquaculture (Phillips, 1995; Tacon, 1996; Tacon and De Silva, 1997; Nunes and Parsons, 1998a). Studies on these subjects will undoubtedly lead to favourable implications in the long-term sustainability of the activity.

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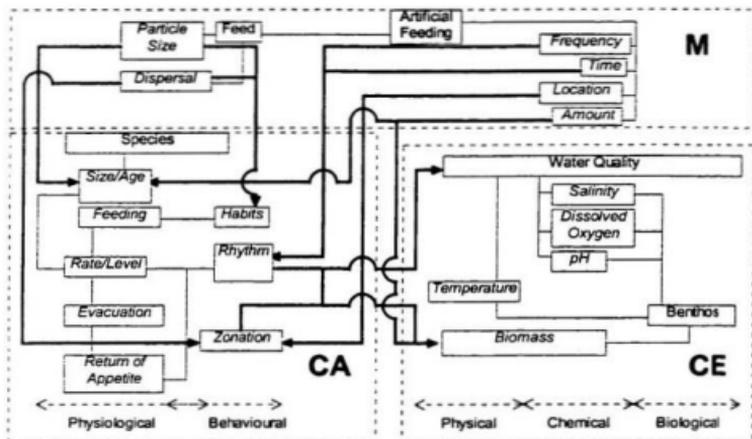
Figure 1.3: A conceptual model of shrimp feeding patterns in a semi-intensive culture system. The model is represented by three components: M, management; CA, cultured animal; and, CE, culture environment. Relationships are indicated by arrows (fine arrows, within components; thick arrows, between components). Source: Nunes and Parsons (1998a).

In the present study, a direct animal approach was taken to investigate the various implications arising from commercial feeding practices (Figure 1.3) on *Penaeus* (*Farfantepenaeus*) *subtilis* feeding. This species has been extensively cultured in many parts of north-eastern Brazil since the mid 1980's (Nunes, 1995) and has also supported a significant offshore fisheries between the Guianas and Brazil. Due to its prominent carnivorous feeding habit, combined with the lack of appropriate commercial diets, attempts to intensify its production in aquaculture systems have failed.

Although the diet of nearly 40 species of penaeids has been described (Dall *et al.*, 1990), with numerous other publications containing descriptions of their feeding behaviour, very little information is available on their food and feeding patterns under culture conditions. In nature, it is suggested that physiological, morphometrical, spatial and temporal factors such as age, sex, moult stage, mouthparts and chelae sizes, habitat, availability of food, time of day and night, season and tide govern and regulate penaeid feeding.

The present study examined the food and feeding patterns of *Penaeus subtilis* under laboratory-controlled and culture conditions. Data was interpreted in the context of penaeid feeding ecophysiology and behaviour and pond ecology. Together the results provide indications and alternatives which may lead to optimisation of the use of both natural and artificial food in *P. subtilis* rearing systems. The objectives of this research investigation are as follows:

1. Describe and determine the handling efficiency and particle size selectivity of *Penaeus subtilis* fed a commercially formulated food. Specifically, this study aimed to determine how significant the effects of shrimp body weight and (or) feed particle

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size are on shrimp handling capacity, in terms of food capture and ingestion success and overall manipulation efficiency. The work also examined *P. subtilis* selectivity according to food particle size;

2. Investigate the feeding levels of *Penaeus subtilis* in response to food dispersal method. Specifically, this work determined shrimp feeding patterns and growth in relation to food concentration versus food broadcast over a complete rearing cycle. The study provided explanations as to why and how food dispersal may affect shrimp feeding behavioural responses. The role of time of feed distribution on food consumption and the possible effects on pond sediment quality produced by feeding method were also examined;

3. Define the effects and relationships of shrimp body size on quantitative feeding and evacuation parameters of *Penaeus subtilis*. This work investigated and determined the possible interactions between shrimp body weight and maximum meal size, food ingestion, return of appetite, faecal production and gastric evacuation. The study presented mathematical models of the relationships observed and determined the time required for shrimp stomach emptying and appetite revival;

4. Investigate the impacts of *Penaeus subtilis* predation and stocking density, and the growth promoting effects of artificial feeding on the population dynamics of benthic polychaetes. This work aimed at examining polychaete population patterns (number and biomass) relative to various culture conditions, such as variations in shrimp stocking density and absence or presence of food supply and shrimp predation over a complete rearing cycle. The research evaluated the extent of the effects of these

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parameters and defined possible environmental interactions, including polychaete abundance and water and soil quality, and lunar cycles;

5. Integrate part of the data derived from this research investigation, structure them into a series of one-dimensional dynamic models and analyse the results. Three models were developed and simulations were performed on the following: (*i*) *Penaeus subtilis* hourly feed intake in relation to shrimp body weight, feed ration and feeding frequency over a 24-h time period; (*ii*) shrimp population feeding levels in response to feed dispersal method over a production cycle; and, (*iii*) polychaete population dynamics in relation to shrimp stocking density, feeding regime and initial polychaete availability over a growth cycle.

CHAPTER 2

FOOD HANDLING EFFICIENCY AND PARTICLE SIZE
SELECTIVITY BY THE SOUTHERN BROWN SHRIMP
Penaeus subtilis FED A DRY PELLETED FEED

2.1 Introduction

In aquaculture systems, the mode of handling of dry pelleted food by *Penaeus* spp. is thought to generate a significant loss of feed and leaching of nutrients (Goldblatt *et al.*, 1979; Csavas, 1994; Goddard, 1996a; Lawrence and Lee, 1997). This is associated with the typical cylindrical shape (Dall, 1992) and size of artificial food (Goddard, 1996a; Nunes, 1996a; Nunes *et al.*, 1997a). In penaeid shrimp, food capture and transfer to the mouthparts is carried out by the first three pairs of chelate pereopods (Hindley and Alexander, 1978; Nunes *et al.*, 1997a), as the second maxilliped endites open to receive it (Alexander and Hindley, 1985). At this stage, the third pair of maxillipeds contract to press food particles against the mouth (Hindley and Alexander, 1978; Alexander *et al.*, 1980), where laceration and trituration of food occur. This is accomplished by the mandibular gnathobases and the mandibles (Alexander *et al.*, 1980). Ingestion is a rapid process [less than 20 s in *P. merguiensis* (Alexander and Hindley, 1985)], and declines as

the foregut fills to capacity (Dall, 1967; Sick and Baptist, 1973; Sick *et al.*, 1973; Hill and Wassenberg, 1987).

Under both culture and natural conditions, juvenile and adult penaeid shrimp are reported to consume a wide range of food particle sizes (Racek, 1959; Condrey *et al.*, 1972; Marte, 1980; Suthers, 1984; Stoner and Zimmerman, 1988; Reymond and Lagardère, 1990; Nunes *et al.*, 1997b) and although some authors report little indication of a change in diet with size of shrimp (Sastrakusumah, 1971; Kuttyamma, 1974; Hunter and Feller, 1987), variations associated with shrimp and relative prey size are often evident (Hall, 1962; George, 1974; Leber, 1983; Wassenberg and Hill, 1987; Stoner and Zimmerman, 1988; Reymond and Lagardère, 1990; Nunes *et al.*, 1997b). These dietary variations are characterised by a decline in the consumption of small food items [*e.g.*, nematodes, foraminiferans (Stoner and Zimmerman, 1988; Nunes *et al.*, 1997b), harpacticoid copepods (Wassenberg and Hill, 1987; Stoner and Zimmerman, 1988; Reymond and Lagardère, 1990; Nunes *et al.*, 1997b)], in favour of larger ones [*e.g.*, bivalves (George, 1974; Marte, 1980; Wassenberg and Hill, 1987), gastropods, ophiuroids (Wassenberg and Hill, 1987), polychaetes (Reymond and Lagardère, 1990; Nunes *et al.*, 1997b), chironomids (Reymond and Lagardère, 1990) and amphipods (George, 1974; Stoner and Zimmerman, 1988)] as shrimp attain larger body sizes. Hence, it is assumed that as shrimp grow, they become capable of more effectively capturing and consuming larger prey (Marte, 1980; Wassenberg and Hill, 1987; Stoner and Zimmerman, 1988; Nunes, 1995).

In other crustaceans, animal and food particle size are reported to affect food detection [copepods (Lillelund and Lasker, 1971)], food selectivity [copepods (Lillelund

and Lasker, 1971; Wilson, 1973), crabs (Williams, 1982; Rheinallt, 1986), caridean shrimp (Pihl and Rosenberg, 1984)], food capture and manipulation [copepods (Lillelund and Lasker, 1971), crabs (Rheinallt and Hughes, 1985; Rheinallt, 1986; Boulding and Labarbera, 1986), lobsters (Lau, 1987; Lee, 1995)], rate and amount of food intake [copepods (Wilson, 1973; Richman *et al.*, 1977), euphausiid (Heyraud, 1979), crab (Rheinallt and Hughes, 1985), lobsters (Lau, 1987; Lee, 1995)] and energy gain maximisation [crabs (Elner and Hughes, 1978)]. Although penaeid shrimp and prey size relationships have been described extensively (Leber, 1983; Wassenberg and Hill, 1987; Stoner and Zimmerman, 1988; Reymond and Lagardère, 1990; Nunes *et al.*, 1997b), little is known about the ability of shrimp to manipulate different food particle sizes. As a result, optimal shrimp feed sizes for use in aquaculture have only been determined empirically (Akiyama, 1993). This information is essential to maximise feed use and shrimp feed intake, reducing the loss of feeds and nutrients in shrimp ponds. The present work investigated the food handling efficiency and size selectivity of juvenile and adult *Penaeus subtilis* fed three commercial dried pelleted feed sizes under laboratory conditions.

2.2 Materials and Methods

2.2.1 Classification of Shrimp and Feed Size

Specimens of *Penaeus subtilis* were collected from nursery and grow-out ponds at a commercial marine shrimp farm (Artemisa Aquicultura S.A.) located on the north-eastern coast of Brazil, Acaraú, Ceará. Animals were transported alive in 50-L covered containers with cooled sea water (20 °C) and constant aeration to a laboratory 4 h distant from the sampling site. Collected animals had been raised in large ponds with access only to naturally occurring food organisms.

In the laboratory, shrimp were classified and arbitrarily divided into the following size groups according to their wet body weight: G_1 = 1.148 to 3.760 g juvenile shrimp (2.217 ± 0.769 g) (mean ± standard deviation); G_2 = 4.178 to 6.995 g juvenile shrimp (5.644 ± 0.734 g); G_3 = 7.035 to 9.855 g pre-adult shrimp (8.315 ± 0.777 g); and, G_4 = 10.036 to 16.493 g adult shrimp (11.811 ± 1.300 g). Groups were held separately in 1,000-L tanks (area of 1.13 m²) equipped with a biological filter and a 5 cm layer of 3-mm sand on the bottom. The tank system had a constant air supply and was illuminated artificially under a 12:12 LD light cycle. Prior to the experiment, shrimp were fed *ad libitum* with a diet composed of fish flesh and formulated dried feed for approximately 15 d.

Formulated diet used was a commercially produced shrimp dry pelleted feed (Ração Sibra para Camarões; Sibra Aquicultura S.A., Propria, Sergipe, Brazil), consisting of

three different sizes: P_1 = crumbles of less than 1 mm length by 1.90 ± 0.32 mm diameter ($\pm$ s.d.); P_2 = broken pellets of 1.31 ± 0.35 mm length by 2.31 ± 0.09 mm diameter; and, P_3 = pellets of 5.50 ± 1.48 mm length by 2.38 ± 0.08 mm diameter. Pellets were cylindrical in shape and crumbles had an irregular form. The feed was formulated and processed to provide the same final chemical composition and texture for all sizes. Feed ingredients included: fish meal, soybean meal, wheat flour, agglutinated wheat flour, meat meal, bone meal, yeast, animal fat, manioc flour, rice meal, peanut flour, oyster flour, fish waste meal, corn flour, salt and mineral supplement.

2.2.2 Feed Proximate Chemical Analysis

Analyses were performed to assess if any possible differences in the chemical composition existed among the three feed particle sizes used in the study, following the methodology described by AOAC (1990). Moisture content was determined by weighing three replicates of 2 g for each of the samples and drying at 105 °C for 24 h. The loss in weight represented the moisture content. Crude protein was determined by the Kjeldahl method [calculated as nitrogen (N) $\times$ 6.25], and ash content by burning in a muffle furnace at 660 °C for 2 h. Lipid was determined gravimetrically following Soxhlet extraction using acetone as the solvent. No direct measurements were made of carbohydrate, which was estimated by difference.

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2.2.3 Experimental Design and Apparatus

Penaeus subtilis feeding behaviour was examined with the aid of a video-camera (Sharp Camcorder model VL-L63B, 43-mm diameter lens F1.6 - F3.2, f = 4.9 - 58.8 mm, x12 power zoom, Sharp do Brasil S.A., Manaus, Amazônas, Brazil). Since manipulation of food by penaeids is carried out under its cephalothorax or thoracic region (Alexander and Hindley, 1985), the camera was installed below a glass aquarium (15 cm height by 24.5 cm length by 15 cm width, volume of 3.76 L), where one shrimp at a time was introduced and allowed to feed.

The aquarium was suspended on a transparent glass base attached to the top of a rectangular aluminium frame (74 cm in height by 38 cm length). On the bottom, a small cart placed on a flat section of wood, held the video system in a vertical position (Figure 2.1). Hence, it was possible to adjust the video-camera horizontally in response to the animal's locomotory movements within the aquarium area. A 15-W white fluorescent light was attached to the top-left side of the frame and directed towards the aquarium. The arrangement allowed detailed examination of the rapid food manipulation activity carried out by the feeding appendages of *Penaeus subtilis*. The aquarium was covered with a dark sheet and the water was constantly aerated. No substratum was placed on the aquarium bottom because bottom-image observations would not be possible. Sea water was maintained at 27.8 ±0.5 °C (± s.d.; n = 198) temperature, 30 ± 2‰ (n = 197) salinity and 8.08 ± 0.14 (n =198) pH.

Figure 2.1: Schematic diagram of apparatus used to record *Penaeus subtilis* feeding behaviour.

Dark Sheet

Aquarium

Penaeus subtilis

24.5 cm

Aeration

15 cm

Feed

Glass Base

Light

74 cm

Aluminium Structure

Video Camera

Cart

38 cm

18
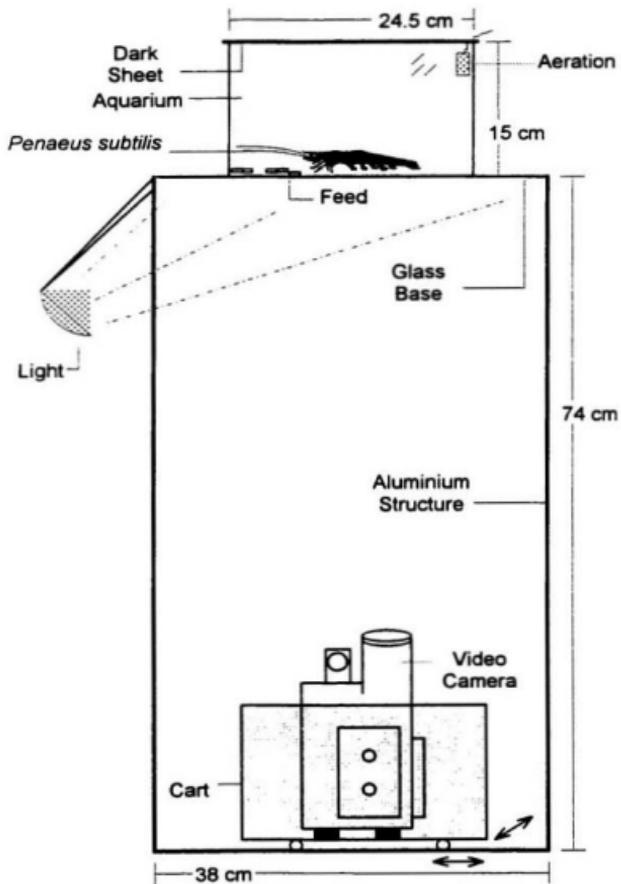
2.2.4 Feed Manipulation and Size Selectivity

Prior to feeding trials, shrimp were starved for 48 h. Animals were individually acclimated to the experimental light conditions and aquarium area and bottom for 48 ± 27 min ($\pm$ s.d.; $n = 193$). Only active and healthy shrimp with completely functional pereopods and maxillipeds were used. Each specimen of *Penaeus subtilis* was videotaped only once. If feed was not detected or no food consumption occurred within the first 5 min of exposure to food, recording was interrupted, and a newly acclimated shrimp used. After recording each shrimp, the water was discarded, and the aquarium washed and filled with new filtered sea water.

Filming was started by introducing a single feed size to a recently acclimated shrimp. Prior to feeding, formulated food was soaked in sea water for 2 min so that it would sink immediately and completely in the aquarium. Food was always administered in excess, in equal quantities to all shrimp size groups (section 2.2.1). The amount of food offered however, varied according to feed particle size [1.008 ± 0.007 g P_1 ($\pm$ s.d.), 1.008 ± 0.008 g P_2 and 2.503 ± 0.070 g P_3]. On average, video-recording for handling trials lasted for 10.75 ± 4.83 min ($n = 158$) from the time feed was first offered to the animal. A minimum of ten different shrimp of each size class exhibiting feeding behaviour responses, *i.e.*, capture, handling and consumption of food, were tested for each feed particle size.

Size selectivity experiments were carried out under identical conditions, except that all three feed sizes, *i.e.*, P_1, P_2 and P_3, were administered simultaneously in equal

amounts. In this case, total ration per animal amounted to 1.5 g [0.507 ± 0.005 g P_1 (± s.d.), 0.507 ± 0.005 g P_2 and 0.508 ± 0.06 g P_3]. Since it is suggested that shrimp chelate pereopods have a particle size discriminating function (Hindley and Alexander, 1978), selectivity was considered here only with regard to those food particles grasped successfully by the feeding appendages of *Penaeus subtilis* and further conducted to the mouthparts.

2.2.5 Feeding Behavioural Analysis

Preliminary video recordings were made of *Penaeus subtilis* food manipulation behaviour. These images were used to identify the different behaviours involved in the feed handling process and to establish an appropriate time period for analysis. Observations showed that feeding activity of this species decreased substantially after 10 min of exposure to food. Thus, only the first 8 min of filming (starting at food detection), was considered for analysis. Detection is defined as the shrimp's successful searching response, after introduction of food into sea water (Hindley, 1975). To determine if food manipulation success changed over the period of feeding activity, the video sequences were divided into two equal 4-min intervals, T_{0-4} (*i.e.*, starting at first detection of food up to 4 min) and T_{4-8} (*i.e.*, interval between 4 to 8 min after first food detection).

Image analyses of *Penaeus subtilis* manipulation behaviour were conducted visually. A hand counter was used to record the following variables: (1) AC = number of attempts (successful or unsuccessful) to capture (catch or grasp) feed particles; (2) PC =

number of feed particles conducted successfully to the pre-oral cavity (formed by the following mouthparts: 1st and 2nd maxillipeds, 1st and 2nd maxillas, paragnath, labrum and mandibles); and, (3) PI = number of movements performed by the mouthparts which may have led to ingestion of food placed successfully in the pre-oral cavity.

Counting was performed three times for each variable in time intervals T_{0-4} and T_{4-8}. This procedure totalled 18 measurements of feed handling for each individual animal (*i.e.*, 3 variables x 3 countings x 2 time periods). Based on these data, a mean value was determined for T_{0-4} and T_{4-8} and the following indices calculated:

$$\text{Capture Efficiency Ratio (CER)} = \frac{PC}{AC} \qquad (2.1)$$

$$\text{Ingestion Efficiency Ratio (IER)} = \frac{PI}{PC} \qquad (2.2)$$

$$\text{Manipulation Capacity Index (MCI)} = \frac{CER + IER}{2} \qquad (2.3)$$

2.2.6 Statistical Analysis

Statistical analyses were performed with the Statistical Package for Social Sciences Windows version, release 7.5.1 (SPSS Inc., Chicago, Illinois, USA). Homogeneity of variance was examined for all data by using Bartlett-Box F and Cochran's C tests. Kurtosis and skewness and their standard error (*i.e.* s.e. kurtosis and skewness) were applied to the data as measures of asymmetry and tests of normality. Based on these results, AC, PC and PI were transformed to a log(x+1) scale in order to normalise and

homogenise the variances and to meet statistical assumptions. Probability of type I error was set at 0.05.

2.3 Results

2.3.1 Chemical Composition of Feed

Apart from ash content, no other significant differences were found in the chemical composition of the three formulated feed sizes used in this study (one-way ANOVAs, $F_{2,5}$ = 179.65, $P < 0.001$, ash; $F_{2,5}$ = 2.06, $P = 0.223$, carbohydrate; $F_{2,6}$ = 2.23, $P = 0.189$, moisture; $F_{2,6}$ = 2.37, $P = 0.175$, lipid; $F_{2,6}$ = 0.91, $P = 0.452$, protein; Table 2.1). Ash content was significantly lower for P_1 compared to P_2 and P_3, respectively (Scheffé's Multiple Range Test, $P < 0.05$, Table 2.1).

2.3.2 Feed Handling

A total of 28.49 h of filming was carried out with 163 different specimens of *Penaeus subtilis*. Of this number, 121 shrimp (74%) displayed some type of feeding behaviour, while the remaining 42 animals showed no response to the food offered. On average, capture of feed occurred within 6 ± 18 s (± s.d.; n = 68) to food exposure, followed by consumption at 12 ± 29 s (n = 118). During the 10-min observation

Table 2.1: Proximate composition of formulated diet used in the study. Feed sizes refer to crumbles of less than 1 mm length by 1.90 mm diameter (P_1), broken pellets of 1.31 mm length by 2.31 mm diameter (P_2) and pellets of 5.50 mm length by 2.38 mm diameter (P_3). Results are presented as mean ± standard deviation, derived from three replicates of each sample. Common letters denote no significant difference among pellets at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.

Feed	Proximate composition (%)				
Size	Moisture	Protein	Lipid	Ash	Carbohydrate
P_1	7.4 ± 0.4 a	44.6 ± 1.9 a	7.1 ± 0.3 a	15.6 ± 0.1 a	25.3 ± 1.8 a
P_2	7.4 ± 0.8 a	45.8 ± 0.5 a	6.8 ± 0.7 a	16.8 ± 0.1 b	23.2 ± 0.8 a
P_3	8.2 ± 0.1 a	44.4 ± 1.3 a	7.6 ± 0.1 a	16.8 ± 0.1 b	23.0 ± 1.3 a

period, the mean duration of feeding was 9.76 ± 3.94 min ($n = 117$).

2.3.3 Feed Particle Size

Statistically significant differences were found in the AC, PC and PI among the three feed particle sizes fed to *Penaeus subtilis* (MANOVA, $F_{2, 696} = 25.35$, $P < 0.001$, AC; $F_{2, 696} = 60.81$, $P < 0.001$, PC; $F_{2, 694} = 48.80$, $P < 0.001$, PI). Mean values of AC, PC and PI declined as feed particle size increased (Figure 2.2).

A statistically higher AC for P_1 and P_2 (Figure 2.2) resulted in an almost intermittent feeding pattern in shrimp. Food capture and transport of granules and small pellets (P_1 and P_2, respectively) to the mouth were carried out by rapid and synchronic movements of the first three pairs of chelate appendages. While grasping of large pellets (P_3) was often achieved only when one or more pairs of chelate pereopods functioned together in the food capturing process, small food particles (P_1 and P_2) could be individually captured and carried to the mouth. As a result, when fed P_1 and P_2, *Penaeus subtilis* displayed a significantly higher PC in comparison to that of P_3 (Scheffé's Multiple Range Test, $P <$ 0.05, Figure 2.2).

Once feed was brought towards the mouth, *Penaeus subtilis* either stored food items in its pre-oral cavity for grinding or ingested the whole feed particle without further manipulation. Food storage was achieved by a simultaneous antero-laterally contraction of the third pair of maxillipeds and the first pair of pereopods. Large pellets (P_3) required a more prolonged manipulation period prior to ingestion, as reflected in the

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Figure 2.2: Comparison of mean AC (number of attempts to capture feed particles), PC (number of feed particles conducted successfully to the pre-oral cavity) and PI (number of movements performed by the mouthparts which may have led to ingestion of food) for three feed particle sizes (P_1 = crumbles of less than 1 mm length by 1.90 mm diameter; P_2 = pellets of 1.31 length by 2.31 mm diameter; and, P_3 = pellets of 5.50 length by 2.38 mm diameter) fed to *Penaeus subtilis*. Values (n) on top of bars indicate number of observations. Vertical bars represent standard error. Common letters within each figure denote no significant difference at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.
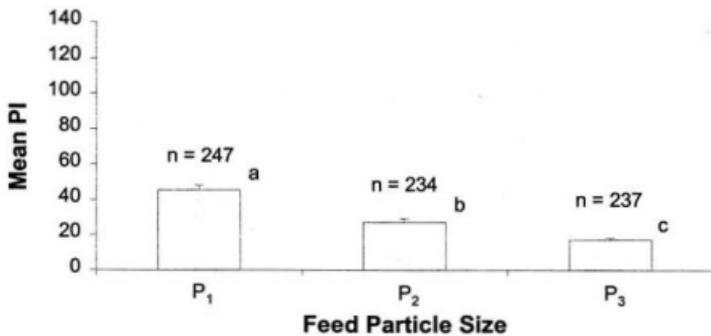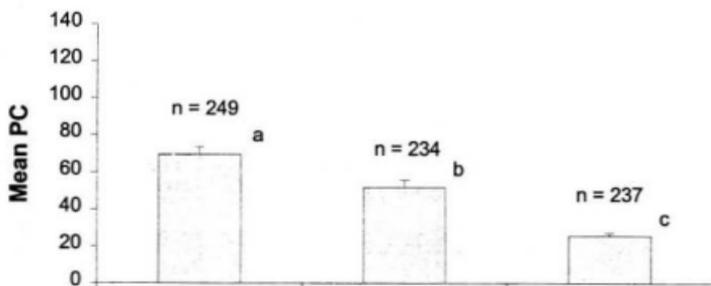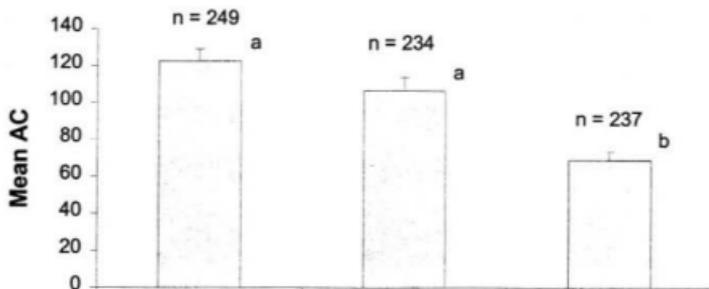
statistically higher PI found for small particle sizes (P_1 and P_2, Scheffé's Multiple Range Test, $P < 0.05$, Figure 2.2).

Capture Efficiency Ratio (CER) was also significantly different among feed particle sizes (one-way ANOVA, $F_{2, 237} = 20.90$, $P < 0.001$), but did not differ significantly between P_1 and P_2 (Scheffé's Multiple Range Test, $P < 0.05$, Table 2.2). Ingestion efficiency (IER) was not statistically different among feed particle sizes (one-way ANOVA, $F_{2, 237} = 3.30$, $P = 0.440$). Manipulation Capacity Index (MCI) was statistically different among feed sizes (one-way ANOVA, $F_{2, 237} = 3.51$, $P = 0.032$) and significantly higher for P_1 (Scheffé's Multiple Range Test, $P < 0.05$, Table 2.2). In general, the higher CER achieved for smaller pellet sizes (*i.e.*, P_1 and P_2), demonstrated that a higher number of attempts to capture food (AC) resulted in an increase in the number of particles successfully conducted to the mouthparts (PC). These combined effects, although not favouring ingestion efficiency (IER) due to a proportionally higher PC compared to PI, led to an enhanced Manipulation Capacity Index (MCI).

2.3.4 Shrimp Size

AC, PC and PI differed statistically among the four size classes of *Penaeus subtilis* (MANOVA, $F_{3, 696} = 15.63$, $P < 0.001$, AC; $F_{3, 696} = 9.87$, $P < 0.001$, PC; $F_{3, 694} = 9.90$, $P < 0.001$, PI). Mean AC was statistically higher for smaller shrimp size groups (G_1

Table 2.2: Handling efficiency indices for *Penaeus subtilis* separately fed three feed particle sizes. Results are presented as mean ± standard deviation. Numbers in parentheses indicate minimum and maximum values. Common letters denote no significant difference among pellets at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.

Feed Particle Size	n	CER	IER	MCI
P_1	81	0.58 ± 0.20a	0.60 ± 0.22a	0.59 ± 0.18a
		(<0.01 - 1.00)	(<0.01 - 1.00)	(<0.01 - 1.00)
P_2	78	0.52 ± 0.19a	0.59 ± 0.24a	0.55 ± 0.18a, b
		(< 0.01 - 0.94)	(< 0.01 - 1.00)	(< 0.01 - 0.96)
P_3	81	0.39 ± 0.16b	0.64 ± 0.24a	0.51 ± 0.18b
		(< 0.01 - 0.77)	(< 0.01 - 1.00)	(< 0.01 - 0.89)

and G_2, Scheffé's Multiple Range Test, $P < 0.05$, Figure 2.3). Such a pattern however, was not evident in PC and PI. As a result, CER, IER (except when contrasting G_1 with G_3) and MCI were not statistically different among shrimp size groups (Scheffé's Multiple Range Test, $P < 0.05$, Table 2.3).

The break-down of data (Figure 2.4) showed that when fed smaller feed particles, mainly P_1, shrimp groups G_2, G_3 and G_4, displayed a significant improvement in CER (one-way ANOVAs, $F_{2, 58} = 3.80$, $P = 0.028$, G_2; $F_{2, 59} = 12.03$, $P < 0.001$, G_3; $F_{2, 57} = 4.80$, $P = 0.012$, G_4; Scheffé's Multiple Range Test, $P < 0.05$, Figure 2.4). Non-statistical differences were found for ingestion (IER) and manipulation (MCI) efficiency ratios for comparisons made within each shrimp group (G_1, G_2, G_3 and G_4) among food particles P_1, P_2 and P_3 (Scheffé's Multiple Range Test, $P > 0.05$, Figure 2.4).

2.3.5 Time Interval

Handling of feed varied between the two time intervals (*i.e.*, T_{0-4} and T_{4-8}) which were analysed (Figure 2.5). Statistically, higher values of AC, PC and PI were found for T_{0-4}, when compared to T_{4-8} (MANOVA, $F_{1, 696} = 52.66$, $P < 0.001$, AC; $F_{1, 696} = 47.24$, $P < 0.001$, PC; $F_{1, 694} = 31.07$, $P < 0.001$, PI). A decline of the order of 33, 31 and 24% was found for AC, PC and PI, respectively, from time interval T_{0-4} to T_{4-8}. In terms of handling efficiency however, no statistically significant differences were observed between the intervals for CER, IER and MCI (*t*-test, df = 238, t = 0.74, $P > 0.05$, CER; *t*-

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Figure 2.3: Comparison of mean AC (number of attempts to capture feed particles), PC (number of feed particles conducted successfully to the pre-oral cavity) and PI (number of movements performed by the mouthparts which may have led to ingestion of food) for four group sizes [G_1 = 1.148 - 3.760 g shrimp (n = 29); G_2 = 4.387 - 6.995 g shrimp (n = 31); G_3 = 7.117 - 9.855 g shrimp (n = 31); and, G_4 = 10.057 - 13.715 g shrimp (n = 30)] of *Penaeus subtilis*. Values (n) on top of bars indicate number of observations. Vertical bars represent standard error. Common letters within each figure denote no significant difference at the α = 0.05 level by Scheffé's Multiple Range Test.
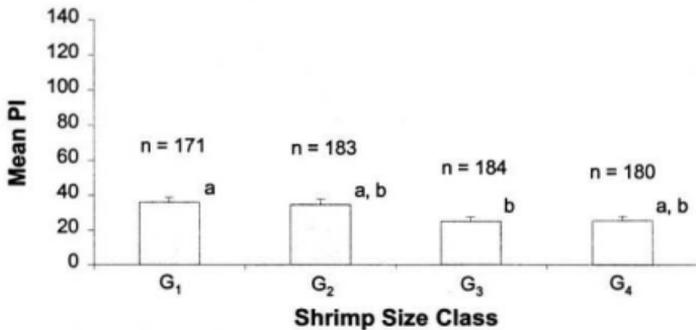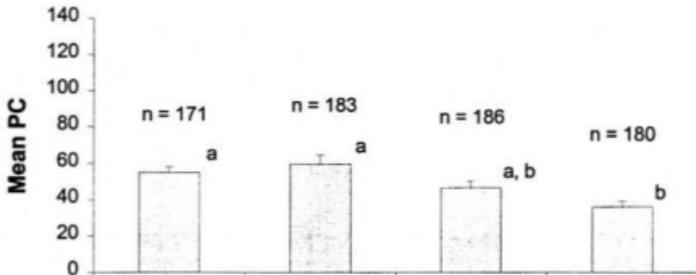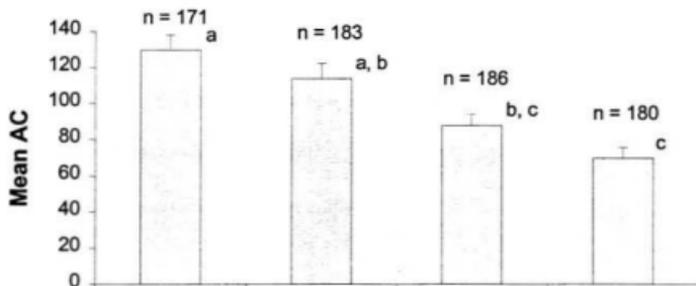
Table 2.3: Handling efficiency indices for four groups sizes of *Penaeus subtilis* fed three feed particle sizes. Results are presented as mean ± standard deviation. Numbers in parentheses indicate minimum and maximum values. Common letters within each column denote no significant difference among shrimp size groups at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.

Shrimp Size Class	n	CER	IER	MCI
G_1	57	0.45 ± 0.12a	0.66 ± 0.20a	0.55 ± 0.13a
		(0.18 - 0.77)	(0.24 - 1.00)	(0.29 - 0.89)
G_2	61	0.50 ± 0.21a	0.60 ± 0.23a, b	0.55 ± 0.19a
		(< 0.01 - 0.99)	(< 0.01 - 1.00)	(< 0.01 - 0.93)
G_3	62	0.51 ± 0.21a	0.54 ± 0.24b	0.52 ± 0.19a
		(< 0.01 - 0.92)	(< 0.01 - 1.00)	(< 0.01 - 0.86)
G_4	60	0.53 ± 0.23a	0.64 ± 0.24a, b	0.58 ± 0.20a
		(< 0.01 - 1.00)	(< 0.01 - 1.00)	(< 0.01 - 1.00)

Figure 2.4: Mean variation in the handling efficiency indices for *Penaeus subtilis* in response to mean shrimp body weight and feed particle size. CER, Capture Efficiency Ratio; IER, Ingestion Efficiency Ratio; and, MCI, Manipulation Capacity Index.

CER

IER

MCI

P₁
P₂
P₃

G₁ G₂ G₃ G₄

Mean Shrimp Body Weight (g)
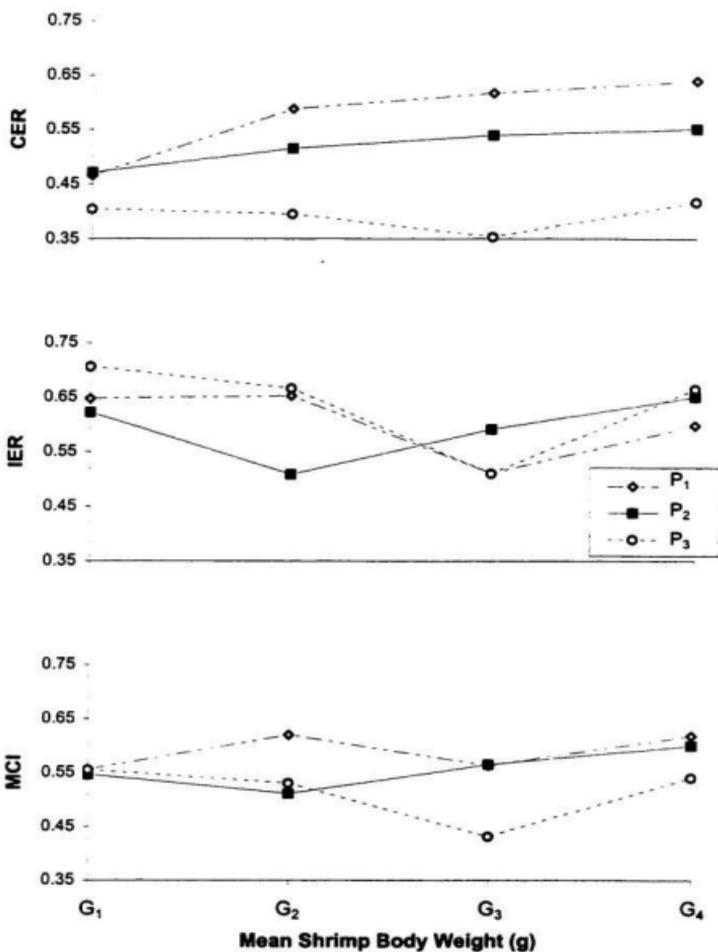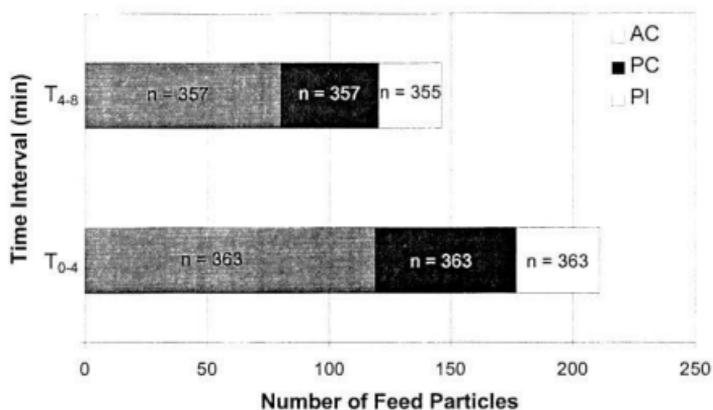
Figure 2.5: AC, PC and PI as a function of the time interval at which images were analysed. T_{0-4} refers to the interval starting at first detection of food by *Penaeus subtilis* up to 4 min. T_{4-8} corresponds to the interval between 4 to 8 min after first food detection by shrimp, n values indicate number of observations.

test, df = 238, t = 0.31, P > 0.05, IER; *t*-test, df = 238, t = 0.60, P > 0.05, MCI; Table 2.4).

2.3.6 Feed Particle Size Selection

Forty-one different specimens of *Penaeus subtilis* were used in feed particle selection trials. Of these, only four individuals did not display feeding behaviour, and were therefore excluded from the analysis of data. Results indicate that the number of feed particles conducted successfully to the mouthparts (PC) was statistically different for each shrimp group (one-way ANOVAs, $F_{2, 177}$ = 92.51, P < 0.001, G_1; $F_{2, 150}$ = 68.38, P < 0.001, G_2; $F_{2, 195}$ = 26.49, P < 0.001, G_3; $F_{2,122}$ = 17.91, P < 0.001, G_4; Figure 2.6). The mean relative PC declined with an increase in feed particle size. Further analysis revealed that the mean PC was not statistically different, except when contrasting feed sizes P_2 and P_3 in groups G_2 and G_3 (Scheffé's Multiple Range Test, P > 0.05, Figure 2.6). Such results indicated that all shrimp groups favoured smaller feed particle sizes, particularly P_1, which showed mean relative PC varying from 59 (G_3) to 81% (G_4).

Table 2.4: Handling efficiency indices for two time intervals (T_{0-4} and T_{4-8}) when
Penaeus subtilis was exposed to formulated food. T_{0-4} refers to the interval
starting at first detection of food up to 4 min. T_{4-8} corresponds to the interval
between 4 to 8 min after first food detection by shrimp. Results are
presented as mean ± standard deviation. Numbers in parentheses indicate
minimum and maximum values.

Time Interval	n	CER	IER	MCI
T_{0-4}	121	0.50 ± 0.17	0.61 ± 0.19	0.55 ± 0.13
		(0.14 - 0.99)	(0.16 - 1.00)	(0.21 - 0.93)
T_{4-8}	119	0.49 ± 0.23	0.61 ± 0.27	0.55 ± 0.22
		(< 0.01 - 1.00)	(< 0.01 - 1.00)	(< 0.01 - 1.00)
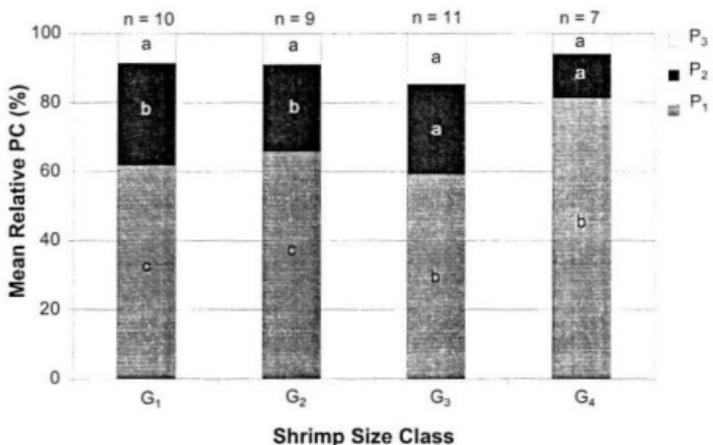
Figure 2.6: Mean relative PC (number of feed particles conducted successfully to the pre-oral cavity) for four group sizes (G_1 = 1.201 - 3.100 g shrimp; G_2 = 4.178 - 6.303 g shrimp; G_3 = 7.224 - 9.302 g shrimp; and, G_4 = 10.347 - 16.493 g shrimp) of *Penaeus subtilis* exposed simultaneously to three feed particle sizes (P_1 = crumbles of less than 1 mm length by 1.90 mm diameter; P_2 = pellets of 1.31 mm length by 2.31 mm diameter; and, P_3 = pellets of 5.50 mm length by 2.38 mm diameter). Values (n) on top of bars indicate number of shrimp analysed. Common letters denote no significant difference at the α = 0.05 level by Scheffé's Multiple Range Test (comparisons within groups only).

2.4 Discussion

2.4.1 Effects of Feed Particle Size

2.4.1.1 Capture Efficiency

In the present study, the statistically lower AC found for P_3 was the result of a longer food retention period in the shrimp's pre-oral cavity due to a larger particle size. One or only a few large pellets were sufficient to overload *Penaeus subtilis* pre-oral cavity. Maintaining several crumbles or small pellets simultaneously against the mouthparts, also appeared to impose more difficulty for shrimp than holding a single large pellet. A combination of these factors likely inhibited new attempts towards the capture of P_3, resulting in a lower AC.

A greater number of successful particle captures (PC) and a significantly higher CER were found when *Penaeus subtilis* was exposed to P_1 and P_2. The capture of small food particles by this species was probably enhanced by the limited opening diameter of its chelae. Although, some authors report that penaeid shrimp are capable of capturing large food material, such as algal mats [*P. setiferus* and *P. aztecus* (Condrey *et al.*, 1972)] and small shrimp [*P. plebejus* and *P. esculentus* (Racek, 1959)], *P. subtilis* exhibited difficulty when coping with large feed particles (*i.e.*, P_3). Similarly, when fed all feed sizes simultaneously (*i.e.*, P_1, P_2 and P_3), this species tended to select small particle sizes, particularly P_1.

In *Penaeus subtilis*, a higher percentage of successful attempts occurred during the capture of P_1 and P_2 (56.10% and 48.60%, respectively) compared with P_3 (36.23%). Feeding optimisation by shrimp was attained by grasping and conducting a greater number of small particles to the mouthparts in a shorter period of time. Small feed particles also required less time and energy expenditure for food laceration and trituration at time of consumption. Such a strategy probably compensated for the proportionally greater energy gain that a single large pellet (P_3) would provide if consumed completely. In general, food capture success appeared to be ultimately related to the shrimp's chaelae diameter. Although the literature lacks morphometric measurements of penaeids chelae, studies with other decapod crustaceans (*i.e.*, crabs; Rheinallt and Hughes, 1985; Rheinallt, 1986) support the observation that the amount of time allocated to and level of success during capturing and manipulation of different food sizes are associated with their chelae.

2.4.1.2 Consumption Efficiency

Over 59% of all food particles placed in the mouth by *Penaeus subtilis* were completely or partially consumed (IER), regardless of the feed size. Large particles, however, were consumed more slowly (PI), although no significant differences were observed for IER. IER data revealed that no differences in ingestion efficiency [*i.e.*, effort allocated to food transport to the mouth (as expressed by PC) versus number of particles ingested], were found among particle sizes of feed.

The consistency obtained in IER among feed sizes was a consequence of the different feed handling modes exhibited by *Penaeus subtilis* in response to food dimension. Storage

of crumbles (P_1) and broken pellets (P_2) in the shrimp's pre-oral cavity was often followed by new attempts at food capture, resulting in the involuntary release or loss of feed which had already been positioned in the mouth. This led to a proportionally higher loss of particles P_1 and P_2 at time of consumption (19.52% and 23.37% loss of all feed particles positioned in the mouth, respectively) when compared to P_3 (11.60%). Small food particles were also reported to be dropped involuntarily after being captured by the crab *Carcinus maenas* (Hughes and Elner, 1979) and *Liocarcinus puber* (Rheinallt, 1986). In *P. subtilis*, the loss of feed after capture may have also been an intentional response, associated with food selectivity, or accidental, due to a prolonged starvation period combined with a high exposure of food. In penaeid shrimp, rejection of food may occur after food items have passed up the pre-oral cavity to the mandibles (Alexander and Hindley, 1985). Deprivation of food affects their level of locomotory activity (Hill and Wassenberg, 1987), which may, in turn, interfere with the food handling process.

Although there was no significant difference found IER among feed particle sizes, when fed P_1 and P_2, a significantly higher food ingestion (PI) was achieved relative to P_3. This observation was probably the result of a positive relationship between the opening diameter of the shrimp's mouth and the size of small feed particles. Administration of crumbles and broken pellets (P_1 and P_2) reduced the period required for manipulation during consumption, allowing at times the ingestion of whole particles without further trituration. This type of feeding behaviour was also reported for *Penaeus merguiensis* feeding on items of less than 1 mm^3 (Alexander and Hindley, 1985). Such observations suggest that the disintegration of dried pelleted food and the period allocated for food

consumption may be significantly reduced if particles are sufficiently small to minimise their manipulation by penaeid shrimp during ingestion.

The combined results of IER and CER for *Penaeus subtilis*, resulted in a significantly higher mean MCI for particle size P_1 [0.59 ± 0.18 (± s.d.)] when compared to P_2 (0.55 ± 0.18) and P_3 (0.51 ± 0.18). Thus, it can be concluded that feed manipulation efficiency in *P. subtilis* was inversely related to food particle size, and that this species preferred crumbles and broken pellets (*i.e.*, P_1 and P_2) to large pellets (*i.e.*, P_3).

2.4.2 Effects of Shrimp Size and Time Interval

The decline in feeding activity (*i.e.*, AC, PC and PI) found for *Penaeus subtilis* from time interval T_{0-4} to T_{4-8} was most likely associated with stomach volume. Penaeid shrimp possess a small stomach volume (Wassenberg and Hill, 1987; Nunes, 1997), which can be filled to capacity within 1 min (Dall, 1967) to 10 min of continuous feeding (Hill and Wassenberg, 1987). The decline in feeding by *P. subtilis* however, did not result in a reduction in food handling efficiency (*i.e.*, CER, IER and MCI) from time interval T_{0-4} to T_{4-8}. This indicated that within the time interval examined, food manipulation efficiency for *P. subtilis* was constant, even after a reduction in feeding activity.

Similarly, *Penaeus subtilis* body size had no significant effect on food handling efficiency. All manipulation indices examined, exhibited no significant differences (except IER between G_1 and G_3). Therefore, within the feed size range used, juvenile shrimp (*i.e.*,

G_1 and G_2) were generally capable of capturing, handling and ingesting food particles as efficiently as larger or adult shrimp (*i.e.*, G_3 and G_4).

Numerically, larger shrimp (*i.e.*, G_3 and G_4) tended to display a more conservative strategy towards food capture (AC and PC). In contrast to other shrimp size groups (*i.e.*, G_2, G_3 and G_4), capture efficiency ratios (CER) in juvenile shrimp (*i.e.*, G_1) did not differ statistically among food particle sizes. This result may be related more to the inadequacy of the overall feed sizes used than the actual ability of juvenile shrimp (G_1) to handle large food particles. Despite the fact that smaller shrimp allocated more effort to food capturing when compared to larger animals (as indicated by AC, G_1 versus G_3), mean PC was not greater (G_1 versus G_3). P_3 also imposed a greater limitation to food capturing success in G_2, G_3 and G_4, as indicated by the significantly lower CER.

In commercial aquaculture operations, cylindrical pellets are widely employed. Sizes greater than 2 mm in diameter by 4 mm in length are recommended for use with shrimp within 3 to 15 g body weight (Akiyama, 1993). It is generally assumed that penaeid shrimp can efficiently use these sizes, although food disintegration due to handling is also hypothesized (Csavas, 1994; Goddard, 1996a). Data collected in the present study indicate that administration of pellet sizes greater than 2.38 mm in diameter by 5.50 mm in length (P_3) to *Penaeus subtilis* ranging from 4.178 (G_2) to 16.493 g (G_4), result in less efficient capture of food. Feed size selectivity for all shrimp groups was also reduced for P_3, suggesting that in aquaculture systems, use of crumbles or broken pellets (*i.e.*, P_1 and P_2, respectively) may be preferable to larger pellets (*i.e.*, P_3) even for pre-adult and adult shrimp (*i.e.*, G_3 and G_4). The potential difficulties shrimp may find in detecting small feed particles in pond systems may be compensated by the fact that in the same amount of food

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(*e.g.*, 1 kg), the number of crumbles (P_1) and broken pellets (P_1) is 14 and 4 times greater, respectively, than large pellets (P_3, pers. obs.). The higher number of feed particles per area of culture should increase the probability of food encounters.

Under the experimental conditions employed, capture efficiency did not generate any detectable effects on shrimp food ingestion (*i.e.*, IER). In aquaculture systems, however, other factors (*e.g.*, presence of sediment, feed dispersal, food concentration, inter-animal behavioural relationships) may increase the significance of feed capture success. Under pond systems, formulated food is less concentrated, food encounters may be lower and partitioning of food resources may occur among the cultured population, thus requiring shrimp to adopt a more effective and less selective feeding strategy.

2.5 Conclusions

Results indicated that *Penaeus subtilis* is selective in regards to feed size. Selectivity by this species tended towards small food particles, specially crumbles, discriminating against large sizes. Small particles were also more easily captured and manipulated, although not producing a significant influence on food consumption. Administration of crumbles and broken pellets however, did reduce the period of food manipulation, at times allowing the ingestion of whole particles without further trituration. Within the feed size range examined, handling of food by juvenile *P. subtilis* was as effective as that of adults. Shrimp manipulation efficiency was not affected by time of food exposure, although a reduction in feeding activity was observed over time.

CHAPTER 3

FEEDING LEVELS OF THE SOUTHERN
BROWN SHRIMP *Penaeus subtilis*
IN RESPONSE TO FOOD DISPERSAL

3.1 Introduction

Over-feeding has been referred to as one of the major causes of organic loading in penaeid shrimp ponds and in estuarine coastal areas adjacent to marine shrimp aquaculture operations (Lin, 1995; Goddard, 1996a,b; Gonzalez-Vila *et al.*, 1996; Macintosh, 1996; Rajendran and Kathiresan, 1996; Goddard and Nunes, 1997; Lawrence and Lee, 1997; Nunes and Parsons, 1998a). As a result, current feeding methods are being designed to improve control over feed inputs. The development of efficient feed management strategies in shrimp farming involves the investigation of aspects related to time of feed distribution, feeding rates, feeding frequency and feed dispersal methods. Such factors are primarily associated with the feeding behaviour of *Penaeus* spp. and the fluctuations of pond environmental parameters.

Under both cultured and natural conditions, food consumption in penaeid shrimp is characterised by irregular patterns (Wassenberg and Hill, 1987; McTigue and Feller, 1989; Reymond and Lagardère, 1990; Nunes *et al.*, 1996). Shrimp feeding rhythms respond to exogenous cyclic cues, such as tides (Sastrakusumah, 1971; Marte, 1980),

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water quality (Sastrakusumah, 1971; Nunes, 1998), natural food availability (Hill and Wassenberg, 1987; Wassenberg and Hill, 1987; Nunes *et al.*, 1996, 1997b) and light intensity (Hughes, 1969; Brisson, 1977; Reymond and Lagardère, 1990), as well as to physiological variables such as moult stage (Bursey and Lane, 1971; Huner and Colvin, 1979; Hill and Wassenberg, 1992) and age (Reymond and Lagardère, 1990; Nunes *et al.*, 1996; Nunes, 1997). In confined systems, artificial feeding may act as a feeding stimulus (Moller and Jones, 1975; Nunes *et al.*, 1996), inducing shrimp to emerge from the substrate (Kutty and Murugapoopathy, 1968; Hughes, 1969) and resume food intake (Moller and Jones, 1975; Nunes *et al.*, 1996). Studies indicate that in ponds, formulated food is better utilised by shrimp when feeding frequency is increased (Sedwick, 1979a; Robertson *et al.*, 1993). In addition, feeding times should be synchronised with the most active feeding times of a particular species (Cuzon *et al.*, 1982; Nakamura and Echavarria, 1989; Nunes *et al.*, 1996).

Traditionally in marine shrimp farms, formulated diets are administered through manual broadcasting from small boats or pond walls. More recently, semi-intensive shrimp farms have implemented a new technique of feed distribution (Salame, 1993; Berger, 1994; Rosenberry, 1994; Jory, 1995; Viacava, 1995; Jory, 1996; Nunes, 1996b; Goddard and Nunes, 1997). The method consists of concentrating dry pelleted food exclusively in feeding trays, as an attempt to reduce organic and nutrient pollution, and increase feed conversion efficiency. The effects of feed concentration versus feed broadcasting on food intake by *Penaeus subtilis* have never been investigated. The objective of the present study was to examine the feeding levels of *P. subtilis* in response to the method of food dispersal.

3.2 Materials and Methods

3.2.1 Study Site and Experimental Design

The study was conducted at a commercial marine shrimp farm (Tecnarão Tecnologia de Camarão Ltda., Ares, RN), located on the north-eastern coast of Brazil (Figure 3.1). The farm comprised a total area (*A*) of 95.68 ha, consisting of 53.51 ha of grow-out ponds (n = 15; mean *A* = 3.58 ha). For the study, a 4.65 ha grow-out pond, operating with a water level between 0.75 and 0.95 m was used (Figure 3.2).

Six open-bottom rectangular enclosures were strategically installed in a V position, facing the water inlet system (Figure 3.2). The enclosures were spaced 10 m apart, and placed 18 m and 33 m from the pond walls (enclosure number 1 and 6, respectively), occupying an individual area of 105 m^2 (7 x 15 m). During enclosure construction, wooden sticks were drilled into the pond bed, until a fixed position was obtained. These were surrounded by a white polyethylene 1.0-mm diameter mesh net with 1.20 m height (Tela Industrial Monofil, Monofil Companhia Industrial de Monofilamentos, Ponta Grossa, Paraná, Brazil), to form the sides. To avoid shrimp from escaping to the pond, the net was buried 0.1 m into the substrate and extended 0.2 m above the water level. Enclosures were installed in the pond before it was filled with water, but after sterilisation and fertilisation procedures.
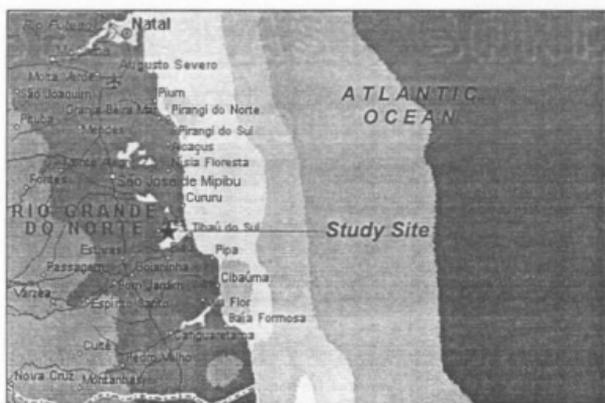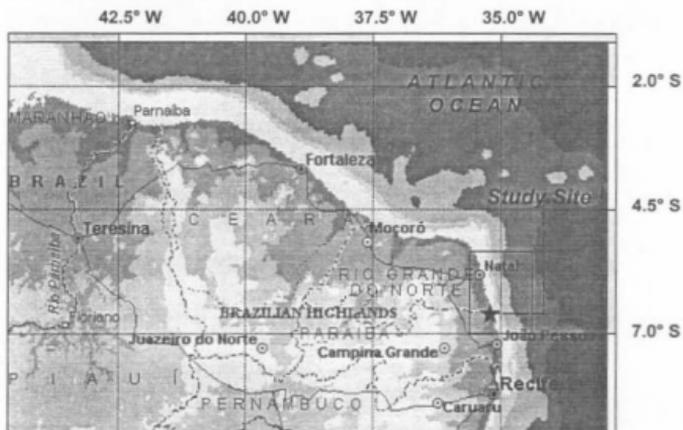
Figure 3.1: Geographical location of the study site in NE Brazil, State of Rio Grande do Norte.
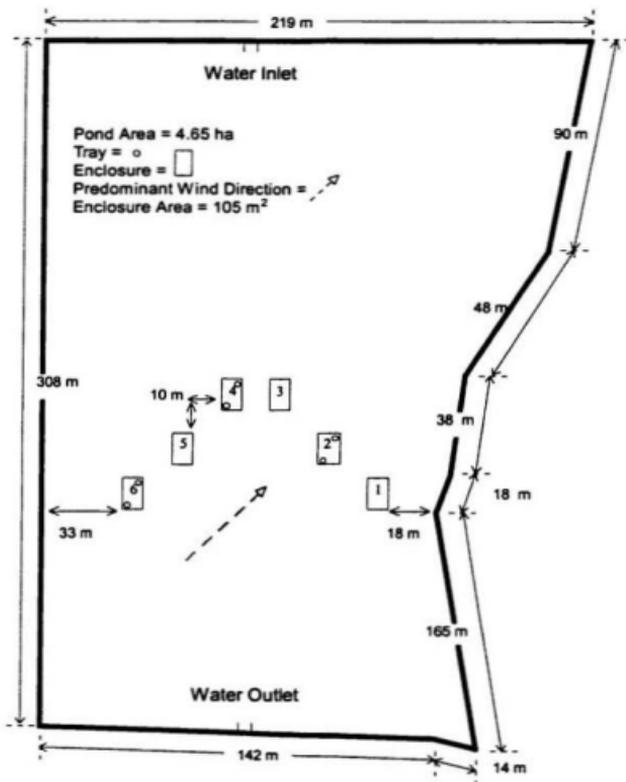
Figure 3.2: Schematic plan of the grow-out pond, indicating location of enclosures and feeding trays.

3.2.2 Pond Preparation, Stocking and Management

Initially, the pond bottom was exposed to the sun for 14 d until the substrate was completely dried. For fertilisation, a combination of triple superphosphate (600 g/ha) and urea (48 kg/ha), was added to the water to achieve a visibility of 35 cm. Concurrently, the pond was treated with sodium silicate at a rate of 750 cm^3/ha to enhance diatom productivity.

The study started on 16 October 1996 (stocking) and extended until 13 January 1997 (harvest). Ten thousand 23-d old post-larval (0.016 g body weight) *Penaeus subtilis*, produced in a penaeid shrimp hatchery (Aquatec Industrial Pecuária Ltda., Canguaretama, Rio Grande do Norte, Brazil), were transported to the farm area in plastic bags containing oxygen. Preceding stocking, post-larvae (PL) were slowly acclimated to temperature and salinity of the pond. A total of 1,500 shrimp was released in each enclosure yielding an initial stocking density of 14.3 PL/m^2. The external area of the enclosures was stocked with PL of the white shrimp *P. vannamei* at a density of 13.95 PL/m^2. No nursery culture phase was used.

Shrimp were fed a dried commercially produced pelleted food (MR-35 Ração Purina para Camarões, Ralston Purina do Brasil Ltda., São Lourenço da Mata, Pernambuco, Brazil). Feed proximate analysis indicated the following chemical composition: 8.7% moisture, 38.8% protein (N x 6.25, dry basis), 7.3% lipid, 12.2% ash and 33.0% carbohydrate. During the initial 39 d of culture, a mixture (1:1 ratio) of broken pellets (1.3 mm length by 2.3 mm diameter) and granules (1.9 mm diameter) was used.

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For the remaining rearing period, shrimp were fed pellets measuring 5.5 mm length by 2.3 mm diameter.

Distribution was conducted in two forms: feed dispersal over the whole area of the enclosure (*Broad*, feed dispersal treatment) and concentration of feed in feeding trays (*Conc*, feed concentration treatment). In each treatment, three replicates (*i.e.*, enclosures) were used. For *Conc* treatment, two feeding trays per enclosure were introduced at the opposite right and left sides. Trays were fabricated from the rubber and steel core sections of tires. They were circular, with an internal diameter of 51 cm ($A = 0.20 \text{ m}^2$), boarders measuring 4 cm in height, with a 1.3 mm diameter mesh net fixed on the bottom. Ropes were attached to each tray to allow their retrieval after immersion in the water.

Feed was administered in equal amounts three times a day, at 0600, 0930 and 1430 h, following feeding schedules of local commercial operations (Goddard and Nunes, 1997). Feeding rates were kept constant for all enclosures. Daily at each feeding time, trays were rinsed, brushed clean and inspected for uneaten feed which was then collected for weighing. Modifications in feed ration were made to all replicates according to average feed consumption observed from trays used in *Conc* treatment. However, feed quantities were always in excess of estimated feed consumption to avoid under feeding.

The abundance of polychaetes in the substrate was used as an indicator of the availability of natural food. The density of polychaetes was determined a day prior to each sampling period, starting on the 19th d of culture (*i.e.*, 20 d after PL stocking) and continuing throughout the complete study period (*i.e.*, 89 d). Collection of polychaetes was conducted with a PVC hand sampler, with a 9.8 cm diameter, ($A = 75.4 \text{ cm}^2$) and a length of 1.20 m. At each sampling period, three sub-samples per enclosure of about 10

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cm or less of upper sediment layer were collected at random, mixed thoroughly and analysed as a single sample (a total of six samples per sampling period, one for each enclosure). Separation and counting of polychaetes were perfomed according to the methodology described by Crockett *et al.* (1988).

Water was exchanged daily, following tidal fluctuations, at a rate of 5% of total pond volume/d. Salinity and transparency were monitored daily at 1000 h with a salinity refractometer (ATAGO Salinity Refractometer, model 2441-WO5) and a Secchi disk, respectively. After harvest, three replicates of substrate samples were randomly collected from each enclosure for chemical analysis. Prior to analysis, replicates were mixed thoroughly amounting to 1 kg of wet sediment/enclosure. Tests followed the methodology described by EMBRAPA (1979).

In the laboratory, soil samples were dried at 60 °C until a constant weight was obtained. Soil pH was measured with a potentiometer in a soil-to-water ratio of 1:1. Organic carbon was determined by oxidation of the organic matter in the soil with a solution of 0.4 N $K_2Cr_2O_7$. Organic matter was measured indirectly by multiplying the figure for organic carbon by 1.724. A neutral 1 N NH_4OAc was used for analysis of calcium, magnesium, potassium and sodium. Calcium and magnesium were determined by EDTA titration, and sodium and potassium by flame photometry. Exchangeable aluminium was measured by titration with neutral $0.1N$ NaOH, using phenolphthalein as indicator. Total nitrogen was determined by the Kjeldahl method. Phosphorus was extracted with a solution of 0.05 N HCl and 0.025 N H_2SO_4, and determined by colorimetry.

3.2.3 Shrimp Collection and Stomach Content Analysis

Collection of shrimp started on day 20 (D_{20}) of culture, *i.e.*, 21 d after stocking of PL, and continued on a 12-d interval until D_{80} of culture. Twenty shrimp per enclosure (1.3% of the initial stocked shrimp population) were sampled 30 min after feed distribution, at 0630, 1000 and 1500 h, totalling 2,160 specimens (*i.e.*, 20 shrimp x 3 collection hours x 6 sampling periods x 6 enclosures) collected during the extent of the rearing period. Shrimp were captured using a nylon cast net ($A = 13.3$ m^2). Immediately after capture, animals were immersed in cold water (1 °C) and kept under low temperature until biometric measurements were conducted within 4 h of collection.

In the laboratory, shrimp were sexed, the post-orbital carapace length (CL) measured and the wet body weight (BW) determined. Stomachs were dissected and weighed to the nearest milligram. Shrimp in proecdysis and ecdysis were not used in stomach content quantification because feeding declines or completely ceases during these stages (Drach and Tchernigovtzeff, 1967). The wet weight of the stomach contents was calculated applying the following formula:

$$W_C = W_S - W_{ES} \qquad (3.1)$$

where, W_C is the wet weight of the stomach food contents (g); W_S is the wet weight of the stomach, including existing food contents (g); and, W_{ES} is the wet weight of the stomach (g), without food contents.

W_{ES} was estimated with specimens of *Penaeus subtilis* with BW ranging from 2.624 to 21.570 g. Shrimp were collected from grow-out ponds and transported alive to

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the laboratory. Animals were stocked in tanks with constant aeration and starved for a 48-h period to allow a complete evacuation of the stomach contents. Following this period, shrimp were sexed, weighed and the CL measured. The empty proventriculus of each shrimp was removed, washed with distilled water, blotted dried and weighed on an electronic balance. Results were used to establish a relationship between W_{ES} and CL.

Identification of food in the stomachs was conducted using the stable carbon isotope mass spectrometry technique, following the methodology described by Nunes *et al.* (1997b). In the present case, the $\delta^{13}C$ examination aimed at providing direct information as to the relative occurrence of natural and artificial food in shrimp stomach contents, calculated as:

$$R_O = \left(\frac{\delta^{13}C_{sc} - \delta^{13}C_n}{\delta^{13}C_a - \delta^{13}C_n} \right) \times 100 \qquad (3.2)$$

where, R_O is the relative occurrence of carbon from artificial food (%); $\delta^{13}C_{sc}$ is the $\delta^{13}C$ of shrimp stomach contents (‰); $\delta^{13}C_n$ is the $\delta^{13}C$ of natural food (‰); and, $\delta^{13}C_a$ is the $\delta^{13}C$ of artificial food (‰). Since studies have indicated that polychaetes are the most significant natural food source in the diet of *Penaeus subtilis* cultured under semi-intensive systems (Nunes, 1995; Nunes *et al.*, 1997b), these organisms were chosen as the representative of pond natural food ($\delta^{13}C_n$) in the $\delta^{13}C$ analysis. Similarly, samples of dried pelleted feed were selected as the other food source ($\delta^{13}C_a$). Polychaete samples were collected after PL stocking, but prior to shrimp first feeding. For $\delta^{13}C_{sc}$, the stomach contents of a total of 27 shrimp per feeding treatment (*i.e.*, 3 shrimp x 3 enclosures/treatment x 3 feeding times) were collected at each sampling period, 30 min after feed distribution (at 0630, 1000 and 1500 h), mixed thoroughly and analysed as a single sample.

3.2.4 Statistical Analysis

Statistical analyses were performed with the Statistical Package for Social Sciences Windows version, release 7.5.1. (SPSS Inc., Chicago, Illinois, USA). Homogeneity of variance was examined for W_C and CL data by using Bartlett-Box F and Cochran's C tests. Kurtosis and skewness and their standard error (*i.e.*, s.e. kurtosis and s.e. skewness) were applied to the data as measures of asymmetry and tests of normality. Based on these results, data were logarithmically transformed to normalise and homogenise the variances and to meet statistical assumptions. W_C was adjusted to a log (W_C +1) scale and CL to a log (CL) scale.

3.3 Results

3.3.1 Water and Sediment Analyses

Salinity levels increased as the duration of the culture period increased, ranging from a minimum of 30‰ to a maximum of 41‰ [38 ± 2‰ (mean ± standard deviation)]. Water transparency (Figure 3.3) ranged from 18 to 40 cm (28 ± 7 cm).

Polychaete density was low and exhibited a high level of variance in all enclosures (324 ± 346 polychaetes/m²; Figure 3.4). A significantly higher (*t*-test, df = 17, P = 0.012) abundance of polychaetes was found in *Broad* treatment enclosures (354 ± 225 polychaetes/m²), when compared to the *Conc* treatment (295 ± 212 polychaetes/m²). In
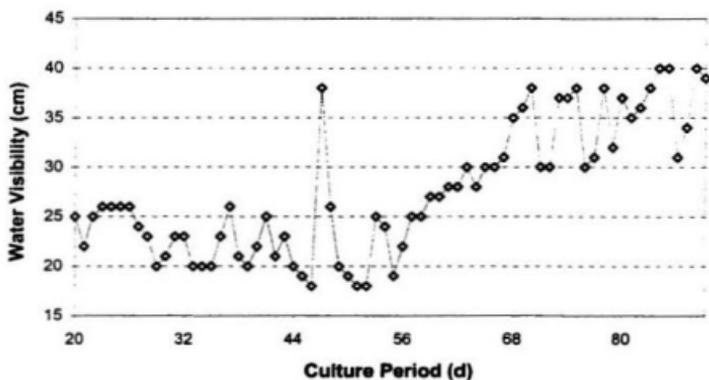
Figure 3.3: Daily fluctuations in water transparency (cm) over the grow-out cycle of *Penaeus subtilis*. Culture period (d) is the number of days after post-larvae stocking.
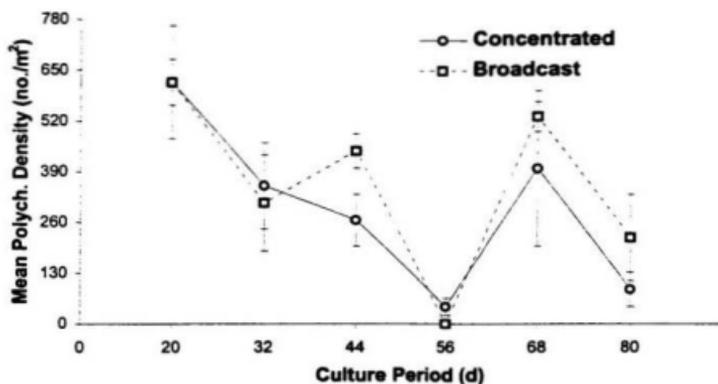
Figure 3.4: Mean polychaete density ± standard error (n = 3) in the pond sediment of six enclosures under two feeding methods (feed concentrated and feed broadcast) during a 89-d grow-out cycle of *Penaeus subtilis*. Values from the sample size of 75.43 cm^2 were converted to m^2.

general, the pattern of variation in the average number of polychaetes displayed a similar trend for both treatments. There was a continuous reduction in polychaete density until D_{56}, followed by an increase and then a recurrent decline on the final sampling day. The following polychaete families were identified in the pond bottom: Spionidae, Nereidae and Capitellidae.

Table 3.1 presents the chemical characteristics of the pond bottom for the six enclosures used in the study. Apart from sodium, no other chemical parameters showed statistically significant differences between feeding treatments. Final mean sodium concentration was statistically lower in the pond sediment when feed was concentrated in a single location compared to the feed broadcast treatment.

3.3.2 Relationship between the Wet Weight of the Empty Proventriculus and the Post-Orbital Carapace Length of *Penaeus subtilis*

Correlation analysis indicated a significant relationship at the $\alpha = 0.01$ level between the wet weight of the empty stomach of *Penaeus subtilis* (W_{ES} in g) and its post-orbital carapace length (CL in mm). The relationship (Figure 3.5) was expressed as a power function by the following equation: $W_{ES} = 4.4 \times 10^{-6} CL^{3.0392}$ ($r = 0.926$, $n = 101$, $P < 0.001$).

The above model was used to estimate the empty stomach wet weight of shrimp collected during the study period, based on measurements of its post-orbital carapace length (CL).

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Table 3.1: Chemical analysis of bottom samples from the grow-out pond after a 89-d growth cycle. Results are presented as means ± standard deviation for treatments feed broadcast and feed concentration. *t*-test data indicate contrasts between treatments.

Variable	Feeding Method Broadcast	Feeding Method Concentrated	*t*-test df	Sig. *P*
pH	8.2 ± 0.1	8.2 ± 0.1	-	-
Organic carbon (%)	1.17 ± 0.11	1.22 ± 0.02	4	0.497
Kjeldahl N (%)	0.09 ± 0.01	0.10 ± 0.01	4	0.374
C:N (%)	13 ± 1	12 ± 1	4	0.519
Organic matter (%)	2.02 ± 0.18	2.10 ± 0.03	4	0.511
Phosphorus (‰)	8 ± 4	6 ± 4	4	0.622
Calcium (meq/100 g)	19.2 ± 1.4	17.3 ± 1.5	4	0.202
Magnesium (meq/100 g)	19.5 ± 1.8	20.9 ± 1.3	4	0.323
Potassium (meq/100 g)	5.89 ± 0.85	5.45 ± 0.07	4	0.424
Sodium (meq/100 g)	37.14 ± 4.63	25.30 ± 0.53	4	0.012
Aluminium (meq/100 g)	<0.01	<0.01	-	-

$$W_{ES} = 4.4 \times 10^{-6} CL^{3.0392}$$
$$r = 0.926; P < 0.001$$
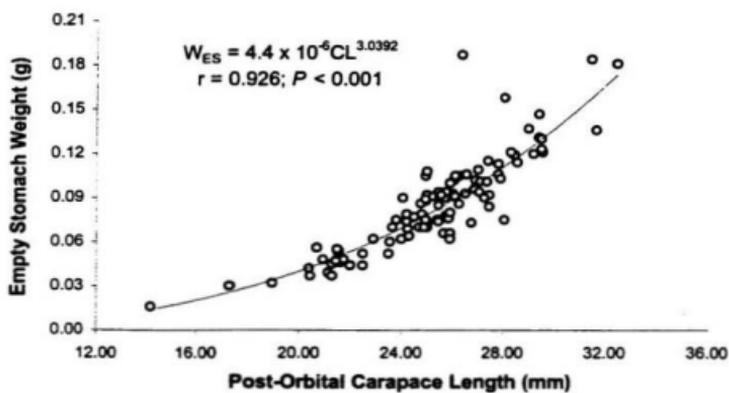
Figure 3.5: Relationship between wet weight of empty stomach, W_{ES} (g) and post-orbital carapace length, CL (mm) of *Penaeus subtilis*.

3.3.3 Growth Determination

Correlation analysis provided evidence of a strong relationship between post-orbital carapace length (CL) and wet body weight (BW) of *Penaeus subtilis* (BW = $0.0023CL^{2.6527}$, r = 0.988, n = 1,814, $P < 0.001$; Table 3.2). Thus, measurements of CL were used for statistical analyses as the main index of growth of *P. subtilis*. Standard error of kurtosis and skewness for CL were 0.115 and 0.057, respectively. Measures of mean growth rate, survival, final biomass and food conversion ratios (FCR) for *P. subtilis* are presented in Table 3.3.

A gradual, but continuous increase in CL and BW was observed throughout the study period (Table 3.2). Overall shrimp growth was statistically different between treatments *Broad* and *Conc* (two-way ANOVA, $F_{1, 1,802} = 11.65$, $P = 0.001$, Table 3.2). Differences however, were very small, as indicated by separate analyses of each individual sampling period (*t*-test, Table 3.2).

Mean CL of males (16.68 ± 3.01 mm) was not significantly larger (MANOVA, $F_{1, 1,786} = 2.33$, $P = 0.127$) than that of females (15.89 ± 3.88 mm). Average CL of shrimp sampled at 0630 h (16.19 ± 3.72 mm), 1000 h (16.18 ± 3.51 mm) and 1500 h (16.26 ± 3.48 mm) were not statistically different (one-way ANOVA, $F_{2, 1,811} = 0.34$, $P = 0.709$).

Table 3.2: Comparison of shrimp biometric data among culture periods (d) and feeding methods. Results are reported as means ± standard deviation for n individuals. Numbers in parentheses indicate minimum and maximum values. *P* values present degree of significance for post-orbital carapace length (CL, in mm) by feeding method. BW refers to shrimp wet body weight (g).

Culture	Broadcast			Concentrated			t-test
Period (d)	n	BW (g)	CL (mm)	n	BW (g)	CL (mm)	Sig. P
20	178	1.544 ± 0.559	11.60 ± 1.72	180	1.376 ± 0.525	11.00 ± 1.77	0.002
		(0.401 - 2.919)	(6.85 - 15.10)		(0.290 - 2.672)	(5.40 - 14.35)	
32	178	2.938 ± 1.108	14.63 ± 2.16	182	2.901 ± 0.990	14.62 ± 1.97	0.899
		(0.811 - 6.075)	(9.00 - 19.50)		(0.800 - 6.456)	(9.40 - 19.20)	
44	145	4.170 ± 1.310	16.66 ± 1.95	180	3.942 ± 1.322	16.36 ± 2.37	0.169
		(1.102 - 8.908)	(10.45 - 20.75)		(0.859 - 8.790)	(9.80 - 26.90)	
56	120	5.338 ± 1.333	18.45 ± 1.70	180	5.206 ± 1.410	18.16 ± 1.94	0.161
		(1.660 - 11.853)	(11.90 - 24.10)		(1.452 - 11.477)	(11.20 - 24.30)	
68	119	6.095 ± 1.292	19.42 ± 1.58	145	5.909 ± 1.458	18.97 ± 1.67	0.028
		(2.266 - 12.836)	(13.70 - 22.55)		(2.906 - 10.667)	(14.30 - 22.55)	
80	88	6.814 ± 1.338	20.41 ± 1.66	119	6.769 ± 1.189	20.18 ± 1.45	0.330
		(3.431 - 11.209)	(15.55 - 24.40)		(3.147 - 9.548)	(14.75 - 23.35)	

Feeding Method

Table 3.3: Growth rate, survival, final biomass and food conversion ratios of *Penaeus subtilis* for treatments feed broadcast and feed concentration over a 89-d rearing cycle. Data presented as means ± standard deviation.

Feeding Method	Growth Rate[a] (g/week)	Survival (%)	Final Biomass (kg)	Food Conversion Ratio
Broadcast	0.668 ± 0.734	69.0 ± 6.9	6.27 ± 2.08	5.00 ± 0.45
Concentrated	0.663 ± 0.764	71.9 ± 3.7	7.27 ± 0.44	4.55 ± 0.30

[a]Sample size of 829 (broadcast) and 986 (concentrated) shrimp.

3.3.4 Feeding Method

Of 2,160 collected specimens of *Penaeus subtilis*, a total of 1,780 animals were in the intermoult stage. From this number, 393 stomachs or 22.08% were empty (*i.e.*, $W_C \leq$ 0.). All stomachs, including those with values of $W_C \leq 0$, were used for quantification analysis of the stomach contents. Standard error of kurtosis and skewness for W_C were 0.116 and 0.058, respectively.

Weight (log transformed) of the stomach contents of *Penaeus subtilis* was statistically different between the two feeding methods examined (MANOVA, $F_{1, 1,744} =$ 150.93, $P < 0.001$). On average, shrimp fed under the *Broad* treatment contained more food in their stomachs (0.012 ± 0.010 g) than those fed under the *Conc* treatment (0.007 ± 0.007 g). Also, relatively more stomachs were empty when feed was concentrated (29.47% or 285 stomachs) than when formulated food was manually broadcast (13.28% or 108 stomachs). Further analysis revealed that broadcasting produced statistically higher stomach content weights in all sampling periods (Figure 3.6).

Stomach content weight was statistically different among the sampling periods (MANOVA, $F_{5, 1,744} = 31.00$, $P < 0.001$). Separate analysis of data showed that differences in the more intermediate stages of culture, particularly D_{32}, D_{44}, D_{56} and D_{68} for feed broadcasting and D_{32}, D_{44} and D_{56} for feed concentration, were not statistically different (Scheffé's Multiple Range Test, $P > 0.05$). This indicated a more uniform pattern of food consumption along the rearing cycle as opposed to an increasing consumption of food with shrimp growth.

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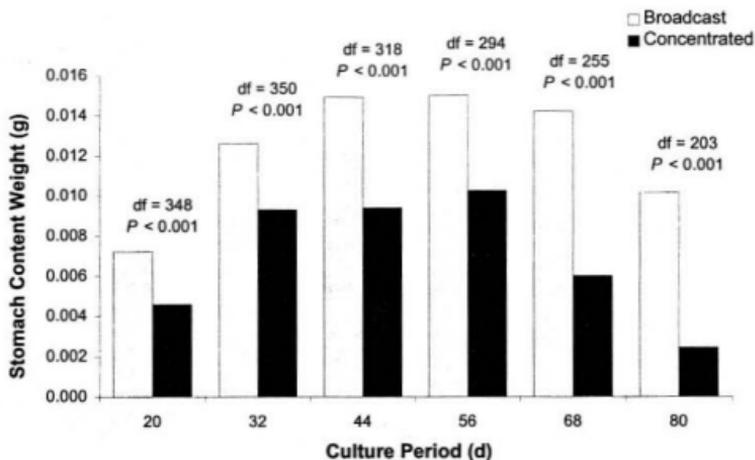
Figure 3.6: Mean stomach content weight (g) 30-min after feeding of *Penaeus subtilis* for two methods of feed distribution (broadcast and concentrated) over a 89-d culture period. Numbers on top of bars indicate results from two-tailed *t*-tests.

No statistically significant differences were found between the stomach content weights of males (0.009 ± 0.009 g W_C) and females (0.010 ± 0.010 g W_C) of *Penaeus subtilis* (*t*-test, df = 1,775, $P = 0.125$).

3.3.5 Time of Feed Distribution

Log transformed stomach content weights were significantly different at 0600, 0930 and 1430 h (MANOVA, $F_{2, 1744} = 20.60$, $P < 0.001$). Mean stomach content weights varied from 0.008 ± 0.009 g at 0600 h and 0.009 ± 0.009 g at 0930 h to 0.011 ± 0.011 g at 1430 h. No statistically significant interaction was found between feeding method and time of feed distribution (MANOVA, $F_{2, 1744} = 0.41$, $P = 0.667$).

A significant interaction however, was observed between sampling period and time of feed distribution (MANOVA, $F_{10, 1744} = 3.55$, $P < 0.001$). Scheffé's Multiple Range Test revealed that food intake at 0600 h was significantly lower compared to the 0930 h and 1430 h in sampling periods D_{20} and D_{56}. In general, feed distribution at early afternoon, *i.e.*, at 1430 h, produced a higher mean stomach content weight for shrimp of 14.63 ± 2.06 mm CL and 16.49 ± 2.19 mm CL (D_{32} and D_{44}, respectively), together with feed distribution at 0930 h in intermediate to late culture periods, *i.e.*, D_{56} and D_{68} (Scheffé's Multiple Range Test, $P < 0.05$). On D_{80}, no statistically significant differences were found for stomach content weights among times of feed distribution (Scheffé's Multiple Range Test, $P > 0.05$). In summary, stomach content weight data indicated that

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shrimp captured in the early and late stages of culture showed lower stomach content weights at 0600, 0930 and 1430 h than in the more intermediate stages (Figure 3.7).

3.3.6 Relative Source of Food in Shrimp Stomach Contents

The stable carbon isotopic composition of *Penaeus subtilis* stomach contents collected over the rearing period, as well as the $\delta^{13}C$ values for artificial and natural food, are presented in Table 3.4. $\delta^{13}C_{sc}$ values gradually became more negative as the culture period progressed, towards the $\delta^{13}C$ of artificial food (*i.e.*, -22.770 ± 0.002‰), reflecting a greater percentage of pelleted feed in shrimp stomach contents.

Calculations revealed that for both methods of feeding (*i.e.*, *Broad* and *Conc*), over half of the food contained in *Penaeus subtilis* stomach contents (30 min after feed distribution) was derived from pelleted feed (except on D_{20}, Figure 3.8). Although the overall relative occurrence of formulated food in the stomach contents of shrimp fed under the *Broad* treatment (73.24 ± 16.10%) was not statistically different (*t*-test, df = 10, *P* = 0.428) from that of the *Conc* treatment (65.84 ± 14.88%), comparisons at each culture period indicated a higher percentage of formulated food for shrimp in the *Broad* treatment (except on D_{32}, Figure 3.8).
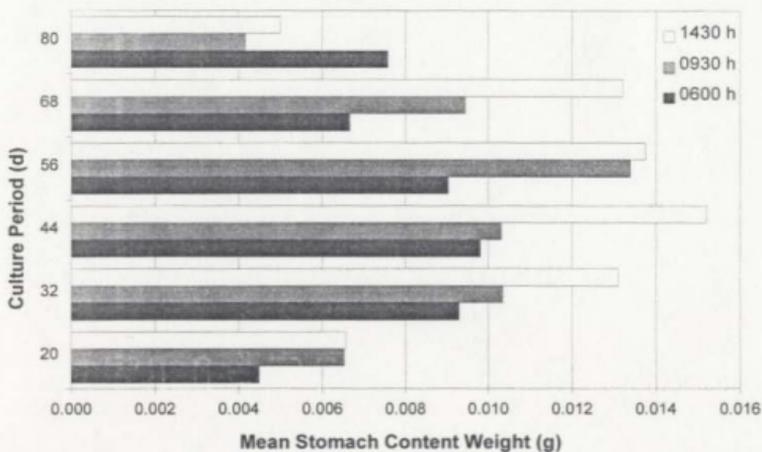
Figure 3.7: Pattern of food intake of *Penaeus subtilis* at three feed distribution times (0600, 0930 and 1430 h) over the course of a 89-d rearing cycle. Culture period represents the number of days of rearing after stocking grow-out pond with post-larvae.

Table 3.4: Carbon isotopic data [mean $\pm$ standard deviation[a] (n^b = 8)] of *Penaeus subtilis* stomach contents ($\delta^{13}C_{sc}$) throughout the rearing period for treatments feed broadcast and feed concentration. Each value represents a mixture of the stomach contents from a total of 27 shrimp. $\delta^{13}C$ of artificial food ($\delta^{13}C_a$) and polychaetes ($\delta^{13}C_n$) were -22.770 $\pm$ 0.002‰ and -17.109 $\pm$ 0.006‰, respectively.

| | $\delta^{13}C_{sc}$ (‰) | |
Culture Period (d)	Broadcast	Concentrated
20	-19.571 $\pm$ 0.006	-19.181 $\pm$ 0.004
32	-20.896 $\pm$ 0.008	-21.433 $\pm$ 0.008
44	-21.460 $\pm$ 0.004	-21.190 $\pm$ 0.011
56	-21.903 $\pm$ 0.005	-21.142 $\pm$ 0.008
68	-21.749 $\pm$ 0.010	-21.315 $\pm$ 0.005
80	-21.950 $\pm$ 0.008	-20.758 $\pm$ 0.005

[a]Refers to analytical variability.

[b]Number of measurements recorded for each sample.

Figure 3.8: Relative occurrence of formulated versus natural food in the stomach of *Penaeus subtilis*, 30-min after feed distribution, as indicated by stable carbon isotope mass spectrometry analysis. Results are presented for feed broadcasting (A) and feed concentration (B) over six sampling periods.

D₂₀ - A   57%   43%
D₂₀ - B   63%   37%

D₃₂ - A   33%   67%
D₃₂ - B   24%   76%

D₄₄ - A   23%   77%
D₄₄ - B   28%   72%

D₅₆ - A   15%   85%
D₅₆ - B   29%   71%

D₆₈ - A   18%   82%
D₆₈ - B   26%   74%

D₈₀ - A   14%   86%
D₈₀ - B   36%   64%

☐ Artificial food   ■ Natural food
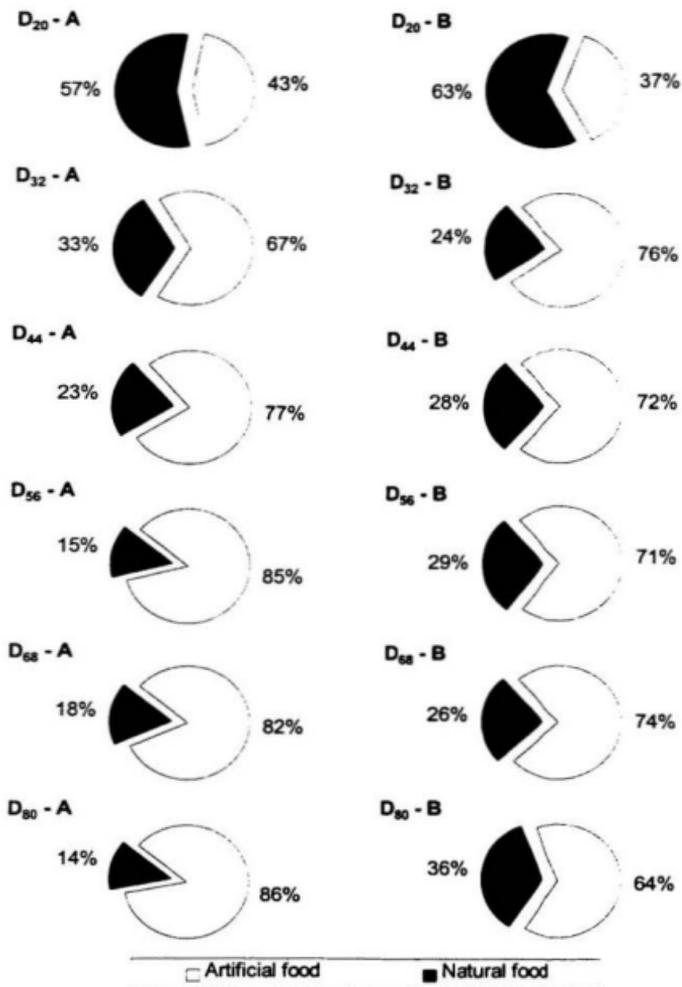
3.4 Discussion

3.4.1 Polychaete Abundance

In the present study, the effects of shrimp predation were probably the leading cause for the continuous declines in polychaete density in the pond. Other studies have also related the reduction in the abundance of polychaetes in ponds to shrimp grazing pressure (Rubright, 1978; Rubright et al., 1981; Ordner and Lawrence, 1987; Wyban et al., 1987; Gonzales, 1988; Lanari et al., 1989; Martins, 1994; Hopkins et al., 1995). The increases detected on D_{68} were most likely a reproduction response of the organism to a high predatory activity by Penaeus subtilis. In marine aquatic invertebrates, low survival of adults due to disease or predation, may result in an increased investment in reproduction (Calow, 1984).

The high level of variance in polychaete density observed for treatments Broad and Conc precludes meaningful conclusions concerning the effects of feeding strategy on polychaete occurrence. Results suggest however, that polychaetes may be used as a reliable indicator of natural food availability for Penaeus subtilis. Polychaetes may be composed of up to 60% protein (pers. obs.), and contribute as much as 32% the diet of P. subtilis (Nunes et al., 1997b). Thus, their abundance in the pond bottom may serve as an indicator the growth potential of this species. Hunter et al. (1987) noted that a major requirement for the effective use of nutritionally incomplete pond feeds is a consistent supply of natural food; otherwise shrimp growth depression occurs. In the present study, the

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low growth rates of *P. subtilis* may be attributed to the reduced availability of polychaetes. Nunes (1995) reported that under densities between 589 ± 236 and 9181 ± 7518 polychaetes/m^2, *P. subtilis* attained a final weight of 14.60 g after a 60-d rearing cycle, with growth rates ranging from 0.98 to 1.82 g/week (stocking density of 10.11 shrimp/m^2, 63% shrimp survival).

3.4.2 Sediment Quality

Sediment chemical analysis indicated statistically higher levels of sodium in the pond bottom of the *Broad* treatment, which may have been derived from uneaten feed. Sediment chemical characteristics however, were considered normal for both methods of feed distribution. Parameters indicative of pond eutrophication such as pH, organic carbon, Kjeldahl nitrogen, Phosphorus and organic matter were not significantly different between treatments and were within levels reported in other studies (Schroeder *et al.*, 1991; Boyd and Pippopinyo, 1994; Gonzalez-Vila *et al.*, 1996; Smith, 1996). Boyd *et al.* (1994) investigating the sediment composition of intensive shrimp ponds in Thailand, reported pH values between 7.42 and 7.56 and organic matter ranging from 1.12 to 1.90%. In general, concentrations of organic matter in ponds between 1 and 5% are considered normal (Queiroz and Boyd, 1997), hence not eutrophic. It is possible that under semi-intensive conditions, feeding rates (30 kg/ha/d of formulated food or less) are not sufficiently high to cause negative effects on sediment chemical quality, even if uneaten feed accumulates. The total amount and composition of organic matter in

sediments from semi-intensive shrimp ponds has recently been found to not change significantly after a grow-out culture period (Gonzalez-Vila *et al.*, 1996). According to Smith (1996) the sediment which accumulates in shrimp ponds is derived primarily from eroded soil of the pond bottom and walls, not from uneaten pelleted feed.

3.4.3 Relative Amount of Artificial Food in *Penaeus subtilis* Stomach Contents

Stable carbon isotope mass spectrometry analysis revealed that, 30 min after feed distribution, over half of the food contained in the stomach contents of *Penaeus subtilis* was derived from artificial food, regardless of the feeding method used (starting on D_{32}). These findings correspond with observations by Nunes *et al.* (1996), who reported that under semi-intensive conditions, this species consumes most pellets within 0.5 h after feed distribution. This feeding behaviour probably results from the reduction of chemoattractability and phago stimulative properties of the feed after immersion in sea water. Cuzon *et al.* (1982) suggested that leaching of soluble nutrients from feed caused a decrease in the level of formulated food consumed by shrimp.

The greater percentage of formulated food and the higher W_C found in *Penaeus subtilis* fed under the *Broad* treatment (except on D_{32}), indicated this method resulted in a greater consumption of feed. Similarly, the lower ingestion of artificial food in the *Conc* treatment, suggests it takes individual shrimp longer to locate feed when it is concentrated in a single location. The time interval from the distribution of the feed to consumption by shrimp, has important consequences on the nutritional quality and value of feed. Within 1

h of water exposure, a dry pelleted shrimp diet can lose 89% of ascorbic acid, 19% of dry matter, 11% of protein and 8% of carbohydrate from its initial composition (Cuzon *et al.*, 1982).

The progressive increase in the percentage of formulated food in the diet of *Penaeus subtilis* over the course of the culture period, probably reflects, in part, the time required for shrimp to become accustomed to formulated food. The increase in feed consumption may have also been the result of a lower availability of prey organisms, such as polychaetes, in the pond substrate. Although some *Penaeus* spp. tend to be more carnivorous as they attain larger body sizes (George, 1974; Das *et al.*, 1982; Stoner and Zimmerman, 1988; Reymond and Lagardère, 1990; Nunes *et al.*, 1997b), in semi-intensive systems, availability of potential prey organisms declines as the culture period progresses (Ordner and Lawrence, 1987; Allan and Maguire, 1992; Hopkins *et al.*, 1995; Nunes, 1995), thus requiring higher supplementation rates of formulated food (Akiyama, 1993; Jory, 1995; Viacava, 1995).

3.4.4 Effects of Feed Dispersal Method on Growth and Food Intake of *Penaeus subtilis*

The final shrimp survival found each of the treatments *Broad* and *Conc*, is comparable to that obtained within the industry (between 50.6 and 85.1% after 80 d of culture; Jory, 1995). The high FCR (between 4.55 ± 0.30 and 5.00 ± 0.45) observed in the present study reflects the provision of excess feed and thus the inability of shrimp to ingest and convert

the quantities of formulated inputs given into body weight. The rations provided were based on observed feed consumption from feeding trays. However, feeding rates always exceeded observed food consumption to avoid the possibility of under feeding.

In general, feed distribution methods did not generate statistically significant differences in shrimp growth between feeding treatments (except on D_{20} and on D_{68}). Under semi-intensive systems, growth as a response to feeding method is a less perceptible and reliable indicator of feed utilisation by shrimp. Under such conditions, insufficiency of pelleted feed may be compensated by a higher grazing activity on natural food. Investigations indicate that in the presence of naturally available pond organisms, such as polychaetes, copepods and remains of aquatic macrophytes, *Penaeus subtilis* derives only ¼ of its carbon growth from pelleted feed (Nunes *et al.*, 1997b).

In the present study, however, when feed was spread over the culture area, individual shrimp had a greater access to formulated food, as observed by the lower variability in stomach content weight [C.V. = 83% (*Broad*) versus 100% (*Conc*)], the higher level of food intake (as indicated by mean stomach content weights) and the higher occurrence of formulated food in the stomach contents of shrimp fed under the *Broad* treatment (as shown by the stable carbon isotope mass spectrometry analysis). In contrast, food intake in the *Conc* treatment was less, possibly due to the limited number of shrimp that could simultaneously access the formulated food at each feeding site. Similar observations were reported for *Penaeus semisulcatus* cultured in tanks (Rasheed and Bull, 1992). Foraging activity of this species was more efficient when food was evenly dispersed over the water surface than presented in a single pile. The authors concluded

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that feeding efficiency could be increased and the socio-behavioural impacts of a high shrimp stocking density reduced by spreading food uniformly over the substrate.

The higher consumption of food for the *Broad* treatment has some behavioural implications. Concentration of formulated food probably caused *Penaeus subtilis* to spend more time searching for the food source. The greater search time, coupled with their non-aggressive habit (Goddard, 1996a,b), resulted in a lower consumption of feed and a greater number of empty stomachs. The characteristic mode of food detection used by *Penaeus* spp. (Hindley and Alexander, 1978), also suggests that a homogenous dispersal of feed favours more encounters with food. Feed concentration may have also intensified inter-animal communication. In aquaculture systems, Lee and Meyers (1997) report that such conditions may suppress the effect of feeding attractants released from feeds. Although penaeid shrimp are not territorial (Dall *et al.*, 1990), they tend to avoid prolonged contact with conspecifics by taking evasive action (Rasheed and Bull, 1992).

In fish and eel culture, a uniform distribution of feed assists smaller individuals in obtaining food and consequently maintaining consistent body sizes among the cultured population (Jobling, 1983; Wickins, 1983). In a study under commercial culture conditions, Nunes (unpublished data) observed that feed concentration progressively increased the disparity of *Penaeus vannamei* body size. Shrimp BW variation ranged from 0.41 g after 2 weeks of culture to 3.02 g after 13 weeks of culture.

3.4.5 Patterns of Food Intake with Shrimp Body Size

No periodicity in food intake along sampling periods could be related to increases in CL. Average W_C was constant throughout the rearing cycle, except when contrasting median (D_{32}, D_{44}, D_{56} and D_{68}) with earlier and later sampling periods (D_{20} and D_{80}). Among shrimp however, there was a high variation in the weight of food content, even when animals of similar sizes were compared. Therefore, a poor relationship between CL and W_C of *Penaeus subtilis* resulted.

Under laboratory conditions, in the presence of pelleted feed alone, both the stomach volume of *Penaeus subtilis* as well as its maximum meal size increase proportionally to increases in its BW and CL (Nunes, 1997; Nunes and Parsons, in review; Chapter 4). However, under natural and culture conditions such an escalating trend in the level of food consumption with penaeid shrimp size has not been reported. McTigue and Feller (1989) observed no clear patterns of variation in the gut content weights with the body size of *P. setiferus* collected from tidal creeks in South Carolina. Nunes (1995) working in a semi-intensive system, concluded that the amount of food consumed by *P. subtilis* did not differ significantly among growth periods, despite constant increases in shrimp body sizes. The author related the lack of correlation between shrimp BW and the amount of food in the stomachs with a reduced availability of food components, such as natural prey and formulated food. Although a reduction in polychaete density was found in the present study, the quantity of formulated food was always maintained above observed levels of shrimp feed consumption. Under culture

conditions, the amount of food ingested relative to shrimp stomach volume, may actually decline as shrimp grow, as observed in the present study (mean W_C/CL, Figure 3.6) and by Nunes *et al.* (1997b). The authors reported that as the stomach volume of *P. subtilis* progressively expanded with growth, relatively less food per stomach volume was consumed (as indicated by stomach content analysis).

The uniformity in levels of food consumption during the rearing cycle was most likely related to physiological changes in *Penaeus subtilis*. In *Penaeus* spp. growth rates, food conversion efficiency and nutritional requirements, all decrease with an increase in body size (Colvin and Brand, 1977; Sedgwick, 1979a; Romero, 1983; Lee and Lawrence, 1985; Dall *et al.*, 1990; Akiyama *et al.*, 1992; Lawrence and Lee, 1997). Thus, under culture conditions, increases in shrimp body size should be accompanied by either a declining or a constant pattern of food intake. In semi-intensive systems, such a pattern in *P. subtilis* is probably balanced by shifts in the nutritional quality of its diet. Nunes *et al.* (1997b) reported gradual declines in the intake of plant material and detritus with growth of *P. subtilis*, combined with an increase in prey consumption, mainly polychaetes.

3.4.6 Food Intake as a Response to Time of Feeding

Penaeus subtilis food intake was significantly influenced by time of artificial feeding. Generally, for both treatments, *i.e.*, *Broad* and *Conc*, feed distribution at 1430 h and at 0930 h had statistically higher stomach content weights when compared to 0600 h. Nunes *et al.*

(1996) proposed that consumption of food during the early morning hours by *P. subtilis* could be suppressed by characteristically lower concentrations of dissolved oxygen (DO).

In semi-intensive shrimp ponds, morning DO is lowest at around dawn, and then progressively increases until reaching a peak in the late afternoon, right before sunset, then declining during night times (Goddard, 1996a; Nunes, 1998). Average levels of DO, at around 0700 h, have been reported to be as low as 3.05 mg/L (Nunes, 1998). Seidman and Lawrence (1985) found that severe hypoxia (DO < 1 mg/L) significantly reduced the growth rates of *Penaeus vannamei* and *P. monodon*. Low levels of food ingestion associated with low DO concentration was suggested as the main cause of poor growth of these species (Lee and Lawrence, 1997). Sandberg *et al.* (1996) examined the critical oxygen levels in relation to the predation efficiency of the brown shrimp *Crangon crangon*, and found a significant reduction in the predation rate at a 30% DO concentration. Because DO concentrations, in semi-intensive shrimp ponds, follow daily cycles which do not change substantially from one site to another (pers. obs.), higher W_C found at 0930 and at 1430 h for *P. subtilis* was possibly enhanced by higher DO levels when compared to 0600 h.

The overall results indicated that feeding levels of *Penaeus subtilis* were more pronounced when food was broadcast over the culture area. Feed distribution method, however, had no detectable impact on shrimp growth and on the chemical quality of the sediment. Feeding at 0930 h and at 1430 h produced a higher food consumption when compared to that at 0600 h, meaning that early morning feeding is not advisable for *P. subtilis*. The high incidence of feed found in shrimp 30-min after its distribution corroborates observations of Nunes *et al.* (1996), who reported that most feed is ingested

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by this species within this period. The low growth rates obtained in the present study emphasize the requirement for a consistent and abundant supply of natural food, particularly polychaetes, when nutritionally incomplete diets are used with pond culture of *P. subtilis*. These results also establish the need for further investigations on the development of feed management strategies that produce a sustainable use of naturally occurring pond food organisms.

3.5 Conclusions

Results from the present investigation indicate that broadcasting was a more effective method of feed distribution with respect to *Penaeus subtilis* food intake. This feeding practice resulted in a greater access and a higher consumption of food among the cultured shrimp population, a lower number of empty stomachs and a greater occurrence of formulated food in the shrimp's diet. Shrimp growth was not significantly different between feeding treatments and no short-term detrimental effects on sediment chemical quality were evident. Feed distribution at 1430 h and at 0930 h produced statistically higher stomach content weights when compared to that of 0600 h.

Feeding levels of *Penaeus subtilis* were almost uniform throughout the rearing cycle, despite progressive increments in shrimp carapace length. As a result, no periodicity in food intake along sampling periods could be related to increases in *P. subtilis* body size. The relative occurrence of formulated feed in the shrimp's diet however, successively increased over the culture period. This study also revealed that 30-

min after feed distribution over half of the food contained in *P. subtilis* stomach contents, was derived from artificial food, regardless of the feeding method used.

CHAPTER 4

SIZE-RELATED FEEDING AND GASTRIC
EVACUATION MEASURES FOR THE SOUTHERN
BROWN SHRIMP *Penaeus subtilis*

4.1 Introduction

Descriptions of the relationship of food consumption to penaeid shrimp size have ranged from a uniform (in cultured conditions, Nunes, 1995; Nunes and Parsons, 1999; Chapter 3), declining (in cultured conditions, Hunter *et al.*, 1987) or undetectable (in the wild, McTigue and Feller, 1989) pattern to an increasing trend (in laboratory-controlled conditions, Sick and Baptist, 1973; Sick *et al.*, 1973; Sedgwick, 1979a; Nunes, 1997). Despite these conflicting reports from studies conducted in different environments, the amount of food eaten by penaeids appears to be ultimately associated with their body size, as shown for the larval to juvenile stages (Sick and Baptist, 1973; Sick *et al.*, 1973; Sedgwick, 1979a; Chu and Shing, 1986; Kurmaly *et al.*, 1989; Wong *et al.*, 1989; Chen and Chen, 1992) and for other crustaceans [euphausiid (Heyraud, 1979), cladoceran (Malhotra and Langer, 1989); copepods (Paffenhöfer, 1971; Mauchline, 1998); *Artemia* spp. (Abreu-Grobois *et al.*, 1991; Nimura *et al.*, 1994), caridean shrimp (Katre and Reddy, 1977; Kumlu and Jones, 1995), crayfish (Villarreal, 1991; McClain, 1995)]. Penaeid aquaculture operations use the wet body weight of shrimp, combined with

estimates of survival and food conversion ratios to determine empirically feeding rates and feeding frequencies. Inherent to these practices is the assumption that as shrimp attain larger body sizes, higher food rations must be provided at more frequent intervals. However, only generic information on penaeid ingestion and foregut clearance rates is available.

Some studies indicate that *Penaeus* spp. can fill and empty their proventriculus rapidly [in 10 min and in 2 to 4 h, respectively (Marte, 1980; Cockcroft and McLachlan, 1986; Hill and Wassenberg, 1987; Hentschel and Feller, 1990)], with defecation starting within 1 to 6 h after feeding (Dall, 1968; Al-Mohanna and Nott, 1987). Food is ingested in small amounts (Sick and Baptist, 1973; Marte, 1980) as foreguts represent only 2 to 3% of their body weight (Wassenberg and Hill, 1987). While their feeding periodicity is still unresolved (McTigue and Feller, 1989; Nunes *et al.*, 1996), in wild and cultured environments food can be found in their stomachs at almost any time (Wassenberg and Hill, 1987; McTigue and Feller, 1989; Reymond and Lagardère, 1990; Nunes *et al.*, 1996), agreeing with the belief that consumption occurs while an earlier meal is still being digested (Hall, 1962; Dall, 1968; Hill and Wassenberg, 1987). Satiation is suggested to be controlled by the loading capacity of their digestive gland [*P. semisulcatus* (Al-Mohanna and Nott, 1987)], where final digestion and absorption of nutrients take place (Dall and Moriarty, 1983; Dall, 1992).

Feeding by penaeid shrimp has been associated with factors such as the period and frequency of exposure to the food [*Penaeus setiferus* (Sick *et al.*, 1973) and *P. merguiensis* (Sedgwick, 1979a), respectively], moulting activity [*P. esculentus* (Hill and Wassenberg, 1992)], ration size [*P. setiferus* (Sick *et al.*, 1973) and *P. merguiensis*

(Sedgwick, 1979a)], light intensity [*P. setiferus* (Sick *et al.*, 1973; McTigue and Feller, 1989)], feed dispersal [*P. subtilis*, (Nunes and Parsons, 1999; Chapter 3)], formulated diet composition and palatability [*P. duorarum* (Sick and Baptist, 1973), *P. merguiensis* (Sedgwick, 1979b), *P. japonicus* (Guillaume *et al.*, 1989), *P. vannamei* (Holland and Borski, 1993) and *P. monodon* (Sarac *et al.*, 1993)], pellet feed size [*P. subtilis* (Nunes *et al.*, 1997a; Nunes and Parsons, 1998b; Chapter 2)], time of day [*P. japonicus* (Reymond and Lagardère, 1990) and *P. subtilis* (Nunes *et al.*, 1996)] and water quality conditions [*P. japonicus* (Liao, 1969), *P. brasiliensis*, *P. paulensis* (Brisson, 1977) and *P. subtilis* (Nunes, 1998)]. Studies attempting to optimise feeding methods in penaeid aquaculture have often relied on results of growth, survival and food conversion ratios (Sedgwick, 1979a; Hernandez-Llamas *et al.*, 1993; Robertson *et al.*, 1993; Viacava, 1995; Cardona and Jory, 1997; Martinez-Cordova *et al.*, 1998a). At present, data relating to maximum ration, ingestion rates, faecal production rates, foregut evacuation and appetite revival are lacking for penaeids, despite their relevance to the development of models aimed at maximising food use in marine shrimp culture systems. The present study was conducted to examine and determine the effects and relationships of shrimp body size on quantitative feeding and evacuation parameters of *P. subtilis*.

4.2 Materials and Methods

4.2.1 Collection of Shrimp and Size Classification

Specimens of *Penaeus subtilis* were collected from nursery and grow-out ponds at a commercial marine shrimp farm (Artemisa Aquicultura S.A.) located on the north-eastern coast of Brazil, Acaraú, Ceará. Animals were transported alive in 50-L covered containers containing constatly aerated, cooled sea water (20 °C) to a laboratory 4 h distant from the sampling site. Collected animals had been raised under extensive conditions (stocking density of 2 shrimp/m², in the presence of only naturally occurring food organisms.

Shrimp were classified and arbitrarily divided according to their wet body weight (BW) into the following size groups: group one (*i.e.*, G_1) = 1.515 to 4.863 g juvenile shrimp (3.229 ± 0.884 g) (mean ± standard deviation); G_2 = 5.125 to 6.998 g juvenile shrimp (6.134 ± 0.519 g); G_3 = 7.028 to 9.981 g pre-adult shrimp (8.754 ± 0.824 g); and, G_4 = 10.105 to 19.640 g adult shrimp (12.727 ± 2.262 g). Each shrimp group from the same cohort was collected on different sampling dates, just prior to the start of each trial.

In the laboratory, 70 to 100 animals within each of the same size ranges were communally held in four 1,000-L tanks (area of 1.13 m²; stocking density between 16 to 22 shrimp/m²) equipped with a biological filter and a 5 cm layer of 3-mm sand on the bottom. The tank system had a constant air supply and was artificially illuminated under a 12:12 LD light cycle. Shrimp were fed *ad libitum* a commercially produced penaeid dried feed (Ração Sibra para Camarões; Sibra Aquicultura S.A., Propria, Sergipe, Brazil).

Animals were kept under these conditions for 10 d to select active and healthy shrimp and to stabilise food intake and evacuation. Only shrimp with functional feeding appendages and with a fully formed and rigid exoskeleton (intermoult stage) were chosen for the study.

4.2.2 Apparatus and Experimental Design

The study was conducted in a closed system, comprised of 50 glass aquariums (15 cm height by 24.5 cm length by 15 cm width, volume of 3.76 L), one biological filter (built with three layers of sand and two layers of oyster shells), and two large tanks for water retention and distribution (Figure 4.1). Water entering each aquarium, passed through a 35-μm Nitex® mesh net and was recirculated on a continuous basis at a rate of 0.48 ± 0.24 L/min. Each aquarium was individually provided with constant aeration and a black polyethylene 1.0 by 1.5 cm diameter mesh on top to prevent shrimp from leaping out. The whole system was covered with dark plastic sheeting and artificially illuminated with six 15-W cylindrical fluorescent white light bulbs positioned 0.9 m from the aquariums. A 12:12 LD light cycle (dark period between 1900 and 0700 h) was used in all trials. Sea water was maintained at a temperature of 29.4 ± 0.7 °C (mean $\pm$ s.d.; n = 464), a salinity of 32 ± 1‰ (n = 464) and pH of 7.92 ± 0.11 (n = 463). After each trial, water in the system was partially exchanged for new sea water.

Figure 4.1: Lay-out of the closed aquarium system used in the study.
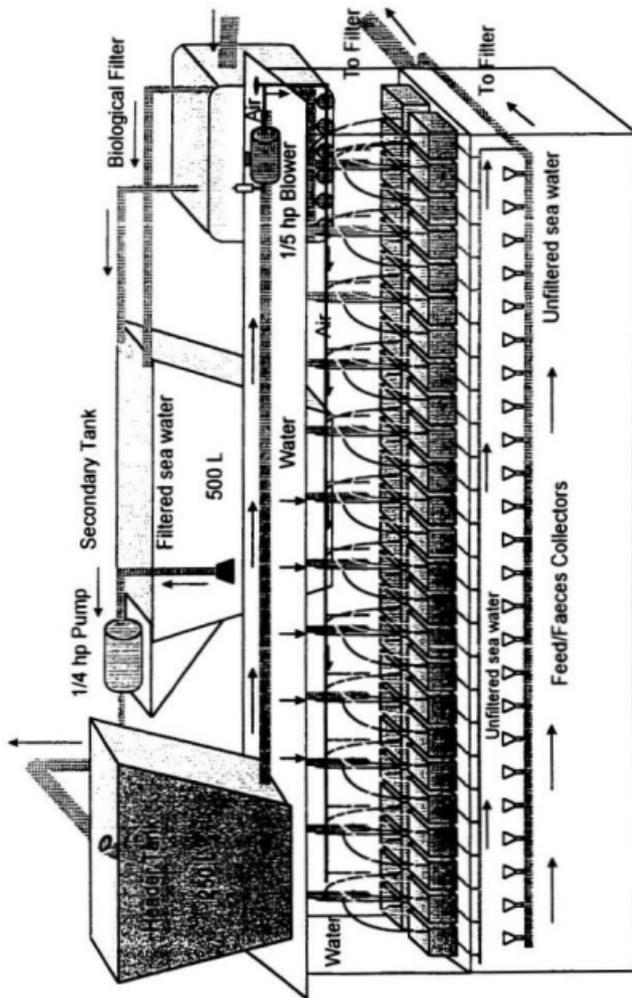
Size groups of *Penaeus subtilis* were tested separately. To avoid conspecific interaction, animals were held individually in each aquarium. Prior to stocking, shrimp were weighed to the nearest milligram and sexed. Animals were then acclimated to the experimental conditions for 2.8 ± 0.5 d. Since under confined systems, *P. subtilis* displays both diurnal and nocturnal food consumption (Nunes *et al.*, 1996), feed was administered only during daylight periods. Shrimp were fed three times daily at 0800, 1200 and 1600 h, for 1 h.

Formulated food was the same as that used during the conditioning phase, composed of 6.2 ± 0.1% (mean ± s.d.; n = 6) moisture, 44.4 ± 0.3% protein (n = 3; N x 6.25, dry basis), 5.2 ± 0.1% (n = 3) lipid, 13.8 ± 0.1% (n = 3) ash and 30.4 ± 0.2% (n = 6) carbohydrate, with 3.5 ± 0.1 kcal/g (n = 6) gross energy content (by combustion in a bomb calorimeter; for ingredient composition see Nunes *et al.*, 1997a and Nunes and Parsons, 1998b; Chapter 2). Previous studies indicated improved food handling efficiency with smaller feed sizes (Nunes and Parsons, 1998b; Chapter 2); therefore, all shrimp groups were only fed crumbles of less than 1 mm length by 1.90 ± 0.32 mm diameter. During feeding, formulated food was soaked in sea water for 1 min to allow an immediate and complete sinking of feed when it was added to the aquaria.

Uneaten feed and (or) faeces were recovered separately by micro-filtering the water using 100% pre-weighed cellulose filters. This was achieved by siphoning the waste material with a 0.5-cm diameter hose into 5-cm diameter PVC funnel-shaped cylinders which held paper filters (Figure 4.1). Each filter was used only once, one for each aquarium. At least ten aquariums per trial were assigned to estimate the amount of feed lost (control aquariums, without shrimp), either as a result of feed dissolution or the food

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recovery procedure. Exuvia and dead shrimp were removed and discarded when observed.

For measurements of food consumption and faecal production, collected feed samples and faeces in pre-weighed filters, respectively, were oven-dried to constant weight and weighed to the nearest milligram. Data collected from shrimp in proecdysis and ecdysis were not used because feeding declines or completely ceases during these stages (Drach and Tchernigovtzeff, 1967). No direct examination of the moulting cycle was made, therefore samples collected 24 h prior to and after the time of observed shedding of shrimp exoskeleton were eliminated from the data analysis.

The amount of food ingested by an individual shrimp was calculated according to the formula:

$$FC = Fl \times (Fo - Fr) \qquad (4.1)$$

where, FC is the dry weight of feed consumed (g), Fo is the dried weight (g) of feed offered/shrimp (*i.e.*, initial weight of feed subtracted from moisture content of artificial food), Fr is the dried weight (g) of feed recovered, and Fl is the proportion dried feed lost in water, given by:

$$Fl = (CFr/CFo) \qquad (4.2)$$

where, CFr is the dried weight (g) of feed recovered from control aquariums and CFo is the dried weight (g) of feed offered/control aquarium (*i.e.*, initial weight of feed subtracted from moisture content of artificial food). Feed loss from control aquariums was estimated every time food consumption of shrimp was assessed. Feed was held alone in control aquariums (*i.e.*, without animals) during the exact duration of time that shrimp

were exposed to the feed. Fl was calculated using the average value obtained for all days that consumption trials were conducted. A body component index was calculated as the ratio of individual measurements of food ingested (FC) or faeces produced to shrimp wet body weight (BW), to standardise values on a body weight-specific basis.

4.2.3 Indices of Food Ingestion and Egestion

Maximum meal of *Penaeus subtilis* [maximum amount of food that can be eaten by one individual (per BW) over one fixed period of time] was determined by measuring the amount of feed consumed per shrimp for a period of one hour. After acclimation, animals were deprived of food for 19 h to allow complete evacuation of their stomachs. Feed was administered in excess (*i.e.*, between 24 to 31% BW), but at different quantities according to shrimp wet body weight [G_1, 0.925 ± 0.003 g of dry feed (mean ± s.d.); G_2, 1.846 ± 0.003 g of dry feed; G_3, 2.768 ± 0.003 g of dry feed; and, G_4, 2.768 ± 0.003 g of dry feed]. Aquaria were continuously monitored for faeces at all times (except at night) to prevent coprophagy during feeding. Three measurements of food consumption per individual were made during three consecutive days. Thus, for four shrimp size groups, a total of 600 samples of uneaten feed were collected (*i.e.*, one measurement/d x 3 d x 50 aquariums x 4 shrimp size groups). Mean maximum meal was determined according to the formula:

$$MM = \sum_N FC/N \qquad (4.3)$$

where, N is the total number of individuals from a specific shrimp size group sampled during the period. Mean maximum meal index was determined as:

$$MMI = \left(\sum_N FC/BW\right)\Big/N \qquad (4.4)$$

All subsequent trials that evaluated appetite revival, ingestion rate, faecal production rate and gastric evacuation were carried out with new specimens of *Penaeus subtilis* [G_1, 3.512 ± 0.642 g BW (mean ± s.d.); G_2, 6.193 ± 0.547 BW; G_3, 8.507 ± 0.802 g BW; and, G_4, 14.010 ± 2.578 g BW)]. These indices were examined on a sequential and continual basis that lasted 14 d for each shrimp size group tested, allowing a 24-h interval between measurement of each parameter. In this case, feeding rates were greater than 1.5 to 2.1 times the estimated average maximum meal (*i.e.*, G_1, 0.285 ± 0.003 g of dry feed; G_2, 0.565 ± 0.003 g of dry feed; G_3, 0.845 ± 0.003 g of dry feed; and, G_4, 0.845 ± 0.003 g of dry feed). Also, shrimp were not deprived of food for more than 5 h prior to any measurement. A satiation ration was given between 0730 and 0800 h, and removed and collectively discarded with any faecal residues after 1-h of exposure. At least 1 h from the recovery of uneaten feed originating from the satiation ration was allowed before the next meal, to generate partial or total evacuation of shrimp stomachs. Measurements of food consumption of recently fed shrimp (*i.e.*, not starved), were designed to replicate wild and culture conditions, under which animals have a continual access to food, either natural or artificial (Wassenberg and Hill, 1987; McTigue and Feller, 1989; Reymond and Lagardère, 1990; Nunes *et al.*, 1996).

Appetite revival [arbitrary food intake after a satiation meal, expressed as the amount of food consumed (per BW) per unit time] was quantified by feeding acclimated shrimp at

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five separate times, at 1000, 1100, 1200, 1300 and 1400 h, for 0.5 h following the removal of an initial 1-h satiation ration at 0900 h. Nine shrimp from the same size range were tested at each time period over 3 d, amounting to a total of 600 samples of feed (*i.e.*, 10 samples/time period x 5 time periods x 3 d x 4 shrimp size groups). Mean appetite revival rate was calculated as:

$$AR = \sum_{n}^{t=i} (FC/0.5)/n \qquad (4.5)$$

where, n is the total number of individuals from a specific shrimp size group sampled at time (t) = i. Mean appetite revival rate index was determined as:

$$ARI = \sum_{n}^{t=i} \left[(FC/0.5)/BW \right]/n \qquad (4.6)$$

Ingestion rate [amount of food eaten per shrimp (per BW) per unit time] was quantified by periodically feeding shrimp for 1 h at three time periods, at 1.5, 4.0 and 6.5 h (1000, 1230 and 1500 h) following the removal of a 1-h satiation ration (at 0830 h), over a 3-d period. There were a total of 1800 measurements, 50 samples/time period x 3 time periods x 3 d x 4 shrimp size groups. In this case, the next meal was provided only after 1.5 h had passed from the time of the recovery of the previous meal, allowing shrimp to partially or completely clear their foreguts between rations. Mean ingestion rate (IR) and mean ingestion rate index (IRI) were determined by the respective equations:

$$IR = \sum_{n}^{t=i} FC/n \qquad (4.7)$$

$$IRI = \sum_{n}^{t=i} (FC/BW)/n \qquad (4.8)$$

Faecal production rate [expressed as the dry weight of faecal matter produced per shrimp (per BW) per h] was measured at each hour over a 3-h interval (*i.e.*, at 1, 2 and 3 h

following a satiation ration), but for one day only. After the removal of uneaten feed (satiation meal), faeces were collected and oven-dried to a constant weight. Mean faecal production rate (FP) was calculated as:

$$FP = \sum_{n}^{t=i} Fr/n \qquad (4.9)$$

Mean faecal production rate index was determined by:

$$FPI = \sum_{n}^{t=i} (Fr/BW)/n \qquad (4.10)$$

Gastric evacuation (GE, the rate of the emptying of the stomach after feeding) was measured following a combination of the methodology described by Elliot (1972), Hill and Wassenberg (1987) and Loya-Javellana *et al.* (1995). Shrimp were sequentially killed at T_0 (*i.e.*, immediately after 1-h of food exposure), T_1 (*i.e.*, 1 h after initial food exposure), T_2 and T_3, after the removal of a 1-h satiation ration. Shrimp were dissected, their anterior proventriculus removed and the stomach repletion index (*i.e.*, degree of stomach fullness expressed in percentage terms) determined according to Lagardère (1972), Poxton *et al.* (1983), Reymond and Lagardère (1990) and Nunes *et al.* (1997b).

4.2.4 Statistical Analysis

Statistical analyses were performed with the Statistical Package for Social Sciences, Windows version, release 7.5.1 (SPSS Inc., Chicago, Illinois, USA). Homogeneity of variance was examined for all data by using Bartlett-Box *F* and Cochran's *C* tests. Kurtosis and skewness and their standard error (*i.e.*, s.e. kurtosis and s.e. skewness) were determined as measures of asymmetry and tests of normality of the data. Based on these

results, MM, MMI, AR, ARI, IR, IRI, FP and FPI were transformed to a log(x + 1) scale to normalise and homogenise the variances and meet statistical assumptions. Probability of type I error was set at $\alpha = 0.05$. A regression analysis was conducted to determine equations that best described the relationships between MM, AR and IR with shrimp BW. Wherever appropriate, the inflection point in each plotted curve was determined by repeatedly calculating the resulting value of the dependent variable for each 0.001 g increase in BW. The point of inflection was defined as the point at which correlation values began to decrease.

4.3 Results

Of the 350 shrimp used in the study, 43 (12%) died and 213 (61%) moulted at a frequency of 6 ± 3 d (mean $\pm$ s.d.; n = 190). Moulting frequency was significantly different among shrimp groups (one-way ANOVA, $F_{3, 186} = 171.11$, $P < 0.001$). Larger shrimp (G_3 and G_4) moulted at a frequency that was significantly lower that that for juvenile shrimp (G_1 and G_2; Scheffé's Multiple Range Test, $P < 0.05$). A total of 3,482 samples of feed and (or) faeces derived from 307 shrimp were collected for quantitative analysis. Estimates of daily feed loss were used to calculate food consumption. Overall, feed loss resulting from feed dissolution in water and the recovery procedure was $33 \pm 15\%$ (mean mean $\pm$ s.d.; n = 411).

4.3.1 Maximum Meal

Standard error of kurtosis and skewness for MM and MMI were 0.238 and 0.119, respectively (Figure 4.2). One-way analysis of variance of log transformed MM indicated significant differences among shrimp groups (one-way ANOVA, $F_{3, 416} = 374.34$, $P < 0.001$), except when comparing G_3 with G_4 (Scheffé's Multiple Range Test, $P = 0.086$, Figure 4.2). Similarly, MMI differed significantly among shrimp groups (one-way ANOVA, $F_{3, 416} = 111.25$, $P < 0.001$, Figure 4.2), but not between G_2 and G_3 (Scheffé's Multiple Range Test, $P = 0.808$, Figure 4.2).

Overall, MM and MMI changed in response to *Penaeus subtilis* body size, showing an increasing (MM) and declining (MMI) pattern, respectively. Thus, although pre-adult and adult shrimp (G_3 and G_4) ingested larger amounts of food (*i.e.*, MM) compared to juvenile shrimp (G_1 and G_2), higher amounts of food consumed per BW (*i.e.*, MMI), were found for smaller animals.

These observations were confirmed by the strong and significant relationship found between *Penaeus subtilis* MM and its BW. The relationship (Figure 4.3) was expressed as a power function by the equation: $MM = 0.0931BW^{0.6200}$ ($r = 0.840$, $df = 418$, $P < 0.001$). In general, results showed that while *P. subtilis* consumed less food per BW in proportion to its size (MMI, Figure 4.2), food consumption (MM, Figure 4.3) increased regularly, each time at a smaller rate (Figure 4.3).

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Figure 4.2: Maximum meal, ingestion rate, appetite revival and faecal production of *Penaeus subtilis* as a function of shrimp size (n refers to number of observations). Common letters denote no significant difference at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.

Maximum Meal

□ MM (g of dried feed ingested/shrimp)
■ MMI (g of dried feed ingested/shrimp BW)

- G_1: n = 157, 0.187, 0.063
- G_2: n = 52, 0.296, 0.050 a
- G_3: n = 88, 0.429, 0.047 a
- G_4: n = 123, 0.400, 0.035 a

Ingestion Rate

□ IR (g of dried feed ingested/shrimp/h)
■ IRI (g of dried feed ingested/shrimp BW/h)

- G_1: n = 396, 0.086, 0.025
- G_2: n = 387, 0.166, 0.027
- G_3: n = 369, 0.194, 0.023
- G_4: n = 291, 0.208, 0.015

Appetite Revival

□ AR (g of dried feed ingested/shrimp/h)
■ ARI (g of dried feed ingested/shrimp BW/h)

- G_1: n = 111, 0.162, 0.047 a
- G_2: n = 117, 0.312, 0.051 a
- G_3: n = 129, 0.351, 0.042 a
- G_4: n = 120, 0.395, 0.029 a

Faecal Production

□ FP (g of dried faeces produced/shrimp/h) x 0.01
■ FPI (g of dried faeces produced/shrimp BW/h) x 0.01

- G_1: n = 111, 0.501, 0.140 a,b
- G_2: n = 111, 0.648, 0.105 a,b
- G_3: n = 111, 0.666, 0.079 a
- G_4: n = 111, 0.465, 0.036 b

Shrimp Size Class

Figure 4.3: Relationship between maximum meal (MM, g of dried feed consumed/shrimp) and wet body weight (BW, g) of *Penaeus subtilis*. Negative and null values were not considered.

4.3.2 Appetite Revival

Standard error of kurtosis and skewness for AR and ARI were 0.223 and 0.112. Log transformed AR and ARI showed significant differences among all shrimp size groups (MANOVA, $F_{3, 357} = 109.40$ and 51.16, respectively, $P < 0.001$, Figure 4.2), except between G_1 and G_2 (Scheffé's Multiple Range Test, $P = 0.116$, Figure 4.2). AR progressively increased as shrimp BW increased, but values started to decline at 12.352 g shrimp (Figure 4.4). The curve and the cubic expression presented in Figure 4.4 depict this relationship, which also agrees with the significant reduction in ARI found for G_3 and G_4 (Figure 4.2). Thus, the patterns of appetite revival with *Penaeus subtilis* BW can be described as successive increments in food consumption until a certain shrimp size, when reduced ingestion rates occur.

Overall, AR was constant during the 5-h period investigated (Scheffé's Multiple Range Test, $P = 0.086$, Figure 4.5), even within the same shrimp size group (Table 4.1). Conversely, ARI showed significant differences between the last time period (*i.e.*, time 5) and the three first ones (times 1, 2 and 3), and among shrimp size groups at each time period (Table 4.1). Therefore, rates of food consumption per shrimp BW were higher when food was provided in shorter time periods (*i.e.*, 1, 2 and 3 h after initial meal) instead of longer ones (*i.e.*, 5 h after initial meal), particularly for shrimp between 2.107 to 9.881 g BW (Table 4.1).

$$AR = 1.38 \times 10^{-4}BW^3 - 0.007BW^2 + 0.109BW - 0.129$$
$$r = 0.764;\ P < 0.001;\ df = 460$$
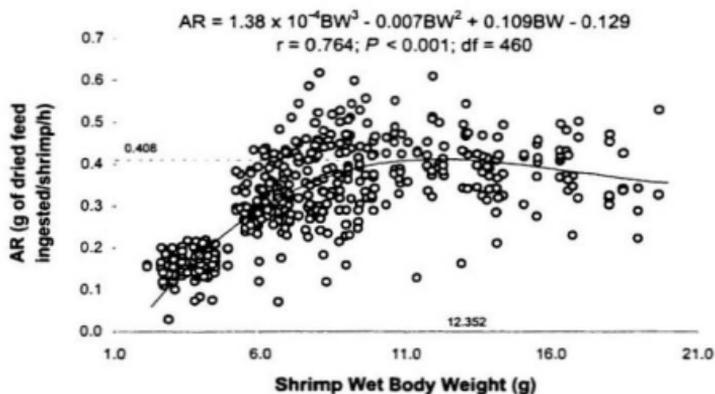
Figure 4.4: Relationship between the rate of appetite revival (AR, g of dried feed consumed/shrimp/h) and wet body weight (BW, g) of *Penaeus subtilis*. Dotted lines indicate point where AR values start to decline in the curve. Negative and null values were not considered.
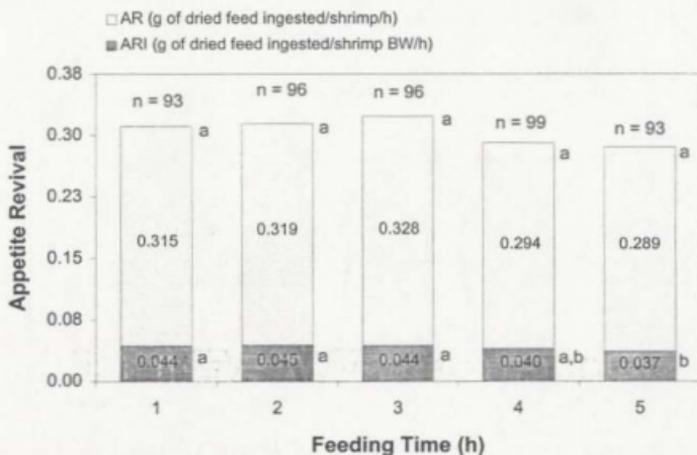
Figure 4.5: Rate of appetite revival (AR) and appetite revival index (ARI) for *Penaeus subtilis* as a function of feeding time (h). Feeding times refer to time food was given following a satiation meal (t = 0; n refers to number of shrimp observed at each time period). Common letters denote no significant difference at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.

Table 4.1: Rate of appetite revival (AR, g of dried feed consumed/shrimp/h) ± s.d. and appetite revival index (ARI, g of dried feed consumed/shrimp BW/h) ± s.d. for four size groups of *Penaeus subtilis* at five time intervals (n denotes total number of observations for each shrimp size class). Shrimp body weight (BW, g) presented as mean ± s.d., with numbers in parentheses indicating minimum and maximum values. Non-significant time periods (horizontal comparisons for AR) and shrimp size groups (vertical comparisons for ARI) are shown in the last column and last line, respectively.

Shrimp Size Group and BW (g)	Index	N	Time Period					Post hoc (time)*
			1	2	3	4	5	
G_1 3.576 ± 0.624	AR	111	0.156 ± 0.034	0.172 ± 0.020	0.170 ± 0.032	0.164 ± 0.027	0.147 ± 0.037	1, 2, 3, 4, 5
(2.107 - 4.863)	ARI		0.050 ± 0.015	0.050 ± 0.009	0.042 ± 0.009	0.051 ± 0.011	0.040 ± 0.011	-
G_2 6.170 ± 0.551	AR	117	0.296 ± 0.052	0.339 ± 0.086	0.343 ± 0.041	0.292 ± 0.115	0.288 ± 0.043	1, 2, 3, 4, 5
(5.125 - 6.980)	ARI		0.050 ± 0.009	0.052 ± 0.013	0.056 ± 0.009	0.048 ± 0.019	0.048 ± 0.009	-
G_3 8.474 ± 0.792	AR	129	0.391 ± 0.116	0.358 ± 0.108	0.373 ± 0.151	0.328 ± 0.147	0.309 ± 0.138	1, 2, 3, 4, 5
(7.028 - 9.881)	ARI		0.047 ± 0.014	0.043 ± 0.014	0.046 ± 0.020	0.037 ± 0.016	0.036 ± 0.016	-
G_4 14.050 ± 2.547	AR	120	0.394 ± 0.080	0.401 ± 0.064	0.407 ± 0.113	0.379 ± 0.072	0.398 ± 0.142	1, 2, 3, 4, 5
(10.269 - 19.640)	ARI		0.029 ± 0.009	0.033 ± 0.007	0.033 ± 0.010	0.027 ± 0.006	0.026 ± 0.012	-
Post hoc (group)*	ARI		1, 2, 3	1, 2, 3	1, 3; 1, 4; 2, 3	1, 2; 2, 3; 3, 4	1, 2; 1, 3	

*Mean difference is not significant at the α = 0.05 level by Scheffé's Multiple Range Test.

4.3.3 Ingestion Rate

Significant differences were found for log transformed IR and IRI (0.129 s.e. kurtosis and 0.064 s.e. skewness) among shrimp size groups (MANOVA, $F_{3, 1371}$ = 288.16 and 100.12, respectively, $P < 0.001$, Figure 4.2). Comparable to the previous parameters, IR increased as BW increased, while IRI declined (Figure 4.2). Over time, there was a significant reduction in IR between the first (ration 1) and last time period (ration 3) examined (Scheffé's Multiple Range Test, $P = 0.008$, Figure 4.6) and between the IRI of the first and the last two (Scheffé's Multiple Range Test, $P < 0.001$, Figure 4.6).

Comparisons made with IR within each group were commensurate with these results (Table 4.2, except for G_4). These results indicate that both IR and IRI values were higher for the first meal (i.e., 1.5 h following the removal of a satiation ration), progressively decreasing with time as more rations were given. Food ingestion for the first ration was also more consistent among shrimp groups (i.e., G_1, G_2 and G_3), except for G_4, which showed significantly lower IRI values at all meals when compared to the remaining size classes (Table 4.2). Overall, results indicated that in a 6.0 h period, the higher the number of rations delivered at consecutive 1.5 h periods, the smaller the IR and IRI values tended to be.

Three cubic equations are presented in Figure 4.7 to express the correlation between IR and BW of *Penaeus subtilis* for each ration examined. For each meal, inflection points were observed where IR started to show reducing tendencies (Figure 4.7, dotted lines). Data revealed that as more rations were given, IR started to decline at a larger shrimp size. Thus, the shrimp size range will be broader at each time period where IR increases proportionally
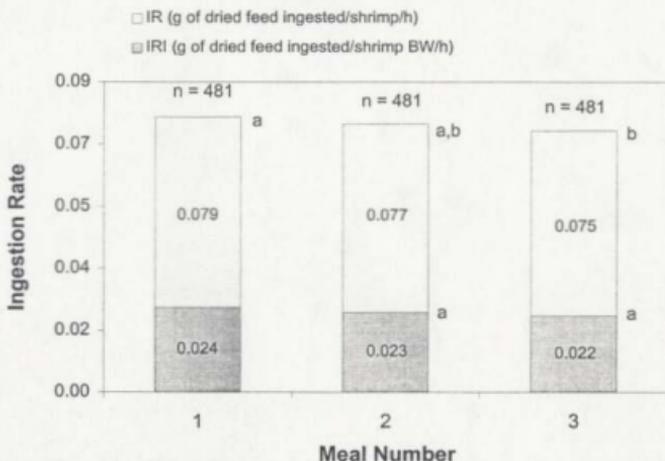
Figure 4.6: Ingestion rate (IR) and ingestion rate index (IRI) for *Penaeus subtilis* as a function of ration given. Meals were delivered for 1 h at consecutive 1.5 h periods, following a 1-h satiation ration (n values indicate the number of shrimp observed at each feeding period). Common letters denote no significant difference at the α = 0.05 level by Scheffé's Multiple Range Test.

Table 4.2: Ingestion rate (IR, g of dried feed consumed/shrimp/h) ± s.d. and ingestion rate index (IRI, g of dried feed consumed/shrimp BW/h) ± s.d. for four size groups of *Penaeus subtilis* for three 1-h rations delivered at consecutive 1.5 h periods (n denote total number of observations for each shrimp size class). Shrimp body weight (g) is presented as mean ± s.d., with numbers in parentheses indicating minimum and maximum values. Non-significant time periods (horizontal comparisons for IR) and shrimp size groups (vertical comparisons for IRI) are shown in the last column and last line, respectively.

Shrimp Size Group	Shrimp Body Weight (g)	Index	n	Ration			Post hoc (time)*
				1	2	3	
G_1	3.552 ± 0.636	IR	396	0.090 ± 0.029	0.086 ± 0.016	0.083 ± 0.020	1, 2; 2, 3
	(2.107 - 4.863)	IRI		0.026 ± 0.010	0.025 ± 0.007	0.024 ± 0.007	-
G_2	6.210 ± 0.536	IR	387	0.176 ± 0.058	0.166 ± 0.032	0.156 ± 0.046	1, 2; 2, 3
	(5.125 - 6.980)	IRI		0.029 ± 0.010	0.027 ± 0.006	0.025 ± 0.008	-
G_3	8.528 ± 0.789	IR	369	0.210 ± 0.092	0.192 ± 0.089	0.179 ± 0.080	1, 2; 2, 3
	(7.028 - 9.881)	IRI		0.025 ± 0.011	0.023 ± 0.011	0.021 ± 0.010	-
G_4	13.954 ± 2.524	IR	291	0.213 ± 0.043	0.200 ± 0.084	0.211 ± 0.074	
	(10.269 - 19.640)	IRI		0.016 ± 0.005	0.015 ± 0.007	0.016 ± 0.006	-
	Post hoc (group)*	IRI		1, 2, 3	1, 2; 1, 3	1, 2	

*Mean difference is not significant at the α = 0.05 level by Scheffé's Multiple Range Test.

Figure 4.7: Relationship between ingestion rate (IR, g of dried feed consumed/shrimp/h) and wet body weight (BW, g) of *Penaeus subtilis* for three meals (M$_1$, M$_2$ and M$_3$) given at 1.5 h consecutive time periods. Dotted lines indicate point where IR values start to decline in the curve. Negative and null values were not considered.

$$IR = 4.80 \times 10^{-5}BW^3 - 0.003BW^2 + 0.050BW - 0.052$$
$$r = 0.779; P < 0.001; df = 451$$

M_3

0.226

14.567

$$IR = 2.85 \times 10^{-5}BW^3 - 0.002BW^2 + 0.050BW - 0.053$$
$$r = 0.822; P < 0.001; df = 448$$

M_2

0.233

13.498

$$IR = 1.23 \times 10^{-4}BW^3 - 0.006BW^2 + 0.079BW - 0.113$$
$$r = 0.814; P < 0.001; df = 446$$

M_1

0.231

11.008

IR (g of dried feed ingested/shrimp/h)

Shrimp Wet Body Weight (g)
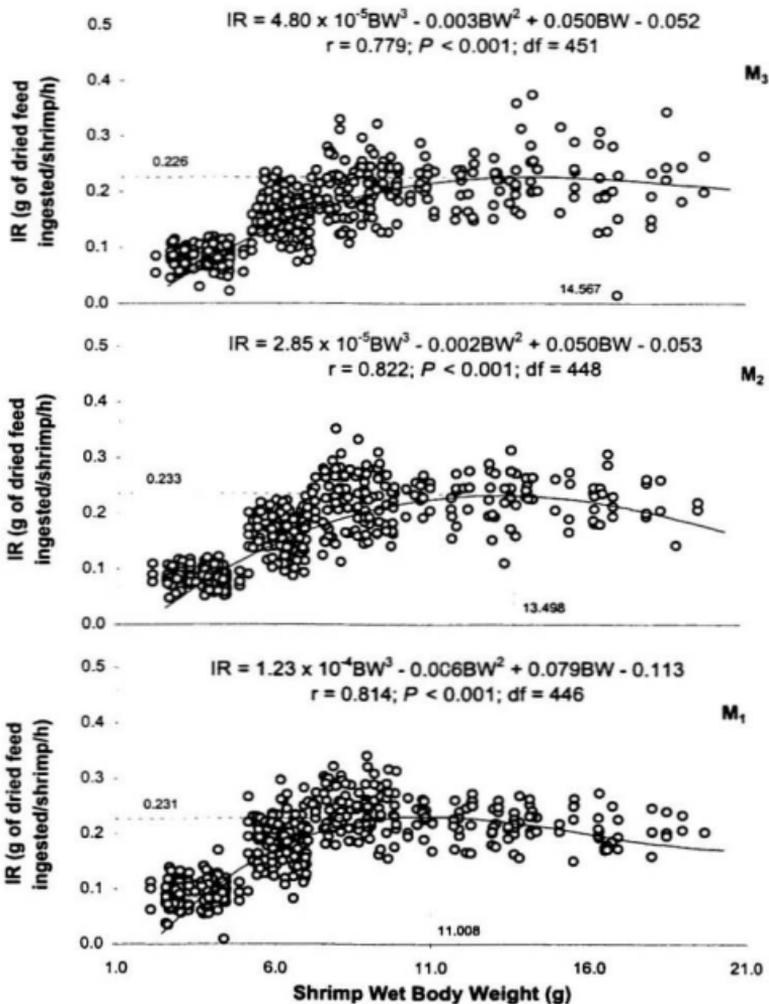
to shrimp BW.

4.3.4 Faecal Production and Evacuation Rates

FP and FPI (0.227 s.e. kurtosis and 0.114 s.e. skewness) differed significantly among shrimp size groups (MANOVA, $F_{3, 436}$ = 6.69 and 36.92, respectively, $P < 0.001$, Figure 4.2). FP was almost uniform among size classes, with significant differences observed between G_3 and G_4 (Scheffé's Multiple Range Test, $P = 0.008$, Figure 4.2). FPI decreased with shrimp size (Figure 4.2), showing higher values for smaller shrimp. Similarly, faecal production decreased with time, with most faeces appearing within the first hour following the recovery of a satiation ration (Figure 4.8). Comparisons between time periods indicated that FP was significantly higher for the first hour (time 1) when compared to the second and third hours (*i.e.*, times 2 and 3), while FPI differed among all time periods (Scheffé's Multiple Range Test, $P < 0.001$, Figure 4.8).

In comparison, nearly all foregut evacuation occurred at the second hour (T_2) following a satiation ration (one-way ANOVA, $F_{3, 148}$ = 62.392, $P < 0.001$, Figure 4.9). Stomach repletion index showed significant differences among all time periods investigated (Scheffé's Multiple Range Test, $P < 0.05$, Figure 4.9). At T_0, *Penaeus subtilis* foreguts were over 91% full. On average, a 22% loss in stomach fullness had occurred by T_1, followed by a 42% and 20% decline at T_2 and T_3, respectively. The overall trend was a slight decline in stomach fullness from T_0 to T_1 and from T_2 to T_3, and a significant reduction from T_1 to T_2 (Figure 4.9).

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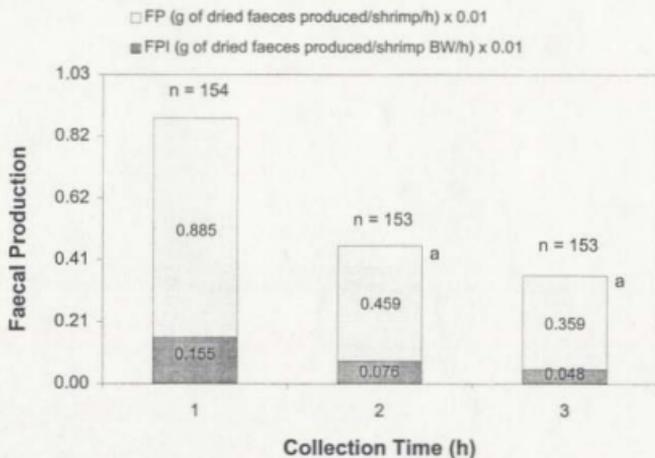
Figure 4.8: Faecal production rate (FP) and faecal production index (FPI) for *Penaeus subtilis* as a function of time (h). Times refer to period of faeces collection following a satiation meal (t = 0), with n values indicating the number of shrimp observed at each time period. Common letters denote no significant difference at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.
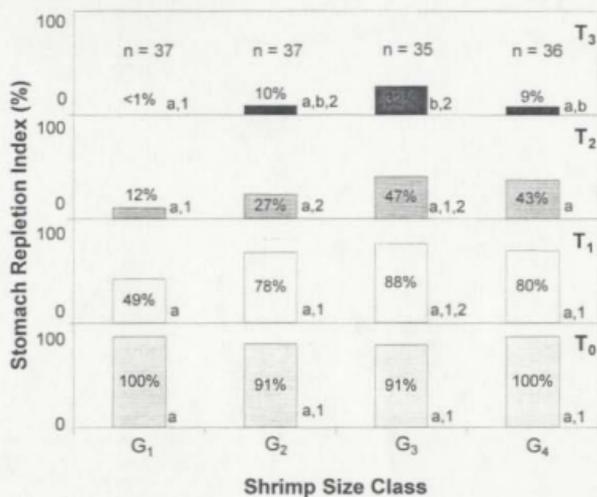
Figure 4.9: Stomach clearance rate for four size groups ($G_1 = 3.707 \pm 0.593$ g; $G_2 = 5.914 \pm 0.990$ g BW; $G_3 = 7.987 \pm 1.719$ g BW; $G_4 = 13.803 \pm 2.604$ g BW) of *Penaeus subtilis* at three time intervals (T_1, T_2 and T_3) as indicated by the stomach repletion index (%). Time intervals refer to period after food collection (T_0; n indicates total number of shrimp sacrificed for each corresponding size group). Common letters (comparisons among groups for each time period) and numbers (comparisons among time periods for each group) denote no significant difference at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.

Comparisons among shrimp groups at each time period revealed no significant differences in foregut evacuation up to T_2 (Scheffé's Multiple Range Test, $P > 0.05$, Figure 4.9). At T_3, G_1 showed a rapid clearance rate when compared to G_3, but did not differ from that of each of the remaining groups (*i.e.*, G_2 and G_4).

4.4 Discussion

4.4.1 Relationship of Amount of Food Eaten with Shrimp Size

Results of food consumption indices (*i.e.*, maximum meal, ingestion rate and appetite revival) revealed that the amount of food ingested by *Penaeus subtilis* is a function of its body weight (BW). Feeding intensity increased progressively with shrimp size, but inversely in percentage terms. As a result, faecal production per BW was greater for smaller shrimp. These relationships are comparable to those derived from other observations in controlled shrimp feeding trials (Sick *et al.*, 1973; Katre and Reddy, 1977; Chu and Shing, 1986; Kurmaly *et al.*, 1989).

The declining consumption of feed relative to a BW increase was also similar to empirical feeding rates used in shrimp farming which reduce in response to body weight gains and to studies with other animal species (Peters, 1983). Under cultured and wild conditions however, penaeid food intake has been reported to present either a constant pattern (Nunes, 1995; Nunes and Parsons, 1999; Chapter 3) or no apparent relationship with shrimp body weight (McTigue and Feller, 1989). Data from the present study along with

other investigations (McTigue and Feller, 1989; Reymond and Lagardère, 1990; Nunes *et al.*, 1996; Nunes, 1998) suggest these discrepancies result from the influence of environmental factors (*e.g.*, variations in water quality, tides, light intensity, natural and formulated food availability) on shrimp feeding activity. Nunes (1998) determined that 26% of the variation in the feeding rhythm of *Penaeus subtilis* cultured in a semi-intensive system was a result of fluctuations in water quality parameters.

On average, the consumption of food by *Penaeus subtilis* was equivalent to 2.3% its BW per hour, while food intake in shrimp with empty stomachs reached 4.9% BW/h. Reports of food consumption for *Penaeus* spp. vary significantly among species, shrimp size, food type and experimental conditions. Marte (1980) reported that in 20 min of continuous feeding, juvenile *P. monodon* (42 to 59 mm carapace length) consumed an average of 0.825 g of prawn meat (dry weight basis). Sedgwick (1979a) found that juvenile *P. merguiensis* (0.5 to 1.3 g) fed on a commercial dry pellet consumed about 12% of their weight per day. In adult *P. esculentus* (15.5 to 25.2 g), daily average food consumption ranged from 0.95 to 2.33 g/d, with crustacean tissue being ingested more than bivalve tissue (Hill and Wassenberg, 1992). Post-larval *P. setiferus* was able to ingest 8.60 mg/BW/h of dried feed (Sick and Baptist, 1973). Under culture conditions, Nunes and Parsons (1999) observed a maximum mean wet stomach content weight of 1.5 mg (or 0.4% BW) for *P. subtilis* after 44 d of rearing. Compilation of data from eight different feeding tables used in semi-intensive and intensive shrimp culture systems (Jory, 1995) reveals average daily feeding rates of 9.7% (1 to 4 g shrimp), 5.1% (5 to 6 g shrimp), 3.8% (7 to 9 g shrimp) and 3.1% shrimp BW (10 to 12 g shrimp), comparable to the MMI figures obtained in this study.

The inflection points shown in the curves presented in Figures 4.4 and 4.7 are likely to be associated with a shift, reported in other investigations, in the feeding behaviour of adult *Penaeus subtilis*. In this species, a more pronounced carnivorous feeding habit starts to occur in adulthood, as first detected by Nunes (1995). Nunes *et al.* (1997b) working with this species in a semi-intensive culture system reported a marked decline in detritus consumption coupled with an increase in prey ingestion at 10.40 g shrimp. The declining slopes (Figures 4.4 and 4.7) may also indicate a reduction in food requirement or an inadequate attractiveness of the formulated food given, particularly for shrimp over 11 g BW. In general, the greater carnivorous tendencies for adult *P. subtilis*, combined with lower levels of feed ingestion indicate that greater attention must be applied to the implementation of feeding programmes and in the formulation of food used in the culture of this species.

4.4.2 Foregut Evacuation, Appetite Revival and Food Administration

In the present study, foregut clearance rates were not significantly different within the size range of shrimp examined (*i.e.*, mean of 3.7 to 13.8 g shrimp). *Penaeus subtilis* stomach fullness reached its lowest peak (13%) after 3 h following food recovery, compared to 25% after 1 h for adult *P. esculentus* (Hill and Wassenberg, 1987) and 5% after 4 h for juvenile *P. monodon* (Marte, 1980). In *P. setiferus*, proventriculus fullness reached a minimum in 2 to 3 h after food ingestion (Hentschel and Feller, 1990). Freshly caught juvenile and adult *Macropetasma africana* completely cleared their foreguts in 2 to

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4 h after capture (Cockcroft and McLachlan, 1986). Marte (1980) hypothesed that for *P. monodon* complete emptying may occur from 5 to 6 h after feeding. At an average gastric evacuation rate of 28% per hour, total emptying of the stomach should occur 4 to 5 h after food recovery for most shrimp sizes groups of *P. subtilis* (except G_1). Although results for *P. subtilis* imply that food intake may be maximised if feed is given at time intervals of 3 h or longer, lengthening hourly food administration periods did not result in higher food consumption levels for any of the shrimp size groups examined. In fact, shorter hourly administration intervals (*i.e.*, 1, 2 and 3 h) favoured higher food consumption per BW than longer ones (*i.e.*, 5 h).

Penaeus subtilis resumed feeding soon after an initial meal was given (*i.e.*, 1 h after the first ration was provided). Since for most shrimp groups, complete stomach emptying may have occurred only after 3 h following food recovery, these results suggest this species may feed while digesting an earlier meal, although at a lower level. Overall, data point to a continuous feeding periodicity in *P. subtilis*, as also suggested for other *Penaeus* spp. [*P. monodon* (Hall, 1962) and *P. esculentus* (Hill and Wassenberg, 1987)]. However, these observations still require confirmation, because feeding cessation intervals cannot be discarded during food exposure or outside the period when feeding measures were made. Nunes *et al.* (1997a) investigated the feeding behaviour of *P. subtilis* and observed shrimp could feed continuously for up to 10 min, but feeding activity decreased substantially after this period. Hill and Wassenberg (1987) reported that *P. esculentus* stopped an average of 6.3 times at a food source during one evening, remaining at most stops less than 5 min, with a few exceeding 20 min (mean duration of 9.3 min for each stop).

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In the present study, as more meals were added, *Penaeus subtilis* ingestion levels declined. Despite the progressive reduction in foregut fullness over time after the consumption of a full meal, refilling of their stomachs could also have occurred following the administration of more food. These results indicate that while the hourly feeding interval between two meals did not affect shrimp food intake, the administration of a third ration played a role in regulating the amount of food consumed, perhaps as a result of foregut fullness. The reduction in food intake was expected as shrimp consume and digest more food, fill their proventriculus, overload their digestive gland with chyme and fine particles (Al-Mohanna and Nott, 1987) and increase their energy reserves (Sedgwick, 1979b). Thus, ingestion in *P. subtilis* may either stabilise or reduce after the amount of food given exceeds a certain level or a specific shrimp body weight. In conclusion, the progressive decline in feeding by *P. subtilis* following the addition of more food was probably a combined result of these factors.

The largest amount of food material was evacuated from *Penaeus subtilis* stomachs between the first and second hours. This occurred in spite of lower FP and FPI observed after the first hour of faeces collection. Due to the significantly higher faecal production within the first hour, food cleared from *P. subtilis* stomachs during this period may have been of higher digestive characteristics. Indigestible or more difficult to digest food materials appear to have a longer residence time in *Penaeus* proventriculus due to difficulties in its mechanical breakdown and further digestion. After feeding on soft tissue, *P. monodon* and *P. esculentus* emptied 56% to nearly 75% of all their foregut contents within 1 h (Marte, 1980; Hill and Wassenberg, 1987), in comparison to the 26% obtained in the present study for *P. subtilis* fed on a commercial pelleted food

In *Penaeus subtilis*, the bulk of faecal production occurred within 1 h following the recovery of food. In contrast to these observations, Al-Mohanna and Nott (1987) reported that the first faeces produced by adult *Penaeus semisulcatus* occurred 6 h after feeding, while the bulk was produced in 10 to 12 h. This prolonged period for faeces production is unusual in penaeids since digestion is normally completed in 4 to 6 h (Dall, 1992). However, at a lower temperature (11 °C) periods as long as 10 h have been reported (Arosemena, 1976). Therefore, the longer period for faeces production to occur may have been due to the comparably lower water temperatures used in their trial (24 to 25 °C versus 29.4 ± 0.7 °C for the present study), although shrimp were apparently fed a more digestible type of food (*i.e.*, cooked prawn flesh).

4.4.3 Implications to Penaeid Feeding Strategies

In aquaculture ponds, feeding of formulated food has been shown to trigger the resumption of *Penaeus subtilis* food intake when food was given at 10 and 14-h intervals (*i.e.*, at 0700 and 1700 h; Nunes *et al.*, 1996). Data from the present study indicated that feeding intensity and resumption of food intake by *P. subtilis* was not markedly controlled by the level of fullness of their stomach or by longer intervals between food administration. While food load occurred progressively as more feed was given and evacuated from their stomachs, feeding continued at reduced levels, even when shorter feed distribution periods were used. Thus, the daily administration of food in shorter feeding intervals, but at continually reduced amounts may be advantageous in the culture

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of *P. subtilis*. In other studies, increases in the frequency of feeding have been shown to increase penaeid food utilisation (Sedgwick, 1979a) and enhance their growth rates (Sedgwick, 1979a, Robertson *et al.*, 1993). However, other factors associated with food load (*e.g.*, digestive gland loading capacity) must be investigated prior to establishing optimal feed distribution intervals in shrimp culture. Differential foregut clearance rates were not observed among shrimp size groups of *P. subtilis*, implying that the number of feedings per day does not necessarily need to increase over the growth cycle in response to higher feeding rates or larger shrimp sizes.

Food consumption indices obtained for *Penaeus subtilis* indicated that shrimp body weight was a reliable indicator of their feeding levels, and thus should continue to be used to assist the estimation of feeding rates in aquaculture operations. The equations presented in the present study should provide accurate estimates of *P. subtilis* feeding levels, but other factors (*e.g.*, environmental variability and its effects), must be considered before they can be applied to culture conditions.

4.5 Conclusions

The results of the present investigation indicate that *Penaeus subtilis* food consumption (maximum meal, appetite revival and food ingestion) is a function of its body weight. Differential foregut clearance rates however, was not observed among different size groups. Feeding intensity increased progressively with shrimp size, but inversely in percentage terms. Larger amounts of food were consumed by pre-adult and adult *P. subtilis*, but juvenile shrimp ingested higher quantities relative to their body weight. On

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average, shrimp food consumption was equivalent to 2.3% of its body weight/h while with empty stomachs food intake reached 4.9% per shrimp body weight.

Food load occurred progressively as more feed was given and evacuated from shrimp stomachs, but feeding still continued at reduced levels. Complete gastric emptying in *Penaeus subtilis* may occur only after 3 h of food recovery, although the bulk of faeces can be produced within 1 h. However, recurrence of feeding occurred soon after an initial meal was given. Data indicated that control of feeding intensity and resumption of food intake by *P. subtilis* were not markedly affected by the level of their stomach fullness or by longer food administration intervals.

CHAPTER 5

EFFECTS OF THE SOUTHERN BROWN SHRIMP, *Penaeus subtilis*, PREDATION AND ARTIFICIAL FEEDING ON THE POPULATION DYNAMICS OF BENTHIC POLYCHAETES IN A TROPICAL MESOCOSM

5.1 Introduction

In extensive and semi-intensive marine shrimp culture systems, natural food can comprise most to all of the shrimp's diet (Boddeke, 1983; Anderson *et al.*, 1987; Reymond and Lagardère, 1990; Bombeo-Tuburan *et al.*, 1993; Nunes *et al.*, 1997b; Focken *et al.*, 1998). In these organically rich ponds, penaeid shrimp graze on a variety of naturally occurring food sources, including detritus, plant material and animal prey (Das *et al.*, 1982; Boddeke, 1983; Reymond and Lagardère, 1990; Bombeo-Tuburan *et al.*, 1993; Nunes *et al.*, 1996, 1997b; Focken *et al.*, 1998). Under these conditions, benthic fauna can be diverse, consisting of several potential shrimp prey species (Rubright, 1978; Rubright *et al.*, 1981; Moriarty *et al.*, 1987). In many areas, polychaetes are reported to be the most predominant benthic macrofauna (Rubright, 1978; Maguire *et al.*, 1984; Ordner and Lawrence, 1987; Martins, 1994; Martinez-Cordova *et al.*, 1997; Nunes and Parsons, 1999; Chapter 3), occurring at high densities throughout the initial stages of the growth

cycle (Crockett *et al.*, 1988; Nunes, 1995). While in marine and brackish water environments, these animals have been extensively used as active indicators of anthropogenic pollution (Lewbell, 1985; Ansari *et al.*, 1986; Tsutsumi, 1987; Holte and Oug, 1996), polychaete abundance in commercial penaeid aquaculture operations, reflects pond productivity and availability of natural food (Crockett *et al.*, 1988; Nunes *et al.*, 1997b; Nunes and Parsons, 1999; Chapter 3), assisting farmers in the optimisation of shrimp stocking, feeding and harvest (Nunes *et al.*, 1997b).

In the fine sediments of ponds, benthic polychaetes feed on particulate organic matter (Olivier *et al.*, 1995) and function in the mixing of substrate particles (Madsen *et al.*, 1997) and recycling of nutrients (Kristensen *et al.*, 1985; Chareonpanich *et al.*, 1994; Mayer *et al.*, 1995), increasing degradation rates and gas exchange between the substrate and water. Under confined shrimp culture systems, polychaetes have been recognised as the most important prey item of several penaeid species (Boddeke, 1983; Nunes *et al.*, 1997b), accounting for as much as 33% of their total diet (*P. subtilis*, Nunes *et al.*, 1997b). In the wild, polychaetes are known to compose the diet of many fish and crustaceans (Botton and Haskin, 1984; Sheridan *et al.*, 1984; Freire and Gonzalez-Gurriaran, 1995; Ellis *et al.*, 1996), including several commercially important *Penaeus* spp. (George, 1974; Marte, 1980; Wassenberg and Hill, 1987; Gleason and Wellington, 1988; Stoner and Zimmerman, 1988). Due to their high nutritional value, particularly in the content of polyunsaturated fatty acids (Dall *et al.*, 1991), they have been used in compounded maturation diets to promote ovarian development of penaeids (Bray and Lawrence, 1992).

Penaeid shrimp can consume polychaetes throughout all stages of their juvenile and adult life (Stoner and Zimmerman, 1988; Nunes *et al.*, 1997b), but grazing rates are

thought to increase progressively as larger shrimp body sizes are attained. Although polychaetes are described as having a rapid life-cycle (George, 1984; Tsutsumi, 1987), combined with an exceptional capacity of recolonisation (Chesney and Tenore, 1985a; Tsutsumi, 1987), in aquaculture ponds, shrimp predation generally results in significant declines in polychaete abundance (Allan and Maguire, 1992, Nunes and Parsons, 1999; Chapter 3), requiring larger amounts of supplemental feed inputs to sustain shrimp growth and survival.

The dynamics of polychaete populations have been studied under laboratory conditions (Chesney and Tenore, 1985a,b), but mainly in organically polluted environments (Lewbell, 1985; Ansari et al., 1986; Tsutsumi, 1987). In mariculture, most studies related to polychaetes have focused on their response to benthic disturbance and organic and inorganic enrichment arising from fish, shrimp or bivalve cultivation (Tsutsumi, 1987; Schafer et al., 1995; Angsupanich and Aksornkoae, 1996; Kaiser et al., 1996; Spencer et al., 1996; Hargrave et al., 1997). In shrimp ponds, few previous investigations have addressed thoroughly their population patterns and the possible interactions with penaeid predation and artificial feeding. This information is essential for the development of management strategies for the enhancement of natural food in penaeid ponds, particularly in less intensive culture systems. The present work examined the impacts of Penaeus subtilis predation and stocking density, and the growth promoting effects of artificial feeding on the population dynamics of polychaetes in a tropical mesocosm.

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5.2 Materials and Methods

5.2.1 Study Site and Experimental Design

This work was conducted at a commercial semi-extensive marine shrimp farm (Artemisa Aquicultura S.A., Acaraú, Ceará), located in the north-eastern region of Brazil Figure 5.1). The farm is situated on the north-western coast of the state, between the parallels 3°49'53"S and 3°50'28"S and the meridians 40°07'15"W and 40°08'13"W. The operation covers a total area (A) of 341.78 ha, which includes 184.30 ha of nursery and grow-out ponds (n = 11; mean A = 14.18 ha), 131.73 ha of mangrove area, and 25.75 ha of inactivated salt pans. The property is surrounded by mangrove vegetation and bordered by the estuary of Acaraú River.

The study was conducted in a 10-ha grow-out pond (Figure 5.2), in which 9-m² (3 x 3 m) open-bottom enclosures, consisting of a grey polyethylene 2.0-mm diameter mesh net with 1.50 m height (Indústria Textil Florence Ltda., Jandira, São Paulo, Brazil), were placed. Enclosure construction followed the methodology described by Nunes (1995), Nunes *et al.* (1996) and Nunes and Parsons (1999). The pond had an irregular shape with a water depth between 0.70 to 0.90 m. Enclosures were lined up in three columns (R, right; C, centre; L, left), each composed of 15 enclosures, arranged 40 m apart and between 30 to 140 m from the pond walls. They were spaced 30 m from the outlet system and positioned at every 20 m towards the central section of the pond.

Figure 5.1: Geographical location of the study site in NE Brazil, State of Ceará.

Figure 5.2: Arrangement of enclosures in grow-out pond used for the study. Shrimp stocking density (5, 10, 15 or 20/m^2) and respective feeding regime are indicated by numbers next to each enclosure (FNS = feed, no shrimp; NFNS = no feed, no shrimp; SNF = shrimp, no feed; FS = feed, shrimp).

Pond Area = 10 ha
Enclosure = □
Enclosure Area = 9 m² (3 x 3 m)
Enclosure Position:
R (right), C (centre), L (left)

□ FNS
□ NFNS
■ NFS
□ FS

Water Inlet

Water Outlet

Wind Direction

NE
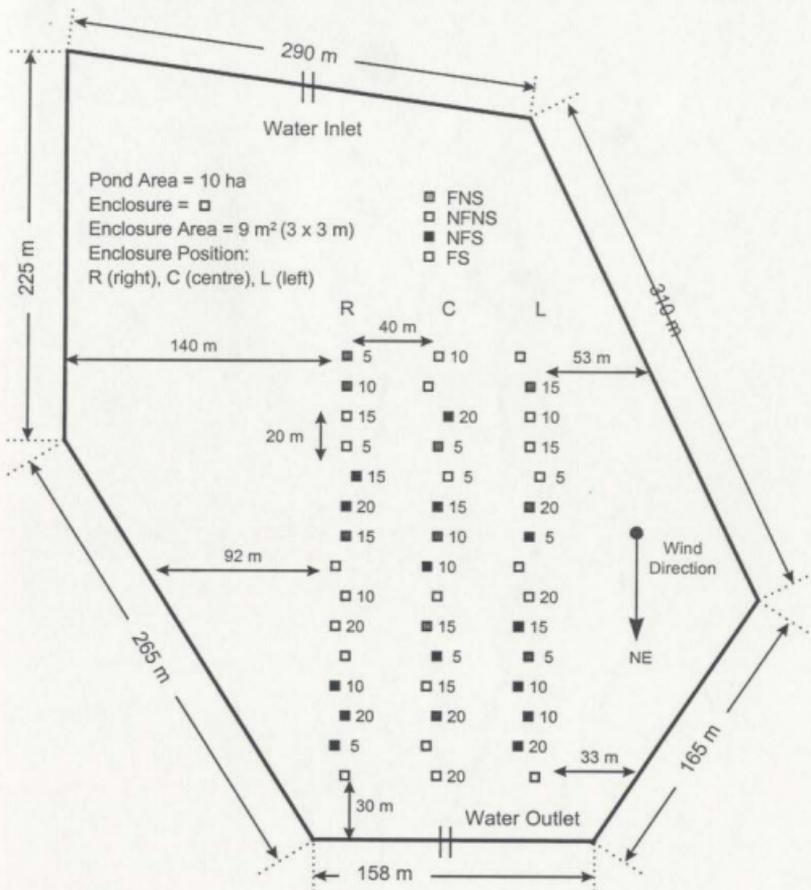
The study consisted of four treatments: enclosures with shrimp and a supply of feed (*i.e.*, FS); enclosures without a supply of shrimp and shrimp (NFNS, control); enclosures with shrimp, but without a supply of feed (SNF); and, enclosures with a supply of feed, but without shrimp (FNS). For enclosures with shrimp (*i.e.*, FS and SNF), four initial shrimp stocking densities were used (*i.e.*, 5, 10, 15 and 20 shrimp/m^2 or FS$_5$, FS$_{10}$, FS$_{15}$, FS$_{20}$ and SNF$_5$, SNF$_{10}$, SNF$_{15}$, SNF$_{20}$).

Feeding rates were constant among FNS and FS enclosures, but the amount distributed varied according to initial stocking density. To examine the possible effects of the amount of feed administered on polychaetes, four feeding regimes were assigned to each of the FS and FNS enclosures (*i.e.*, FNS$_5$, feeding for 5 shrimp/m^2; FNS$_{10}$, feeding for 10 shrimp/m^2; FNS$_{15}$, feeding for 15 shrimp/m^2; and, FNS$_{20}$ feeding for 20 shrimp/m^2). Triplicate enclosures (except NFNS, 9 enclosures) were used for each treatment, shrimp density and (or) feeding regime, giving a total of 45 enclosures [*i.e.*, 4 stocking densities x 3 treatments (FNS, FS, SNF) x 3 replicates + 9 controls (NFNS)]. Enclosures were distributed in a random fashion throughout each column of the experimental lay-out (Figure 5.2), installed prior to fertilisation procedures, but after pond sterilisation.

5.2.2 Pond Preparation

After shrimp harvest of a previous growth cycle, the pond was sun-dried for one week to eradicate undesirable species. Remaining water puddles were sterilised with 500

L of hypochlorite for 2 d. Initial examination of pond substrate indicated depleted to very low levels of polychaete abundance. Under these conditions, inoculation of polychaetes was conducted according to local commercial procedures.

A total of 0.14 m^3 of substrate with 31,163 ± 11,852 (mean ± s.d.; n = 3) polychaetes were uniformly broadcast in each enclosed area. Animals were transplanted from nearby brackish water lagoons [8.35 ± 0.04 pH (mean ± standard deviation; n = 3), 20 ± 1 ‰ (n=3) salinity and 25 °C temperature] during a 7-d period. To allow sufficient time for polychaete settling and growth, pond water level was exchanged after sterilisation, increased and kept between 10 to 20 cm for 15 d. Subsequently, water depth was gradually increased to 50 cm for fertilisation with 240 kg of water-dissolved chicken manure. Five days after organic fertilisation, water level was raised between 80 and 90 cm and exchanged at a constant rate of 5 to 10% pond volume/d until shrimp stocking.

5.2.3 Shrimp Stocking, Feeding and Pond Management

Initially, shrimp larvae of *Penaeus subtilis* were captured in the estuary of Acaraú River and reared for 30 d in round 0.25-ha nursery earthen ponds. Throughout this period, shrimp were fed twice daily with a mixture of a commercial formulated feed and minced fish flesh. The study started on 28 May 1998 (shrimp stocking) and extended until 30 July 1998 (harvest). Post-larvae (PL) of 0.20 ± 0.04 g (mean ± s.d.; n = 11) were slowly acclimated and released into FS and SNF enclosed areas during early morning. The area

outside the enclosures was also stocked with PL of *P. subtilis* at a density of 2 PL/m². No artificial feeding was carried out during rearing of shrimp in the outside region.

A dried commercial pelleted food, type Ralston Purina MR-35 (Agribrands Purina do Brasil Ltda., São Lourenço da Mata, Pernambuco, Brazil), was broadcast by hand twice daily at 1000 and 1500 h (to the FNS and FS enclosures only). During the initial 19 d of culture, only feed in powder form was used. This was followed by a mixture (1:1 ratio) of powder and granules (1.9 mm diameter; from D_{20} to D_{30}), 70% pellets (1.3 mm length by 2.3 mm diameter) and 30% granules (from D_{31} to D_{40}) and only pellets for the remainder of the culture cycle. Proximate analysis of the feed indicated the following chemical composition: $9.0 \pm 1.0\%$ (mean ± s.d.; n = 18) moisture, $40.5 \pm 1.2\%$ protein (N x 6.25, dry basis; n = 6), $7.5 \pm 1.2\%$ lipid (n = 6), $11.0 \pm 0.3\%$ ash (n = 6) and $32.0 \pm 1.1\%$ carbohydrate (n = 18). Feeding protocols followed commercial feeding practices (Jory, 1995). Feed quantities were adjusted at each 12-d period based on shrimp average body weight. Feeding amounts increased proportionally to the length of the rearing period, with rates as follow: from day 1 to day 19, 15% shrimp body weight/d; 8% shrimp body weight/d from day 20 to day 40; and, 5% shrimp body weight/d from day 41 to day 60. Shrimp growth measurements (post-orbital carapace length, CL) were only conducted with a small number of enclosures, in order to reduce disturbance to the pond substrate and the cultured population. Shrimp were sampled with a cast net at each 10-d rearing period, starting on day 29 (D_{29}) of culture (*i.e.*, 29 days after shrimp stocking). Following measurements, all collected animals were returned to their respective culture areas.

Water was exchanged daily basis in accordance with tidal fluctuations at a rate of 3% of total pond volume/d. Water quality was monitored daily at 1030 and 1530 h. Dissolved

oxygen (DO) and temperature (YSI 55, Yellow Springs Instruments, Yellow Springs, Ohio, USA), salinity (Atago Salinity Refractometer, model 2441-WO5, Atago Co., Tokyo, Japan) and pH (Water Resistant Microprocessor pH Meter, Hanna Instruments Ltd., Leighton Buzzard, Bedfordshire, England) measurements were taken in triplicate twice daily (morning and afternoon). Water transparency was measured with a Secchi disk in triplicate once daily at 1030 h. After harvest, shrimp from each enclosed area were counted and measured. Carapace length (CL) measurements were converted to body weight (BW) by using the equation given by Nunes (1995), where BW = $0.0008CL^{2.9619}$ (r = 0.991).

5.2.4 Polychaete Collection, Examination and Identification

The collection of benthic polychaetes began 2 d prior to shrimp stocking (*i.e.*, D_{stock}). Sampling continued every 10 d throughout the rearing cycle. Since substrate sieving, separation and first counting of polychaetes were conducted with live animals to avoid fragmentation of specimens, collection was carried over a 3-d period, starting with enclosures from column L (left, first day), C (centre, second day) and then R (right, third day).

On each sampling day, polychaetes with and other existing macrobenthic fauna were collected using an acrylic hand-operated sampler measuring 5.1 cm of internal diameter (*A* = 20.43 cm^2) and 63.7 cm in length. The device consisted of a core tube of 50.7 cm in length connected to a brass core head (13.0 cm) possessing a flutter valve which sealed

during retrieval. On the core head, an 83.7 cm length aluminium support was securely attached to allow substrate penetration and retrieval of device. The complete apparatus weighed about 2 kg and was operated from a boat to reduce interference with the pond bottom. During collection, the sampler penetrated between 15 to 20 cm of the pond bottom, but only about 15 cm of the upper sediment layer was retained for analysis.

At each sampling period, a total of 5 to 6 substrate samples were taken randomly from individual enclosures, starting at 0530 h. Core samples were transferred to transparent plastic bags containing sea water and immediately brought to a laboratory for analysis. In the laboratory, each substrate sample was elutriated and sieved through three mesh size nets of 2.83 mm, 1.83 mm and 500-μm (Hubbard Scientific Co., Illinois, Chicago, USA). This procedure effectively separated polychaetes and other macrofauna from larger and smaller particles. Polychaetes were isolated with forceps from the retained 500-μm portion of the sample, individually counted, and stored in plastic vials containing 70% ethanol for subsequent analysis.

Later, samples were re-examined for identification of polychaetes and recounting. During this process, samples were washed with distilled water into a Petri dish, and polychaetes were individually recounted and identified using a dissecting microscope with x20 or x40 magnification. Polychaete families were taxonomically identified according to Fauchald (1977) and Amaral and Nonato (1996). Concurrently, separated animals from replicates were mixed, oven-dried to a constant weight and weighed to the nearest milligram. Polychaete biomass (except for D_{stock}) was calculated by dividing the total dry weight (g) obtained from mixed samples by the total number of replicates collected for each enclosure at each sampling period. Polychaete density was determined by calculating the

average number of collected animals (first and second countings, including replicates). Both polychaete biomass and density were converted from the sample size of 20.43 cm² to m².

From this data and after taxonomic classification of polychaetes and the determination of their numerical abundance in each enclosure, the following indices were calculated (Reymond and Lagardère, 1990; Nunes *et al.*, 1997b):

$$Cn = (100 \times p)/P' \qquad (5.1)$$

where, Cn is polychaete frequency (%), p is the total number of each specific polychaete family and P' is the total number of polychaetes observed in all samples. Polychaete occurrence index (f, %) was calculated as:

$$f = (100 \times N_p)/N' \qquad (5.2)$$

where, N_p is the number of enclosures with each specific polychaete family and N' is the total number of enclosures. From f, three categories of family were determined: prevalent or main family, where $f \geq 50\%$; secondary family, where $10\% < f < 50\%$; and, accidental family, where $f \leq 10\%$.

5.2.5 Soil Analysis

A day prior to shrimp harvest (D_{63}), three replicates or more of substrate samples (15-cm deep each) were randomly collected from each enclosure for chemical analysis. Prior to examination, replicates were mixed thoroughly amounting to 1 kg of wet sediment/enclosure. Tests followed the methodology described by EMBRAPA (1979). In

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the laboratory, soil samples were dried at 60 °C until a constant weight was obtained. Particle size composition was determined by sieving soil samples for particles coarser than 0.05 mm. The pipette method was applied to silt and clay-sized particles. Dispersion was accomplished by the addition of 1 N NaOH. Soil pH was measured with a potentiometer in a soil-to-water ratio of 1:1. Organic carbon was determined by oxidation of the soil organic matter with a solution of 0.4 N $K_2Cr_2O_7$. Organic matter was measured indirectly by multiplying the figure for organic carbon by 1.724. A neutral 1 N NH_4OAc was used for analysis of calcium, magnesium, potassium and sodium. Calcium and magnesium were determined by EDTA titration, and sodium and potassium by flame photometry. Exchangeable aluminium was measured by titration with neutral 0.1 N NaOH, using phenolphthalein as indicator. Total nitrogen was determined by the Kjeldahl method. Phosphorus was extracted with a solution of 0.05 N HCl and 0.025 N H_2SO_4, and determined by colorimetry. Soil particle-size texture was classified using the triangular diagram according to the USDA (1951).

5.2.6 Statistical Analyses

Statistical analyses were performed with the Statistical Package for Social Sciences Windows version, release 7.5.1. (SPSS Inc., Chicago, Illinois, USA). Homogeneity of variance was examined for all data by using Bartlett-Box F and Cochran's C tests. Kurtosis and skewness and their standard error (*i.e.*, s.e. kurtosis and s.e. skewness) were applied to the data as measures of asymmetry and tests of normality. Based on these

results, data were transformed to a log(x + 1) scale in order to normalise and homogenise the variances and to meet statistical assumptions. Probability of type I error was set at 0.05.

5.3 Results

5.3.1 Shrimp Growth and Survival, and Water and Soil Quality

Penaeus subtilis final body weight ranged from a minimum of 4.2 g (SNF$_{20}$) to a maximum of 9.3 g (FS$_5$, Table 5.1). Final survival ranged from 42% (FS$_{10}$) to 69% (FS$_{15}$). Shrimp growth was treatment dependent (*i.e.*, SNF versus FS; Table 5.1), while overall survival was similar among treatments. On average, a higher growth rate was found for FS treatments (8.3 g) when compared to SNF enclosures (6.0 g). Both *P. subtilis* growth and survival decreased as initial stocking density increased (except SNF$_{10}$ and FS$_{20}$, Table 5.1).

Rainfall was scarce during the complete rearing cycle (Figure 5.3). A total of only 67.0 mm of precipitation fell during the 2.5-month study period, decreasing salinity levels at some instances. While temperatures remained relatively uniform during the rearing cycle [29.2 ± 2.6 °C (mean ± s.d.); n = 280], salinity (44 ± 2 ‰; n = 335) varied significantly (one-way ANOVA, $F_{5, 329}$ = 90.27, $P < 0.001$), showing an increasing trend as the culture period progressed. Dissolved oxygen(6.13 ± 1.61 mg/L; n = 282), water visibility (42 ± 5 cm; n = 184) and pH (8.57 ± 0.34; n = 334) were within normal levels

Table 5.1: Mean final growth (g) and survival (%, values in parentheses) of *Penaeus subtilis* for treatments SNF (shrimp and no feed) and FS (feed and shrimp) at four stocking densities.

Treatment*	Shrimp Initial Stocking Density			
	5 shrimp/m²	10 shrimp/m²	15 shrimp/m²	20 shrimp/m²
SNF	6.2 g (66%)	7.4 g (58%)	6.1 g (48%)	4.2 g (54%)
FS	9.3 g (42%)	8.2 g (42%)	7.1 g (69%)	8.5 g (43%)

*Sample size of 272 (SNF) and 264 (FS) shrimp.

Figure 5.3: Daily mean physical and chemical water quality parameters (*i.e.*, pH, salinity, dissolved oxygen, temperature and water visibility) of shrimp pond enclosures over the rearing cycle of *Penaeus subtilis*. Measurements were taken at least once a day. Rainfall data provided by Fundação Cearense de Meteorologia e Recursos Hídricos (FUNCEME).

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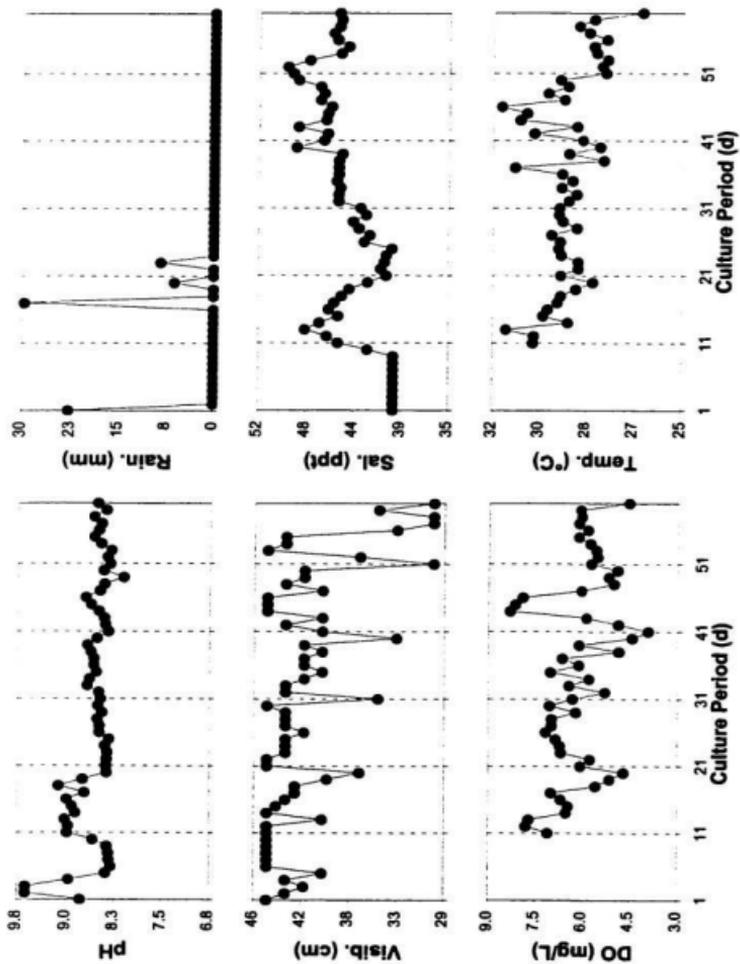
and presented no clear patterns or pronounced variations over the rearing cycle.

Since the physical profile of sediment is known to affect polychaete abundance (Decho *et al.*, 1985), statistical tests were conducted to assess if significant variations existed among treatments. One-way ANOVA conducted for each chemical and physical variable of pond sediment (Table 5.2) indicated no significant differences among enclosures ($P > 0.05$). In general, soil texture fell within the sandy clay loam class ($33 \pm 13\%$ coarse sand, $16 \pm 9\%$ fine sand, $27 \pm 13\%$ silt and $25 \pm 9\%$ clay).

5.3.2 Polychaete Analysis

A total of 1,631 substrate samples of 20.43 cm^2 containing 20,283 polychaetes were collected for analysis. All samples were numerically dominated by polychaetes, although amphipods were sometimes observed at very low numbers. Determination of polychaete density (PD) prior to shrimp stocking (*i.e.*, D$_{stock}$) indicated mean levels of 954 ± 832 polychaetes/m^2 (mean $\pm$ s.d.; n = 269). Initial PD did not differ statistically among treatments FNS, FS, SNF and NFNS (D$_{stock}$, Scheffé's Multiple Range Test, $P > 0.05$; Table 5.3), but changed significantly from other culture periods (Scheffé's Multiple Range Test, $P < 0.05$; Table 5.3).

Table 5.2: Final chemical composition and relative representation of particle size of sediment from pond bottom of enclosures FNS (feed and no shrimp), NFNS (no feed and no shrimp), SNF (shrimp and no feed) and FS (feed and shrimp). Last column indicates significance level of one-way ANOVA.

Variable*	Enclosures				ANOVA
	FNS	NFNS	SNF	FS	Sig. P
Coarse Sand (%)	31 ± 16	34 ± 13	35 ± 14	31 ± 11	0.839
Fine Sand (%)	18 ± 12	14 ± 5	18 ± 12	13 ± 7	0.501
Silt (%)	25 ± 12	27 ± 14	23 ± 9	31 ± 15	0.464
Clay (%)	26 ± 11	25 ± 5	25 ± 7	25 ± 12	0.998
pH	8.1 ± < 0.1	8.0 ± < 0.1	8.0 ± < 0.1	8.0 ± 0.1	0.174
Total C (%)	0.87 ± 0.35	0.82 ± 0.17	0.80 ± 0.28	0.95 ± 0.28	0.617
Kjeldahl N (%)	0.08 ± 0.03	0.08 ± 0.02	0.08 ± 0.03	0.09 ± 0.03	0.723
C:N (%)	10 ± 1	10 ± 1	10 ± 1	10 ± 1	0.250
Organic matter (%)	1.50 ± 0.60	1.42 ± 0.30	1.38 ± 0.48	1.63 ± 0.48	0.616
Phosphorus (‰)	4 ± 5	5 ± 7	5 ± 5	3 ± 4	0.701

*Each variable correspond to a total of 45 measurements.

Table 5.3: Polychaete density (mean number/m² x 10^2 ± s.d.) at the bottom of FNS, FS, SNF and NFNS enclosures. Values in parentheses indicate minimum and maximum values (n refers to number of observations for each treatment). Common letters denote non-significant differences at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test. Lowercase and uppercase letters refer to vertical and horizontal comparisons, respectively.

Culture Period (d)	Polychaete Density (number/m² x 10²)*			
	Treatment			
	FNS	FS	SNF	NFNS
D_{stock}	10 ± 8 A	11 ± 9 A	8 ± 8 aA	9 ± 8 A
	(< 1 - 39)	(< 1 - 34)	(< 1 - 34)	(< 1 - 29)
D_{10-12}	61 ± 53 aA	52 ± 39 aA	34 ± 36 bB	33 ± 33 aB
	(< 1 - 220)	(< 1 - 176)	(< 1 - 201)	(< 1 - 127)
D_{20-22}	52 ± 42 aA	49 ± 43 aA	25 ± 27 ab	42 ± 33 abA
	(< 1 - 201)	(< 1 - 220)	(< 1 - 98)	(< 1 - 108)
D_{30-32}	57 ± 42 aA	54 ± 67 aAB	33 ± 33 bB	45 ± 38 abAB
	(< 1 - 191)	(< 1 - 333)	(< 1 - 137)	(< 1 - 157)
D_{40-42}	109 ± 89 bA	57 ± 65 aABC	55 ± 77 bC	72 ± 54 bAB
	(< 1 - 357)	(< 1 - 323)	(< 1 - 279)	(< 1 - 230)
D_{50-52}	141 ± 108 bA	100 ± 125 aB	60 ± 71 bB	125 ± 65 cA
	(< 1 - 470)	(< 1 - 563)	(< 1 - 333)	(< 1 - 289)
D_{60-62}	154 ± 113 bA	125 ± 166 aB	73 ± 99 b	127 ± 80 cAB
	(< 1 - 519)	(< 1 - 636)	(< 1 - 396)	(< 1 - 348)
N	435	435	436	325

*Values from the sample size of 20.43 cm² were converted to m².

5.3.3 Trends over the Rearing Cycle

Standard error of kurtosis and skewness for polychaete density were 0.121 and 0.061, respectively. Lowest PD were found just prior to shrimp stocking [D_{stock}, 10 ± 8 polychaetes/m^2 x 10^2 (mean ± s.d.); n = 269], in contrast to the highest number recorded at the end of the rearing period (D_{60-62}, 119 ± 124 polychaetes/m^2 x 10^2; n = 225; Figure 5.4). Transformed PD was statistically different among sampling periods (one-way ANOVA, $F_{6, 1624} = 75.51$, $P < 0.001$). A *posteriori* analysis showed that differences concentrated mainly among three stages of culture, an initial (D_{stock}, D_{10-12}, D_{20-22} and D_{30-32}), an intermediate (D_{40-42}) and a final period (D_{50-52} and D_{60-62}; Scheffé's Multiple Range Test, $P > 0.05$; Figure 5.4). Therefore, a constant pattern in polychaete numerical abundance existed until the intermediate stage of the rearing cycle (51 to 57 d after polychaete inoculation) when the density began to increase.

Examination of PD variations for each treatment over the course of the rearing cycle indicated the existence of various patterns (Table 5.3). While the control treatment (*i.e.*, NFNS) displayed PD changes similar to the overall trend, other treatments presented different variations. PD pattern for FNS was limited to two stages, initial (D_{10-12}, D_{20-22} and D_{30-32}) and final (D_{40-42}, D_{50-52} and D_{60-62}; Table 5.3), whereas in FS polychaete densities were uniform throughout the rearing period (except for D_{stock}; Table 5.3). Similarly, PD was maintained almost uniform in SNF enclosures, except for a significant drop detected at D_{20-22} (Table 5.3). In all cases, PD increased as the rearing cycle progressed.
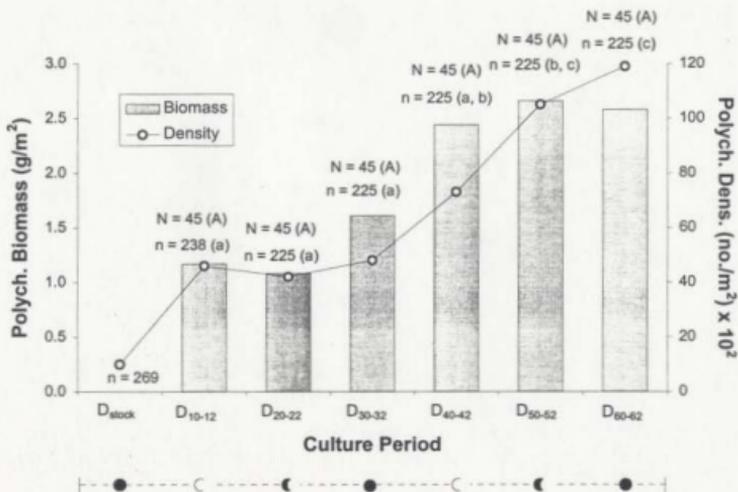
Figure 5.4: Overall mean polychaete density (number/m^2 x 10^2) and biomass (g of dried polychetes/m^2) in relation to the rearing period. Common letters in parentheses indicate non-significant differences at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test. Uppercase letters refer to comparisons for biomass and lowercase for density. Number of observations at each culture period are indicated by N (biomass) and n (density). Lunar cycles are shown on the bottom.

Similarly to PD, polychaete biomass (PB, 0.296 s.e. kurtosis and 0.149 s.e. skewness) differed statistically among culture periods (one-way ANOVA, $F_{5, 263} = 3.261$, $P < 0.001$). However, a *posteriori* analysis could not detect any significant differences at the $\alpha = 0.05$ level (Scheffé's Multiple Range Test). This suggests only slight variations in PB among culture periods, insufficient to generate statistical variations when examined by a conservative *post-hoc* test. Analysis of correlation between PD and PB indicated a significant, but poor relationship at the 1% level (Pearson coefficient of correlation = 0.375; n = 534). Although population growth (*i.e.*, PD and PB) appeared to be synchronous with the lunar cycle, particularly the new moon, no statistically significant increments were detected during these stages (Figure 5.4).

5.3.4 Effects of Shrimp Predation

Overall, polychaete numerical abundance and biomass differed significantly among treatments FNS, NFNS, SNF and FS (one-way ANOVA, $F_{3, 1627} = 39.55$, $P < 0.001$ for PD; and, one-way ANOVA, $F_{3, 265} = 6.37$, $P < 0.001$ for PB; Figure 5.5). SNF enclosures showed statistically lower levels in PD when compared to the remaining treatments (Scheffé's Multiple Range Test, $P < 0.05$; Figure 5.5). This indicated a strong role of shrimp predation on the number and density of polychaete, particularly when feed was not provided. This effect was also clear in an early stage of culture (*i.e.*, D_{20-22}; SNF, Scheffé's Multiple Range Test, $P < 0.05$; Table 5.3). SNF also showed a significantly lower PB when compared to FNS (Scheffé's Multiple Range Test, $P < 0.05$; Figure 5.5),
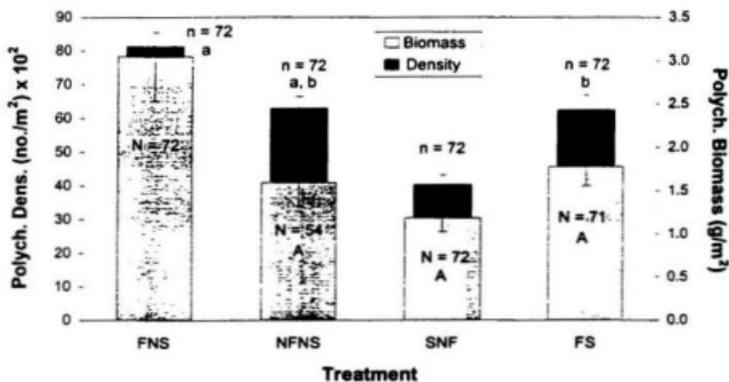
Figure 5.5: Differences in total mean polychaete density (number/m^2 x 10^2) + standard error (s.e.) and biomass (g of dried polychaetes/m^2) - s.e. among treatments FNS (feed and no shrimp), NFNS (no feed and no shrimp), SNF (shrimp and no feed) and FS (feed and shrimp). Number of observations are indicated by N (biomass) and n (density). Common letters (uppercase refers to biomass and lowercase refers to density) denote no significant difference at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.

but it did not differ from NFNS and FS (Scheffé's Multiple Range Test, $P > 0.05$; Figure 5.5). On the other hand, FS presented a significantly lower PD than FNS enclosures (Scheffé's Multiple Range Test, $P < 0.05$; Figure 5.5).

Shrimp predation effect on polychaetes were confirmed by contrasting with (SNF and FS) and without (FNS and NFNS) shrimp treatments (Figure 5.6). In general, there was a significantly higher PD (t-test, df = 1524, $P > 0.001$; Figure 5.6) and PB (t-test, df = 267, $P = 0.005$) in treatments without shrimp (PD, 73 ± 80 polychaetes/m^2 x 10^2; PB, 2.41 ± 0.31 g of dried polychaetes/m^2), when compared to shrimp treatments (49 ± 80 polychaetes/m^2 x 10^2; PB, 1.48 ± 0.14 g of dried polychetes/m^2). Differences in PD became evident starting on D$_{20-22}$ of culture (Figure 5.6) and continued until D$_{60-62}$. The statistically lower PD in the initial stages of culture reflects an early predatory effect of *Penaeus subtilis* on polychaetes, mainly when formulated food was not provided.

Comparisons among shrimp stocking densities indicated significant differences in FS enclosures (FS, one-way ANOVA, $F_{3, 431} = 28.84$, $P < 0.001$; Figure 5.7). On average, the higher the shrimp density, the lower PD was found (FS, 15 and 20 shrimp/m^2; Scheffé's Multiple Range Test, $P > 0.05$; Figure 5.7). In SNF enclosures, however, a similar effect could not be detected (SNF, one-way ANOVA, $F_{3, 432} = 1.38$, $P = 0.248$; Figure 5.7), suggesting that PD may had already reached critical levels for all shrimp stocking densities. No statistical differences were found in PB among shrimp densities in enclosures FS (one-way ANOVA, $F_{3, 67} = 1.53$, $P = 0.214$) and SNF (one-way ANOVA, $F_{3, 68} = 2.22$, $P = 0.094$).

Figure 5.6: Changes in mean polychaete density (number/m^2 x 10^2) ± s.e. in enclosures with (SNF and FS) and without (FNS and NFNS) shrimp and with (FNS and FS) and without (SNF and NFNS) feed. Days represent culture period after shrimp stocking (D$_{stock}$). Numbers next to lines indicate level of significance from two-tailed t-tests.
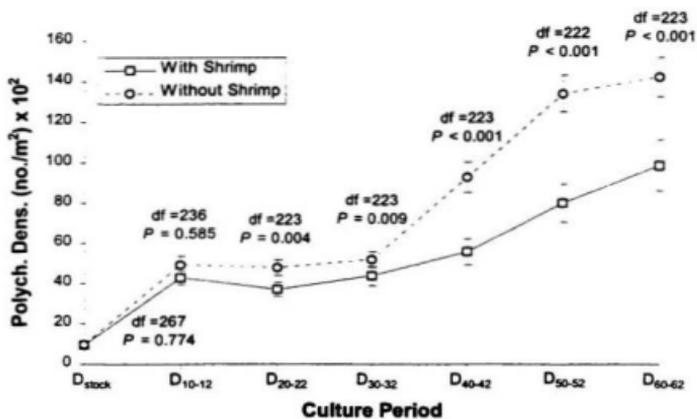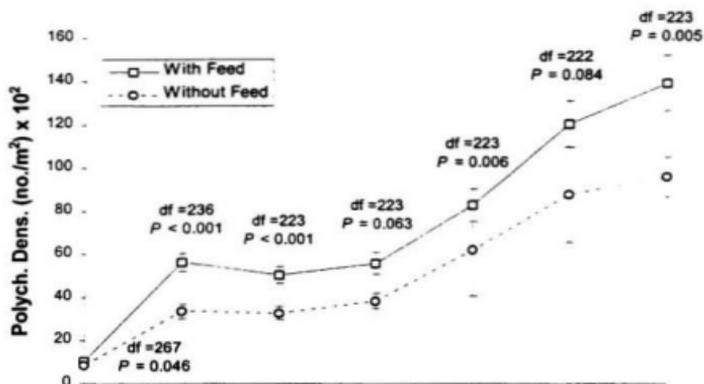
Figure 5.7: Comparison of total mean polychaete density (number/m^2 x 10^2) ± s.e. in relation to four shrimp and (or) feed densities among treatments FNS (feed and no shrimp), FS (feed and shrimp) and SNF (shrimp and no feed). Feed density refer to feeding regime used for each respective shrimp stocking density (number of shrimp/m^2). Values (n) on top of bars indicate number of substrate samples analysed. Common letters denote no significant difference at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test.

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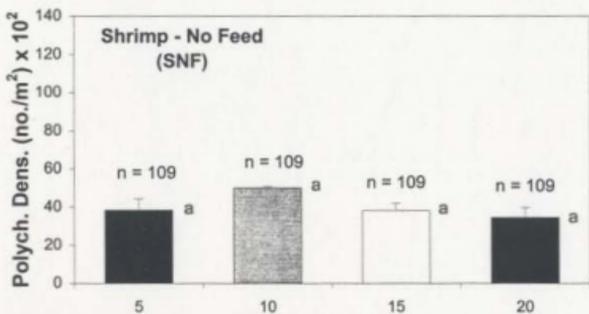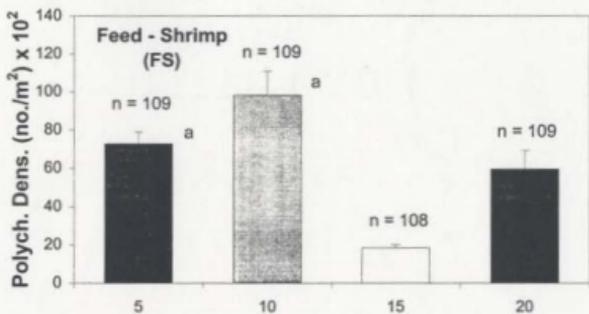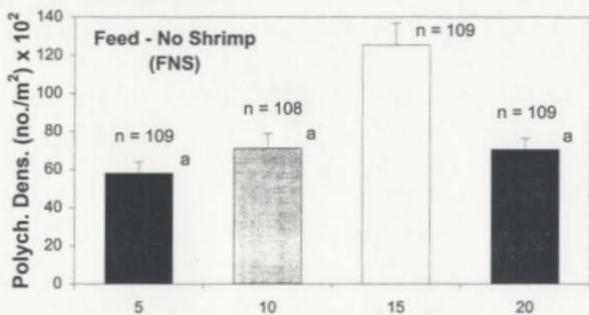
5.3.5 Effects of Artificial Feeding

In contrast to enclosures without feed supply (SNF and NFNS), treatments with feed (FNS and FS) presented a statistically higher PD (t-test, df = 1629, $P < 0.001$; Figure 5.6) and PB (t-test, df = 267, $P = 0.002$). A significantly greater PD was found for enclosures with feed for most culture periods (Scheffé's Multiple Range Test, $P < 0.05$; Figure 5.6). PB was also significantly greater in treatments with feed (2.40 ± 0.28 g of dried polychetes/m²) than those without feed (1.36 ± 0.15 g of dried polychetes/m²). Overall, PD showed significantly higher levels for FNS and FS enclosures when compared to the SNF treatment (Scheffé's Multiple Range Test, $P < 0.05$; Figure 5.5). In the case of PB, the effect of artificial feeding was highest when shrimp were not present (*i.e.*, FNS enclosures; Scheffé's Multiple Range Test, $P < 0.05$; Figure 5.5), indicating a high predatory behaviour of *Penaeus subtilis* on polychaetes in the absence of supplemental feed.

Within FNS enclosures, the effect of artificial feeding on PD was highest for FNS$_{15}$ treatments (Scheffé's Multiple Range Test, $P < 0.05$; Figure 5.7). Under the same feeding regime, FS$_{15}$ enclosures produced the lowest PD levels among all other shrimp densities (*i.e.*, FS$_5$, FS$_{10}$ and FS$_{20}$; Scheffé's Multiple Range Test, $P < 0.05$; Figure 5.7). No statistical differences could be detected in PB among feeding regimes in enclosures FNS (one-way ANOVA, $F_{3, 68} = 0.87$, $P = 0.459$).

5.3.6 Family Classification and Abundance

Six different polychaete families were found within enclosed areas used for the study. They were identified as follows: Spionidae, Eunicidae, Capitellidae, Pilargidae, Nereidae and Sabellidae. Their overall numerical representation was statistically different (one-way ANOVA, $F_{5, 1608} = 396.98$, $P < 0.001$; Figure 5.8). Scheffé's Multiple Range Test ranked (1) Spionidae (Cn = 52.3%) as being the most prominent family, followed by: (2) Capitellidae (Cn = 37.9%); (3) Eunicidae (Cn = 6.8%); and, (4) Nereidae (Cn = 2.7%), Pilargidae (Cn = 0.3%) and Sabellidae (Cn < 0.1%). Visual observations indicated that most spionids, capitellids and nereids had comparatively smaller body sizes than the eunicids. Some eunicids reached 10 cm or more in length.

Only small changes in polychaete frequency and occurrence patterns occurred throughout the production cycle (Figure 5.8). The dominance of Spionidae and Capitellidae over other families was evident starting in the early stages of culture, while Eunicidae became more numerically abundant in the intermediate stage (Table 5.4). The overall density of Spionidae and Capitellidae was also statistically greater than the other families, regardless of the treatment used (Scheffé's Multiple Range Test, $P < 0.05$). In general, Spionidae, Capitellidae and Eunicidae fell within prevalent or main families (*i.e.*, f ≥ 50), Pilargidae and Nereidae (10% < f < 50%) as secondary families and Sabellidae as an accidental family (f ≤ 10%). Within each family, Capitellidae, Pilargidae and Sabellidae showed a constant numerical abundance over the rearing cycle (Table 5.4). A significant
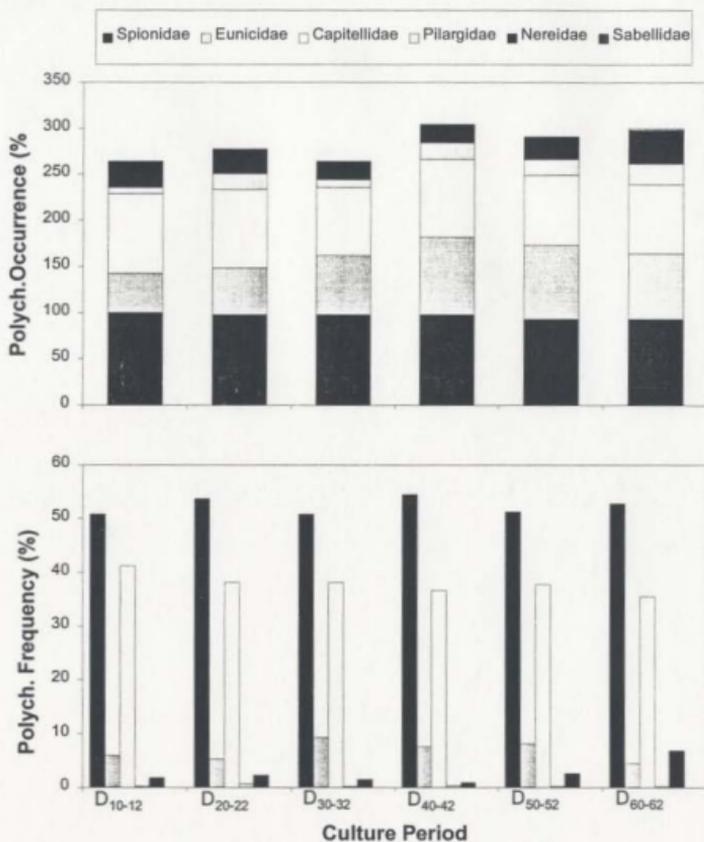
Figure 5.8: Frequency (%) and occurrence (%) of polychaetes by family over a 62-d rearing cycle of *Penaeus subtilis* in pond enclosures.

Table 5.4: Numerical representation of polychaetes [(number/m^2) ± s.d.] by family over a 62-d shrimp rearing cycle (n refers to number of observations at each culture period). Each observation represents a mixture of five to six substrate samples of 306 cm^3. Common letters denote non-significant differences at the $\alpha = 0.05$ level by Scheffé's Multiple Range Test. Small letters indicate horizontal comparisons among culture periods for each family, while uppercase letters refer to vertical comparisons among families at each culture period.

Family	Polychaete Density (number/m²)*					
	Culture Period					
	D_{10-12}	D_{20-22}	D_{30-32}	D_{40-42}	D_{50-52}	D_{60-62}
Spionidae	1968 ± 1170 a	1987 ± 1204 a	2178 ± 1375 a	3583 ± 3152 ac	4914 ± 3891 bc	5986 ± 4851 bc
Eunicidae	235 ± 685 aA	196 ± 377 aA	396 ± 573 abA	494 ± 700 ab	773 ± 803 bc	514 ± 578 acA
Capitellidae	1610 ± 2286 a	1410 ± 1694 a	1630 ± 2159 a	2413 ± 3186 a	3627 ± 4371 a	4023 ± 5521 a
Pilargidae	8 ± 33 aA	24 ± 54 aA	9 ± 28 aB	26 ± 64 aA	20 ± 45 aA	29 ± 59 aAB
Nereidae	73 ± 157 abA	83 ± 196 abA	64 ± 162 abAB	64 ± 230 aA	250 ± 881 abA	778 ± 2242 bAB
Sabellidae	< 1 aA	< 1 aA	< 1 aB	< 1 aA	< 1 aA	2 ± 15 aB
n	45	45	45	45	45	44

*Values from the sample size of 20.43 cm² were converted to m².

increase was observed for Spionidae and Eunicidae in the final stages of culture (*i.e.*, D_{50-52}; Scheffé's Multiple Range Test, $P < 0.05$; Table 5.4), while Nereidae had only slight changes (between D_{40-42} and D_{60-62}; Table 5.4).

5.4 Discussion

5.4.1 Environmental Parameters and Shrimp Performance

All water quality parameters analysed were within levels considered normal for penaeid shrimp culture (Nunes, 1996c; Nunes and Sandoval, 1997; Nunes, 1998). In some instances, salinity was higher than desirable levels, but average values (44 ± 2 ‰; n = 335) were within limits found in other studies conducted at the same site and during the same season (Nunes *et al.*, 1996; Nunes, 1998). It is also unlikely that the increasing salinity levels during the production cycle depressed polychaete growth, because increments in their population size were observed.

Results of chemical analysis of the pond sediment were comparable to those of other investigations in shrimp ponds (Boyd *et al.*, 1994; Boyd and Pippopinyo, 1994; Gonzalez-Vila *et al.*, 1996; Smith, 1996; Queiroz and Boyd, 1997; Nunes and Parsons, 1999; Chapter 3). The general textural classification (*i.e.*, sandy clay loam) of the soil suggests a good water retention capacity. The observed content of silt (25%) and sand (49%) reflects a normal soil structure (Guillet and Rouiller, 1982), with textural characteristics similar to those of other aquaculture ponds (Boyd and Pippopinyo, 1994;

Smith, 1996). The absence of significant variations in soil particle size among treatments indicates that sediment physical profile had no influence on the differences found for polychaete abundance.

The average survival of *Penaeus subtilis* observed in SNF and FS treatments, is comparable to normal levels obtained in the industry (between 54.0 and 88.1% after 60 d of culture; Jory, 1995). In SNF, some of the low growth and survival values found for *P. subtilis* (SNF$_{15}$ and SNF$_{20}$) were the result of higher shrimp stocking densities, coupled with the absence of external food supply. In general, shrimp mean body weights increased with feed inputs and at lower shrimp stocking densities. Maguire and Leedow (1983) and Allan and Maguire (1992) observed a similar shrimp density and growth interaction for *Metapenaeus macleayi* and *P. monodon*, respectively. In both studies, the authors reported that as shrimp density increased, average shrimp weight gain decreased, although survival was unaffected by stocking density. In another study however, Apud *et al.* (1981) found that stocking density affected both survival and growth of shrimp.

5.4.2 Polychaete Families

In the present study, taxonomic representation of polychaetes in the pond bottom was not diverse. Data indicated the predominance of spionids and capitellids over other families throughout all stages of the growth cycle. These families have also been more frequently reported to occur in shrimp ponds (Maguire *et al.*, 1984; Ordner and Lawrence, 1987; Martins, 1994; Nunes and Parsons, 1999; Chapter 3), although, in some cases,

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families such as Nephtyidae and Opheliidae, have also been noted to be abundant (Maguire *et al.*, 1984). These observations support the results of other studies that show the high capacity of spionids and capitellids to recolonise defaunated azoic areas and to rapidly restore previous population sizes (Tsutsumi and Kikuchi, 1983; Chesney and Tenore, 1985a; Tsutsumi, 1987).

Capitellids, spionids and nereids have all been found in the stomach of penaeid shrimp (Gleason and Wellington, 1988; Stoner and Zimmerman, 1988; Reymond and Lagardère 1990). The disproportional larger body sizes observed for eunicids in the present study, may explain why this family has not been reported to occur in the diet of penaeids. Although *Penaeus* spp. are known to consume a wide range of prey sizes (Racek, 1959; Condrey *et al.*, 1972; Marte, 1980; Suthers, 1984; Stoner and Zimmerman, 1988; Reymond and Lagardère, 1990; Nunes *et al.*, 1997b), in the presence of various food sizes they discriminate against large items (Nunes and Parsons, 1998b; Chapter 2). Overall, results suggest that further investigations aimed at increasing polychaete population levels in shrimp ponds should mainly focus on opportunistic families, such as spionids and capitellids. Their ability to restore and maintain high population sizes during the growth cycle, even under continuous shrimp predation pressure was confirmed in the present study. Their apparent adequate body sizes for shrimp handling and consumption should also bring more favourable results.

5.4.3 Polychaete Population Patterns

Variations in polychaete density (956 to 11,921 polycahetes/m^2) and biomass (1.17 to 2.58 g/m^2) over the production cycle are comparable to estimates in a study by Crockett *et al.* (1988), which indicated density levels varying between 1,919 to 22,311/m^2 and dry biomass between 0.119 to 3.870 g/m^2. The trend in polychaete density for the control treatments indicated that if left undisturbed (*i.e.*, without shrimp predation or addition of feed inputs), pond polychaete populations have the potential to significantly increase during a rearing cycle (NFNS, Table 5.3). According to Chesney and Tenore (1985a), when conditions favour development, population growth of opportunistic polychaetes explodes and tends to overshoot the carrying capacity of the environment.

However, in the present study, increases in population number did not produce higher levels of total polychaete biomass. Although size-frequency distribution data were not collected, these results suggest that natural recruitment of the polychaete population occurred over the study period. Within a 2.3-month period, Tsutsumi (1987) reported two mass recruitments in a population of *Capitella capitata* growing near fish cages. George (1984) stated that some mangrove-dwelling capitellid polychaetes may become gravid in 3 to 5 weeks after larval metamorphosis and spawn 1.5 weeks later.

In the present study, observed peaks in the overall polychaete density suggest that spawning occurred from the intermediate towards the final stages of culture (*i.e.*, D_{40-42} to D_{60-62}; Figure 5.4). In other investigations (Nunes, 1995; Martinez-Cordova *et al.*, 1998b; Nunes and Parsons, 1999; Chapter 3), a similar effect was found between 5 to 10 weeks

after the initial stocking of shrimp. Nunes and Parsons (1999) reported a significant peak in polychaete density on the 68th day of a shrimp rearing cycle after observing continual 12-d declines. In another study, Nunes (1995), working in a semi-intensive shrimp culture system, found an increase from 9 ± 4 polychaetes/m^2 x 10^2 (40th day of culture) to 92 ± 75 polychaetes/m^2 x 10^2 (50th day of culture). It appears that in shrimp ponds, prominent increases in polychaete density are mainly the result of their reproductive rhythm, although factors such as low survival of adults due to predation has been suggested to accelerate this process (Nunes and Parsons, 1999; Chapter 3).

In the presence of shrimp, polychaete densities and biomass remained relatively uniform throughout the rearing cycle (SNS and FS, Table 5.3). This pattern disagrees with the general perception that polychaete density and biomass in shrimp ponds, successively decline as the culture period progresses (Martinez-Cordova *et al.*, 1998b; Nunes and Parsons, 1998a). In fact, many cultured *Penaeus* spp., including *P. subtilis* (Nunes, 1995; Nunes *et al.*, 1997b), acquire a more pronounced carnivorous habit as larger body sizes are attained [*e.g.*, *P. monoceros* (George, 1974), *P. monodon* (Marte, 1980), *P. brasiliensis* (Stoner and Zimmerman, 1988); *P. japonicus* (Reymond and Lagardère, 1990)]. Such a feeding behaviour combined with a larger population biomass of shrimp usually result in an increased grazing pressure over naturally occurring prey species (Nunes and Parsons, 1999; Chapter 3). Ordner and Lawrence (1987) studying the benthic infaunal communities of shrimp ponds, reported that in the presence of shrimp, polychaete densities started to decline after the third week of culture. Martins (1994) reported bi-weekly polychaete biomass measurements from 83 commercial production

cycles of seven semi-extensive (*i.e.*, 2 to 4 shrimp/m^2; none to low supplemental feeding) polyculture ponds of *P. subtilis*, *P. brasiliensis* and *P. schmitti*. In agreement with the present study, Martins' (1994) data did not show any meaningful reductions in polychaete biomass throughout grow-out cycles, or of a depleted polychaete biomass prior to shrimp harvest.

On the other hand, in the present study, artificial feeding did not produce progressive increments in polychaete density patterns (FNS), as would be expected with the continual increases in feed ration. The only major temporal peak observed for treatment FNS was from D_{30-32} to D_{40-42}. This peak appears to correspond to the event found during the same period for the control treatment (NFNS). Thus, rather than a promoting effect generated by artificial feeding, such an increase is more likely to be related to the timing of polychaete recruitment. The low impact of feed on promoting polychaete growth patterns is explained by the small variations in feed quantity that occurred throughout the rearing cycle. Although the amount of feed given increased during the course of the study, differences among sampling dates may not have been sufficiently large to produce a significant effect on polychaete density patterns.

In general, increases in polychaete density over the production cycle can be attributed to their initial successful colonisation prior to shrimp stocking and their reproduction. The absence of other predominant macrofauna or competitive species in the sediment may have also favoured their growth. These results indicate that in shrimp ponds, polychaetes can successfully and rapidly recolonise previously depleted areas and maintain high levels of growth even under continuous grazing pressure by shrimp. Such observations fit into the general description of polychaetes life-history and behaviour as

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opportunistic and r-strategistic animals (Grassle and Grassle, 1974; Rosenberg, 1976; McCall, 1977; Tsutsumi, 1987).

5.4.4 Impact of *Penaeus subtilis* Predation on Polychaete Abundance

Penaeus subtilis grazing pressure was the primary cause for a reduced polychaete growth in enclosures SNF and FS when compared to other treatments (*i.e.*, NFNS and FNS). This influence could be detected for both polychaete biomass (SNF versus FNS) and density (SNF versus other treatments; and, FS versus FNS). The effects of shrimp predation on polychaetes have been identified in both cultured (Rubright, 1978; Rubright *et al.*, 1981; Moriarty *et al.*, 1987; Wyban *et al.*, 1987; Gonzales, 1988; Lanari *et al.*, 1989; Martins, 1994) and natural environments (Kneib, 1985; Leber, 1985; Nilsson *et al.*, 1993) and under laboratory-controlled conditions (Bonsdorff and Pearson, 1997). In shrimp ponds, polychaete populations have been reported to decrease from a total of 45,000 animals/m^2 to less than 10 animals/m^2 (Hopkins *et al.*, 1995), changing the community structure of these systems (Moriarty *et al.*, 1987).

In the present study, *Penaeus subtilis* predatory behaviour on polychaetes closely follows the descriptions of its feeding habits and diet. Stoner and Zimmerman (1988), examining the food of *P. subtilis* in a mangrove-fringed estuary, found that capitellid polychaetes composed 20 to 38% of its diet on a biomass basis. Under semi-intensive culture conditions, Nunes *et al.* (1997b) characterised this species as having a benthic omnivorous opportunistic feeding habit, favouring polychaetes as its main prey during all

stages of the growth cycle. The authors also encountered an intense predation on polychaetes by juvenile *P. subtilis*. These observations are commensurate with the reduced polychaete levels detected within 20 to 22 d after the start of the study.

Penaeus subtilis grazing rates on polychaetes increased at higher stocking densities of shrimp (FS). This predator-prey effect was particularly noticeable at densities of 15 and 20/m². In model farming ponds, Allan and Maguire (1992) found declines in the numerical abundance of polychaetes (1241, 263, 132 and < 1 animals/m²) with comparable increases in *P. monodon* stocking densities (5, 15, 25 and 40/m²). Although in the present study, polychaete population was not depleted at high shrimp stocking densities, mean levels were comparable to those found for the SNF treatment (*i.e.*, with shrimp and no feed supply). Therefore, under increased shrimp stocking densities (*i.e.*, FS₁₅ and FS₂₀), artificial feeding only partially reduced *P. subtilis* predatory potential on polychaetes and poorly enhanced their abundance, although larger amounts of nutrients are expected to be produced under these conditions (Briggs and Funge-Smith, 1994).

Reasons that can account for the low impact of artificial feeding on the overall culture system, include an inefficient feeding programme (*e.g.*, through deficient amounts of formulated food), and food attractiveness. The recognition of polychaetes as an important prey for *Penaeus subtilis* (Stoner and Zimmerman, 1988; Nunes, 1995; Nunes *et al.*, 1997b), suggests the need to use, in aquaculture systems, feeds with improved attractability to allow an equal balance between shrimp feed consumption and polychaete predation. This is mainly important for larger shrimp because a carnivorous habit becomes more evident (Nunes, 1995; Nunes *et al.*, 1997b), possibly making polychaetes more susceptible to shrimp attacks.

Overall polychaete density and biomass in SNF treatments revealed prohibitive polychaete levels when no external food was supplied (as also found for shrimp final body weights, Table 5.1), regardless of shrimp stocking density (SNF). Although under culture and natural conditions *Penaeus subtilis* is known to feed on other epifauna and macrobenthos, such as amphipods, copepods, foraminiferans, nematodes and molluscs (Stoner and Zimmerman, 1988; Nunes *et al.*, 1997b), in the present study, observations of the pond substrate (first 10 to 15 cm layer) showed a rare occurrence of other potential prey items. This situation may have led the species to concentrate its predatory efforts on polychaetes exclusively, even at lower stocking densities (SNF).

5.4.5 Polychaete Growth Interactions with Artificial Feeding

Artificial feeding influenced polychaete numbers/density in all enclosures receiving external food supply (*i.e.*, FNS and FS). When exposed to feed inputs, polychaete population size was higher both in density (FNS and FS) and biomass (FNS). Although only a slight sediment organic and nutrient enrichment was found for the pond bottom of these treatments (as seen for organic matter, carbon and nitrogen levels on Table 5.2), polychaetes were likely to have taken advantage of external food sources by either feeding directly on fresh pellets or on decomposed food stuffs.

Organic-related polychaete growth responses have been well documented in the literature, particularly for polluted environments (Lewbell, 1985; Ansari *et al.*, 1986; Tsutsumi, 1987). In aquaculture ponds, a feed promoting effect was reported on benthic

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macroinvertebrates, mainly oligochaetes (Wahab and Stirling, 1991). In shrimp ponds, artificial feeds can form the base of the food chain (Moriarty et al., 1987), adding in excess of 130 g C, 12 g N, and 3 g P/m²/d to sediments (Schroeder et al., 1991). It is estimated that only 10 to 15% of the organic carbon and 20 to 70% of the nitrogen and phosphorus in feed are converted to shrimp flesh and removed from ponds at harvest (Briggs and Funge-Smith, 1994; Boyd, 1995). The remaining organic matter and nutrients are deposited on the pond bed, increasing the lipid content of the soil by more than 20% during one growth cycle (Gonzalez-Vila et al., 1996). In the present study, remains of uneaten feed in FS enclosures were possibly responsible for maintaining polychaete densities at higher levels when compared to SNF treatment.

The presence of feed in FS enclosures also appeared to have partially relieved, as discussed in the previous section, the predatory impact of *Penaeus subtilis* on polychaetes. Larger amounts of feed, either generated a higher polychaete density (FNS$_{15}$) or an equal effect in comparison to treatments with lower feed rations (FNS$_{20}$ versus FNS$_5$ and FNS$_{10}$).

This study has shown that *Penaeus subtilis* predation, shrimp stocking density and external food supply are major factors to be considered when establishing sustainable limits for polychaete use as a naturally occurring food source in aquaculture systems. Shrimp stocking density greatly affected polychaete numerical abundance and biomass, while their population patterns appeared to be dictated by other environmental and endogenous cues. Artificial feeding promoted the growth of polychaete populations even in the presence of shrimp, but was not effective in alleviating grazing pressure at higher stocking densities of *P. subtilis* (i.e., 15 and 20/m²). Although the present feeding regimes used did not produce

differences in polychaete abundance, the amount and particularly the quality of feed should be more precisely evaluated in its potential to minimise and compensate for shrimp predation impacts on pond macrobenthic fauna. Further investigations in extensive and semi-intensive systems should concentrate on developing management strategies for inoculation and restoration of polychaete populations in shrimp ponds, mainly during the various stages of the growth cycle (*e.g.*, through alternating fencing of strategic pond areas).

5.5 Conclusions

The results of the present investigation indicated that *Penaeus subtilis* predatory pressure combined with the use of external food supply significantly impacted the abundance of benthic polychaetes. This influence varied according to shrimp stocking density and feeding regime. When left undisturbed, the polychaete population in the pond significantly increased during the rearing cycle. In comparison, under shrimp predation and without feed supply, polychaete population levels were significantly reduced, regardless of shrimp stocking density. Artificial feeding promoted higher densities of polychaetes even when shrimp were present, but this effect was not observed at increased stocking densities (*i.e.*, 15 and 20/m^2). Larger amounts of feed however, either generated a higher polychaete density or an effect equivalent to that of treatments with a lower feed supplement. This increase occurred in spite of only a slight sediment organic and nutrient enrichment resulting from artificial feeding.

In the presence of shrimp and a supply of feed, polychaete density and biomass were relatively uniform throughout the rearing cycle. This uniformity was attributed to an initial successful polychaete colonisation prior to shrimp stocking and their rapid reproductive cycle. Although taxonomic representation of polychaetes in the pond bottom was not diverse, their density and biomass were comparable to those of other investigations (Crockett *et al.*, 1988). Overall, polychaetes successfully and rapidly recolonised a previously depleted culture area and maintained high levels of growth even under continuous shrimp grazing pressure.

CHAPTER 6

A COMPUTER-BASED STATISTICAL MODEL OF THE FOOD AND FEEDING PATTERNS OF THE SOUTHERN BROWN SHRIMP *Penaeus subtilis*

6.1 Introduction

In less intensive shrimp culture systems, an intrinsic relationship exists among polychaete availability (as natural food), *Penaeus subtilis* feeding and overall system management. In these confined environments, feeding maximisation is pursued by periodical monitoring of environmental and shrimp population conditions. Feeding rates are determined based on estimates from empirical feeding tables or by assessment of food consumption from feeding "trays". Under these changing environmental and management conditions, forecast of natural food sustainability throughout the growth cycle is difficult, and is generally based on intuition and practice.

At present, some of the parameters that govern *Penaeus subtilis* feeding and the dynamics of benthic polychaetes in shrimp ponds have been quantified (Nunes and Parsons, in review and in press; Chapters 4 and 5, respectively). However, this information is scattered and diverse and the interactions are difficult to conceptualise. Integration through computer modelling allows synthesis of data and simulations to be performed, facilitating the design and understanding of the system structure and its

relationships. In aquaculture, modelling has been used to describe and predict pond environmental variability (Piedrahita *et al.*, 1984; Klemetson and Rogers, 1985; Catchcart and Wheaton, 1987; Smith and Piedrahita, 1988; Boyd, 1991; Losordo and Piedrahita, 1991; Brown, 1995; Yi, 1998; Zhu *et al.*, 1998), pond water requirements (Nath and Bolte, 1998) and material cycling (Svirezhev *et al.*, 1984), and the effects of biotic and abiotic factors on fish growth (Cuenco *et al.*, 1985a,b; Diana *et al.*, 1991; Liu and Chang, 1992). In shrimp culture, models have addressed the economic feasibility of farming operations (Hanson *et al.*, 1985) and the effects of biological and environmental factors on shrimp production and profitability (Griffin *et al.*, 1981; AQUACOP *et al.*, 1988; Whitson *et al.*, 1988). However, despite the relevance to maximisation of feed use, models designed to predict shrimp food intake and polychaete dynamics in ponds are not yet available.

Modelling is used to formulate, examine and improve hypothesis and theories, and identify specific areas of research needs. Aquaculture models can provide a working tool to evaluate the consequences of various management strategies, assisting in the decision-making process of commercial operations. In shrimp culture systems, feed management influences penaeid feeding patterns by the frequency and amounts of external feed supply and the method of food dispersal. Shrimp body weight and environmental variables, such as time of day, regulate feed intake. In shrimp ponds, the dynamics of polychaete populations may be affected by initial population size, shrimp stocking density and feed supply. The objective of the present study was to develop and simulate one-dimensional dynamic models for: (1) *Penaeus subtilis* hourly feed intake in relation to shrimp body weight, feed ration and feeding frequency over a 24-h time period; (2) shrimp population

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feeding levels in response to feed dispersal method over a production cycle; and, (3) polychaete population dynamics in relation to shrimp stocking density, feeding regime and initial polychaete availability over a growth cycle.

6.2 Materials and Methods

6.2.1 Model Development, Simulation, and Statistical and Sensitivity Analysis

Three models based on the food and feeding patterns of *Penaeus subtilis* were developed and simulated separately using a dynamic modelling language called STELLA® II, software version 3.0.7 for Windows (High Performance Systems, Inc., Hanover, New Hampshire, USA). Euler's algorithm was used as the numerical integration method.

Parameters employed in simulations were estimated based on relationships derived from the literature. In cases where only data were available, regression analysis was performed with the Statistical Package for Social Sciences, Windows version, release 7.5.1 (SPSS Inc., Chicago, Illinois, USA). Probability of type I error was set at 0.05.

Sensitivity analyses were conducted to determine which inputs in each model contributed most to output variability. Model parameter sensitivity was evaluated with either the one-at-a-time sensitivity technique or a sensitivity index (Hamby, 1994). In the first method, a sensitivity ranking was obtained by varying each parameter by 50% while holding all others constant, and then quantifying the percentage change in the model

output relative to baseline values. The sensitivity index was calculated by varying one input parameter at a time over its entire range of possible values (*i.e.*, from its minimum to its maximum value), and determining the output percentage difference. The sensitivity index (SI) was calculated using:

$$SI = \frac{P\max - P\min}{P\max}$$ (6.1)

where, $P\max$ and $P\min$ represent the minimum and maximum output values, respectively, resulting from varying the input over its entire range (Hamby, 1994).

6.2.2 Model Structure and Parameter Estimation

6.2.2.1 *Penaeus subtilis* Ingestion and Evacuation Rates

The present model simulated *Penaeus subtilis* ingestion and evacuation rates based on data from Nunes and Parsons (in review; Chapter 4). The model was divided into three sections: feed management, shrimp internal feeding control and feed loss (Figure 6.1). The feed management sector was arranged to allow simulations to transpire in response to modifications in its two major components: *feed ration* (*R*, g/shrimp) and *feeding frequency* (*F*, number of feedings/d). The total amount of feed administered to shrimp (*Tr*, in g of feed/shrimp/h) was calculated as:

$$Tr = \frac{RF}{Fi}$$ (6.2)

Figure 6.1: Structure and sectors of *Penaeus subtilis* model of ingestion and evacuation rates. Note the relationships between components.
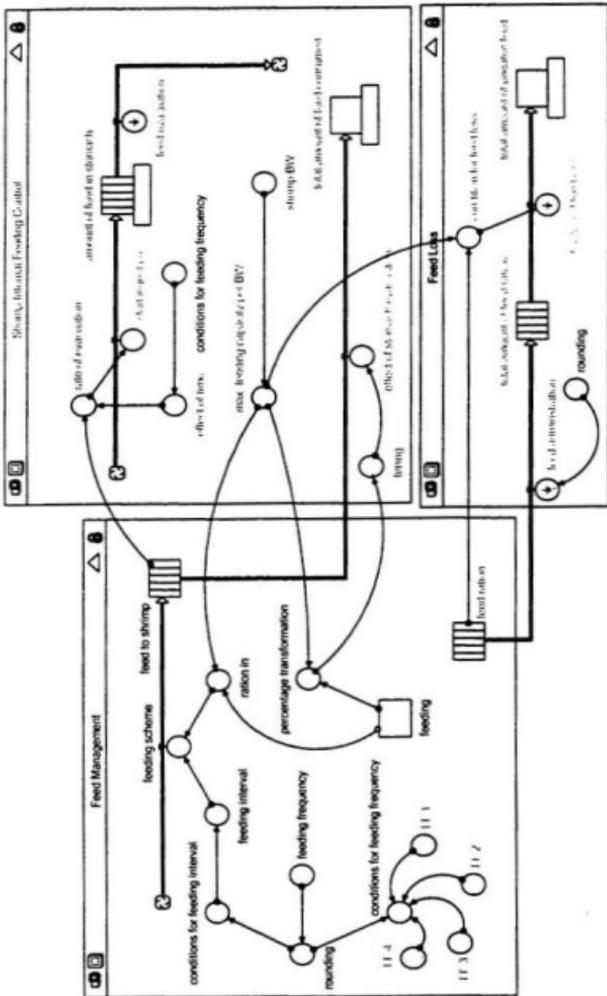
where Fi are pre-established feeding intervals (*i.e.*, duration of feeding and intervals between feedings, in h) for a given *feeding frequency*.

The sector on shrimp internal feeding control (Figure 6.1) held pre-established relationships of food intake with shrimp body weight (maximum feeding capacity per body weight) and rates of evacuation (*effect of stomach evacuation* with time). As in the previous sector, shrimp body weight (*shrimp BW*, in g) could be modified in conformance to values set. The maximum feeding capacity of *Penaeus subtilis* per body weight was calculated following quantification made by Nunes and Parsons (in review; Chapter 4). The authors described the changes in *P. subtilis* feeding rates for three consecutive meals using the functions:

$$IR = 1.23 \times 10^{-4}BW^3 - 0.0057BW^2 + 0.0791BW - 0.1126 \qquad (6.3)$$

$$IR = 2.85 \times 10^{-5}BW^3 - 0.0025BW^2 + 0.0498BW - 0.0533 \qquad (6.4)$$

$$IR = 4.80 \times 10^{-5}BW^3 - 0.0028BW^2 + 0.0497BW - 0.0517 \qquad (6.5)$$

where, IR is ingestion rate per hour (g/h) and BW refers to shrimp wet body weight (g). Assuming that under cultured conditions shrimp have continual access to either natural or artificial food, the maximum food intake of *P. subtilis* for a given BW was expressed as the mean IR value generated by the three previous equations. The absolute amount of feed ingested by shrimp (*ration in*) was given by these conditional expressions:

$$Ri = IR \quad \text{if } Tr > IR \qquad (6.6a)$$

$$Ri = Tr \quad \text{if } Tr \leq IR \qquad (6.6b)$$

where, Ri is the amount of feed ingested by shrimp (g/h) after food administration. The rate of gastric evacuation (% of food evacuated from the stomach per h) was calculated

using data of hourly stomach fullness determined for *P. subtilis* (Nunes and Parsons, in review; Chapter 4). The change in the degree of gastric evacuation (*Se*, in %) over time (*t*, in h) was defined as:

$$Se = 0.4871t^2 + 27.169t - 24.381 \quad (P < 0.001; r = 0.754; n = 145) \qquad (6.7)$$

This equation indicates that complete stomach emptiness in *P. subtilis*, feeding on pelleted food is reached at 4.26 h. Thus, the amount of feed passing through *P. subtilis* stomach is expressed as follows:

$$\frac{dFs}{dt} = Ri \times \lambda \qquad (6.8)$$

where, dFs/dt (g/h) is the *amount of feed in stomach* at time i and λ is the fraction (dimensionless) generated by $Se/100$. Consequently, the *total amount of feed consumed* (*Fc*) was given as:

$$Fc = \sum \frac{dFs}{dt} \qquad (6.9)$$

where, Fc (g/d) is equal to the accumulations in dFs/dt (g/h) over time (h).

The feed loss sector (Figure 6.1) determined the total amounts of unconsumed feed whenever excess feeding occurred. The estimation of the *total amount of uneaten feed* (*Fu*, g/d) was based on the shrimp's hourly capacity (IR) to ingest the quantities of food administered (*Tr*) given a certain *feeding frequency* and *shrimp BW*. Therefore,

$$Fu = 0 \qquad \text{if } Tr \leq IR \qquad (6.10a)$$

$$Fu = \sum \frac{dTr}{dt} - IR \qquad \text{if } Tr > IR \qquad (6.10b)$$

Appendices A, B and C present the descriptions and conditions for each variable and the specific commands employed in the model. For simulations, dt interval (the time

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interval between calculations) was set as 0.1, using a time step of 1 h and a length of simulation of 24 h.

6.2.2.2 Effects of Shrimp Predation on the Population Dynamics of Polychaetes

This model simulated the effects of *Penaeus subtilis* predation on the population dynamics of polychaetes based on data adapted from Nunes and Parsons (in press; Chapter 5). The model was composed of three sections: shrimp population, polychaete population and shrimp grazing pressure (Figure 6.2). In the shrimp population sector (Appendix D), numerical changes in population size were estimated based on empirical survival rates used in the culture of *P. vannamei*. Jory (1995) presented six representative survival tables for this species cultured under semi-intensive conditions. These data were used to obtain a regression relationship, as follows:

$$S = 0.0009t^2 + 0.03757t - 93.946 \ (P < 0.001; \ r = 0.787; \ n = 151) \qquad (6.11)$$

where, S is survival (%) and t is the culture period (d). In this case, the survival of *P. subtilis* was assumed to be unaffected by shrimp stocking density as found for other penaeids cultured under densities between 5 and 40 shrimp/m^2 (Maguire and Leedow, 1983; Allan and Maguire, 1992; Hernandez-Llamas *et al.*, 1993).

Total shrimp population size (*Ps*, total number of shrimp/culture area in m^2) was determined as:

Figure 6.2: Structure and sectors of the model on the effects of shrimp predation on the population dynamics of polychaetes. Note the relationships between components.

Shrimp (Aid-eng) Pacticage

pond area in hectares

changes in No shrimp per m²

No polychaetes grazed per m²

No polychaetes preyed per shrimp BW

shrimp predation factor on polychaetes

polychaete individual dry weight

changes in shrimp population

condition for simulation pause

polychaete density per m²

changes x polychaete population per m² after shrimp predation

maximum daily consumption per shrimp BW

contribution of polychaetes

external feed supply

shrimp growth

round external feed supply

total No shrimp remaining

harvest rate

changes in shrimp population

harvest

effect of mortality

pond area in hectares

total shrimp population

initial shrimp density in No per m²

regeneration fraction

Polychaete Population

polychaete density per m²

initial shrimp density in No per m²

regeneration

conditions for regeneration factor

regeneration fraction

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$$Ps = 10,000 \, DA \qquad (6.12)$$

where D is the initial shrimp density (number/m^2) and A is the total area used for culture (ha). Thus, the *total No of shrimp remaining* (*NR*, number shrimp alive per total culture area in m^2) was given by:

$$NR = \frac{S}{100} \times Ps \qquad (6.13)$$

Therefore, the *changes in shrimp population* was expressed as:

$$\frac{dPs}{dt} = NR \qquad (6.14)$$

where dPs/dt are the changes in the number of shrimp per day (number of shrimp/total culture area in m^2/d).

The polychaete population sector simulated numerical variations in the density of polychaetes per area of culture (in m^2). These temporal changes were based on data given by Nunes and Parsons (in press; Chapter 5). The authors quantified 10-d consecutive numerical variations in a polychaete population under the effect of shrimp predation and artificial feeding. The average daily percentage increase (*Ip*) in their abundance (Nunes and Parsons, in press; Chapter 5) is presented on Table 6.1. These values were inserted in the model as constant fractions to reflect the daily population *regeneration factor* (*i.e.*, *Rg* = *Ip*/100). The model defined a single *Rg* input through a logical function, which evaluated its choice based on the management treatment used (*i.e.*, initial number of shrimp stocked per area of culture and feeding regime). The increase in *polychaete density per m^2* (dDp/dt, number/m^2) was expressed by the differential equation:

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Table 6.1: Percentage daily increase in the number of polychaetes per m^2 in response to shrimp stocking density and feeding regime as observed by Nunes and Parsons (in press).

Feeding Regime	Shrimp Density (number of shrimp/m^2)				
	None	5	10	15	20
with feed	-	1.1%	3.6%	2.4%	2.4%
without feed	3.4%	4.0%	3.6%	0.5%	0.5%

$$\frac{dDp}{dt} = Dpi \times \omega \qquad (6.15)$$

where, Dpi is the initial number of polychaetes prior to shrimp stocking (no/m^2) and ω (dimensionless) is $Rg + 1$ (Appendix E).

The sector on shrimp grazing pressure accounted for the effects of shrimp predation and artificial feeding on polychaetes, and the results of the previous sectors to calculate the overall changes in the abundance of polychaetes (Appendix F). Since quantitative estimates of daily shrimp grazing rates on polychaetes are lacking in the literature, the shrimp predation effect was given as the shrimp daily ration (Dr, g/d), calculated as the mean IR value produced by Equations (6.3), (6.4) and (6.5), multiplied by a factor of four (dimensionless). This latter parameter expressed the number of times shrimp could fill and empty their stomachs completely in 24 h, assuming that feeding is not a continuous process. A growth curve for *Penaeus subtilis* was plotted with data given by Nunes (1998). The following quadratic function was obtained to express *shrimp growth* (*SG*, g) over time (*t*, d):

$$SG = -0.001t^2 + 0.2654t + 1.0015 \ (P < 0.001; r = 0.957; n = 264) \qquad (6.16)$$

To estimate the relative amount of polychaetes in the shrimp's diet, a fraction defined as the *percentage contribution of polychaetes* (*Pc*, dimensionless), was assigned to each feeding treatment used. Nunes *et al.* (1997b) quantified the diet of *Penaeus subtilis* cultured under semi-intensive conditions. The authors found that 32.55% of the shrimp's total diet during a growth cycle was composed of polychaetes. Under conditions where no food supply is used, the contribution of polychaetes was assumed to be 50%. Therefore,

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$$Pc = 0.33 \quad \text{if } Fr \geq 0.5 \qquad (6.17a)$$

$$Pc = 0.50 \quad \text{if } Fr < 0.5 \qquad (6.17b)$$

where, Fr is the feeding regime used, given as $Fr \geq 0.5$ when supplementary feed is used and, $Fr < 0.5$ when there is no feed supply. The *No of polychaetes preyed per shrimp BW* (Np, number of polychaetes/shrimp body weight) was expressed as:

$$Np = \frac{Dr \times Pc}{\psi} \qquad (6.18)$$

where, ψ indicates *polychaete individual dry weight* (g) given as 0.7×10^{-3} (n = 266; Nunes and Parsons, in review; Chapter 4). Np was converted to *No of polychates grazed per m^2* (NPm, number/m^2) by multiplying the resulting value by the *changes in No of shrimp per m^2* (NSm):

$$NSm = \frac{dP/dt}{A \times 10,000} \qquad (6.19)$$

Finally, the changes in *polychaete population per m^2 after shrimp predation* (dPd/dt) was calculated as:

$$\frac{d\,Pd}{d\,t} = Dp - NPm \qquad (6.20)$$

For simulations, d*t* interval was set as 0.5, using a time step of 1 d and a length of simulation of 100 d.

6.2.2.3 *Penaeus subtilis* Feeding Response to Feed Dispersal

The present model described *Penaeus subtilis* feeding response to feed dispersal based on data from Nunes and Parsons (1999). The model was divided into two sectors: shrimp population and shrimp feeding response. A description of the sector on shrimp population was presented in the previous section (Appendix D; Figure 6.3). The shrimp feeding response sector was subdivided into three sub-models each referring to a certain period of the day (*i.e.*, morning, mid-day and afternoon). These sub-models were developed to reflect the shrimp's feeding response to time of day and feed distribution method. For each, a relationship was obtained between shrimp stomach weight (*SW*) and shrimp body weight (*BW*; Table 6.2). The relationships were based on data adapted from Nunes and Parsons (1999), who measured the stomach content weights of *P. subtilis* under culture conditions. In their work, the shrimp population was submitted to either two feed distribution methods for a complete production cycle. The present model applied a conditional expression (Appendix G) based on the feeding method set during simulations to determine an applicable equation, as follows:

$$\text{Equations (6.22a), (6.22c) and (6.22e)} \quad \textit{if Fm} = 1 \qquad (6.21a)$$

$$\text{Equations (6.22b), (6.22d) and (6.22f)} \quad \textit{if Fm} = 2 \qquad (6.21b)$$

where, Fm (dimensionless) is the feed dispersal method used, given as $Fm = 1$ when feed is broadcast and $Fm = 2$ when feed is concentrated. The total shrimp population stomach weight (*PSW*, g/d/total culture area in m^2) was expressed as:

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Figure 6.3: Structure and sectors of the model on *Penaeus subtilis* response to feed dispersal (shrimp feeding response sector only). Sector on shrimp population same as presented in Figure 6.2.

Shrimp Feeding Biosystem
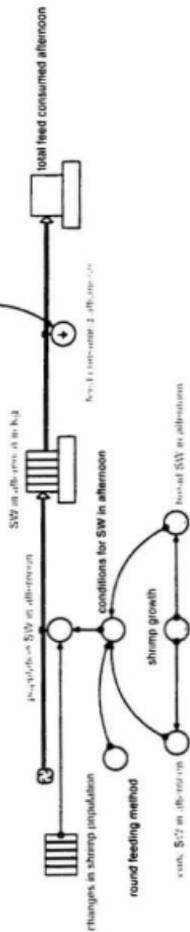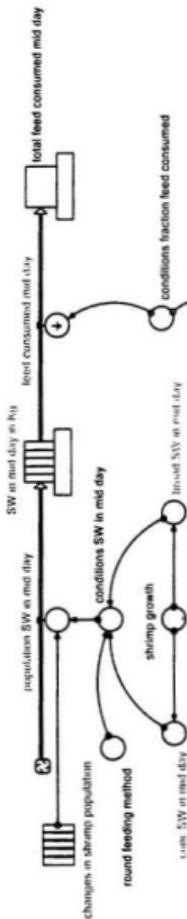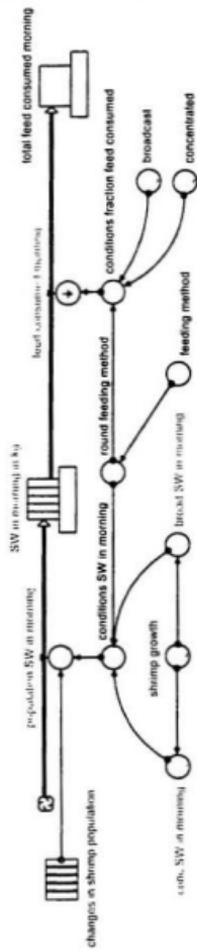
Table 6.2: Power functions obtained between *Penaeus subtilis* stomach weight (*SW*, in g) and shrimp body weight (*BW*, in g) in relation to time of day and feed dispersal method. All equations were significant at the 0.1% level (r is the coefficient of correlation, n refers to number of observations and Eq. Provides the reference number to the equation). Data from Nunes and Parsons (1999).

Time	Feed Dispersal Method	
	broadcast	*concentrated*
Morning	$SW = 0.0094BW^{0.8704}$	$SW = 0.0070BW^{0.9381}$
	(r = 0.858; n = 269; Eq. 6.22a)	(r = 0.885; n = 325; Eq. 6.22b)
Mid-day	$SW = 0.0105BW^{0.8523}$	$SW = 0.0088BW^{0.8136}$
	(r = 0.864; n = 272; Eq. 6.22c)	(r = 0.823; n = 325; Eq. 6.22d)
Afternoon	$SW = 0.0092BW^{0.9764}$	$SW = 0.0088BW^{0.8603}$
	(r = 0.897; n = 270; Eq. 6.22e)	(r = 0.815; n = 317; Eq. 6.22f)

$$PSW = SW\frac{dP}{dt} \qquad (6.23)$$

The *PSW* was divided by 1,000 to convert the results to kg. In order to define the percentage amount of feed present in the stomach contents, data indicating the relative occurrence of artificial food in *P. subtilis* stomachs was used (Table 6.3). Therefore, the total amount of feed consumed (*Tf*, kg) at a certain time of the day calculated as:

$$Tf = \sum_i PSW \times \xi \qquad (6.24)$$

where, ξ is the correspondent fraction (dimensionless) from Table 6.3 at time i (d). The model ran simulations with a *dt* interval of 0.5, using a time step of 1 d and a length of simulation of 80 d.

The three models described above are completely illustrated in an electronic format (Appendix H) using STELLA® II environment, software version 3.0.7 for Windows (High Performance Systems, Inc., Hanover, New Hampshire, USA).

6.3 Results

6.3.1 Shrimp Hourly Feed Ingestion

Simulations of *Penaeus subtilis* hourly feed ingestion (d*Fs*/d*t*) are shown in Figure 6.4. Figures 6.4A (under fed), 6.4B (optimum) and 6.4C (over fed) present the simulation results of shrimp food intake relative to changes in *feed ration* (R). Data indicated that maximisation of feed consumption was achieved when R was equivalent to

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Table 6.3: Percentage occurrence (%) of artificial food in *Penaeus subtilis* stomach contents (30-min after feed distribution) over an 80-d production cycle. Data compiled from Nunes and Parsons (1999).

Culture Period (d)	Feed Dispersal Method	
	broadcast	*concentrated*
20	43.5%	36.6%
32	66.9%	76.4%
44	76.9%	72.1%
56	84.7%	71.2%
68	82.0%	74.3%
80	85.5%	64.5%

Figure 6.4: Simulations of *Penaeus subtilis* feed ingestion (d*FS*/d*t*, g/h) in response to *feed ration* (*R*, g/shrimp), *feeding frequency* (*F*, number of feedings/d) and shrimp body weight (*BW*, g). *Fc* and *Fu* indicate the results of the *total amount of feed consumed* (g/d) and the *total amount of uneaten feed* (g/d), respectively.

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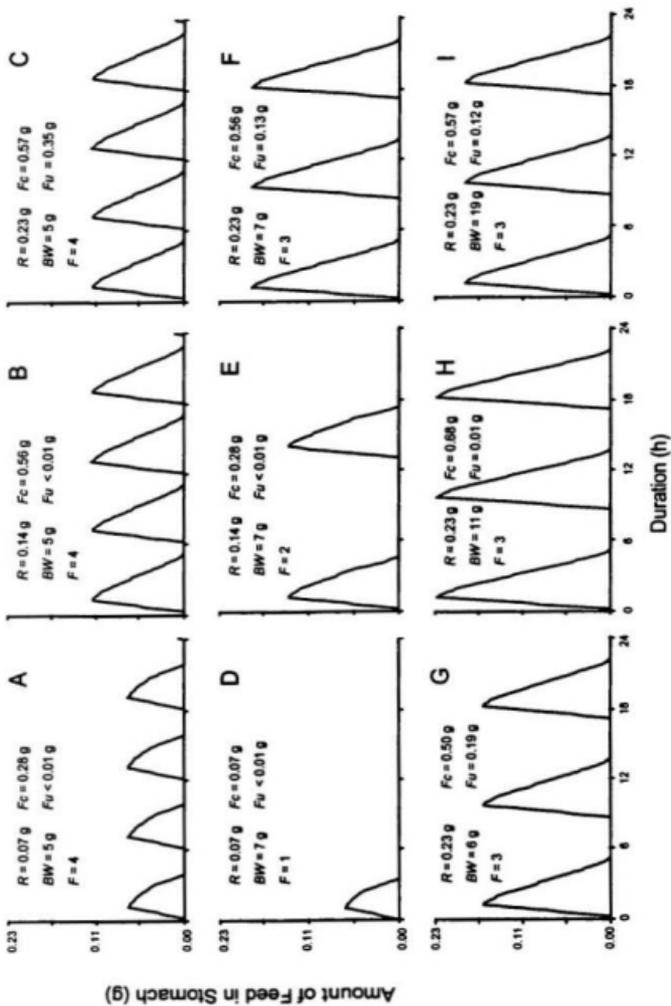
the ingestion rate per hour (IR), and *feeding frequency* was set at four times/d (Figures 6.4B). At its maximum IR, shrimp feeding and evacuation process lasted 5.26 h, thus allowing four feeding intervals to occur within a 24-h period. With R lower than IR values, results showed shorter feeding and evacuation periods and reduced food consumption levels (*Fc*; Figure 6.4A). At each feeding sequence, the model predicted that ingestion peaked only up to the respective maximum IR (determined based on the shrimp body weight). Excess food (*Tr* > IR) was ignored during ingestion and computed as the *total amount of uneaten feed* (*Fu*; Figure 6.4C). Therefore, the *total amount of feed consumed* (*Fc*) increased with R, but remained unchanged when R was higher than maximum IR. At this point, *Fu* increased and reflected feed loss and over feeding.

Shrimp food intake also reacted directly to *feeding frequency* (*F*). As *F* reduced, a lower number of feeding sequences occurred over the simulation period (Figures 6.4D, 6.4E and 6.4F). Simultaneous increments in both *feed ration* (*R*) and *F*, resulted in a more efficient food intake (*Fc*; Figures 6.4D and 6.4E), except when R exceeded IR (Figure 6.4F). Food intake levels progressively increased with shrimp body weight (*BW*), but declined after reaching its peak at 11 g shrimp (*Fc*; Figures 6.4G, 6.4H and 6.4I). When fed the same R, a 19 g shrimp consumed more feed and produced a lower *Fu* than a smaller one with 2 g. The model predicted that if R was kept constant as larger shrimp body weights were attained, either under or excess feeding occurred at some stage of the growth process.

Sensitivity analysis indicated that the total amount of feed consumed (*Fc*) was mostly affected by changes in *feed ration* (*R*), when compared to *feeding frequency* (*F*) and shrimp body weight (*BW*, Table 6.4). Both R and *BW* had a greater effect on the total

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amount of uneaten feed (Fu) in contrast to F. In general, this model was able to correlate all the components contained in its structure, producing expected changes in *Penaeus subtilis* hourly feed ingestion and quantitatively predicting the outputs of an assigned feeding regime.

6.3.2 Polychaete Population Dynamics

The simulated numerical responses of polychaete population to shrimp predation (as reflected by variations in stocking density, D), feeding regime (Fr) and initial polychaete density (Dpi) are presented in Figure 6.5. Dpi affected polychaete population development by restricting its numerical growth throughout the production cycle (Figure 6.5A). The model predicted that under a density-equivalent predatory pressure of 5 shrimp/m^2, an initial polychaete population of 1,000 individuals/m^2, was depleted in less than 25 d, regardless if supplementary feeding was used. Under the same feeding regime and stocking density, but with a Dpi of 5,000 individuals/m^2, the polychaete population showed an increasing growth tendency. However, a final polychaete population size and growth pattern similar to the control treatment (*i.e.*, without shrimp and feed supply) was only obtained by increasing Dpi to 10,000 individuals/m^2 (Figure 6.5A).

At a higher shrimp stocking density (*i.e.*, $D = 20$ shrimp/m^2), the effects of shrimp predation and artificial feeding on polychaetes became more noticeable (Figure 6.5B). External feed supply was able to improve polychaete growth under a Dpi of 3,500

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Table 6.4: Sensitivity analysis of the total amount of consumed (*Fc*) and uneaten (*Fu*) feed to model parameter variability. The sensitivity index (Equation 6.1) was calculated for the entire parameter variability range.

		Sensitivity Index (SI)	
Parameter[a]	Variability	*Fc*	*Fu*
R	0.01 - 0.23	0.93	1.00
F	1 - 4	0.75	< 0.01
BW	2 - 20	0.75	1.00

[a] *R = feed ration* (g/shrimp), *F = feeding frequency* (number of feedings/d) and *BW =* shrimp body weight (g).

Figure 6.5: Simulation of the changes in *polychaete population per m² after shrimp predation* (dPd/dt, number/m²) over a culture period (d) in response to initial polychaete density (Dpi, number/m²), feeding regime (Fr, dimensionless), shrimp stocking density (D, number/m²) and total culture area (A, ha). Dotted lines (control) represent simulations without shrimp and feed supply.

A

Dpi = 1,000 polyc./m² (control)
(a) Dpi = 1,000 polyc./m²; Fr = 1
(b) Dpi = 5,000 polyc./m²; Fr = 1
(c) Dpi = 10,000 polyc./m²; Fr = 1

D = 5 shrimp/m²
A = 5 ha

B

Dpi = 3,300 polyc./m² (control)
(a) Dpi = 3,500 polyc./m²; Fr = 1
(b) Dpi = 5,500 polyc./m²; Fr < 0.5
(c) Dpi = 10,000 polyc./m²; Fr < 0.5

D = 20 shrimp/m²
A = 5 ha

C

Dpi = 3,000 polyc./m² (control)
Dpi = 3,000 polyc./m²; A = 6.5 ha; Fr = 1
(a) D = 5 shrimp/m²
(b) D = 10 shrimp/m²
(c) D = 15 shrimp/m²
(d) D = 20 shrimp/m²

Polychaete Density (no./m²) x 10³

Culture Period (d)

individuals/m^2 and a shrimp density of 20 shrimp/m^2. Without feed supply, polychaete was depleted within less than 25 d, even when Dpi reached 5,500 individuals/m^2. Under these conditions, Dpi could only avoid complete polychaete depletion when significantly increased. At 10,000 individuals/m^2, the population reached detrimental levels in the initial stages of culture, but it was able to recover and maintain a constant pattern until the end of the production cycle (Figure 6.5B). In contrast to these observations, a polychaete population explosion was detected for the control treatment when Dpi was set as 3,300 individuals/m^2.

Among the shrimp stocking densities investigated (*i.e.*, 5, 10, 15 and 20 shrimp/m^2), 10 shrimp/m^2 failed to reflect properly the effects of shrimp predation. Under this density and with feed supply, the population numerical pattern was similar to the control treatment (Figure 6.5C). All other shrimp densities influenced polychaete growth either by restricting substantial numerical increments or by completely eliminating the initial polychaete population (Figure 6.5C). Sensitivity analysis revealed that shrimp stocking density (D) produced greater and inverse effects in the model outputs when compared to the initial polychaete density (Dpi; Table 6.5). A 50% increase in D reduced the final polychaete population to 85.5%, while the same percentage increment in Dpi raised the final number of polychaetes by 36.6% (D = 5 shrimp/m^2 and Dpi = 3,000 polychaetes/m^2 as baseline values).

Table 6.5: One-at-a-time sensitivity analysis of final polychaete population (number/m^2) to model parameters based on a fixed percentage variation. Negative values indicate reduction in final polychaete density.

		Percentage Change in Baseline Value	
Parameter[a]	Baseline Value	+ 50%	- 50%
D	5	- 85.5	69.2
Dpi	3,000	36.7	- 58.2

[a]D = shrimp stocking density (number/m^2) and Dpi = initial polychaete density (number/m^2).

6.3.3 Shrimp Feeding Levels in Response to Feed Dispersal

Over the 80-d simulated duration of culture, a 30% decline in the initial shrimp population occurred (Figure 6.6). Under the model assumptions, final populations were estimated at 350,000 and 1,4000,000 shrimp for initial densities of 5 and 20 shrimp/m², respectively. The reductions in shrimp population however, did not produce lower *PSW* over the culture period. In all simulations, the trend was an increased *PSW* as the time period progressed.

As expected, the model predicted an increase in the total amount of feed consumed (*Tf*) at higher shrimp stocking densities (*D*). Total feed consumption also differed among time periods. For the broadcast *feeding method* (*Fm*), lowest *PSW* and *Tf* were detected for the morning period, followed by the mid-day and afternoon. When feed was concentrated (*Fm* = 2), *PSW* and *Tf* values were highest in the afternoon and morning, and lowest in the mid-day. More feed was also consumed when the broadcast method (*Fm* = 1) was used in the simulations. *Tf* values were 21, 31 and 35% higher (morning, mid-day and afternoon, respectively) for feed broadcast in comparison to the concentrated method. Analysis of sensitivity indicated that the *D* variability range produced 74% more variation in *Tf* outputs when compared to feed dispersal method (*Fm*, Table 6.6).

Figure 6.6: Simulation of the changes in the total stomach weight of a shrimp population (PSW, kg/d/total culture area in m^2) during a growth period (d) in response to time of the day, feeding method (Fm, dimensionless) and shrimp stocking density (D, number/m^2). Tf indicates the results of total amount of feed consumed (kg) for each plotted curve. Total culture area (A) was 10 ha.

Figure axes: Population Stomach Weight (kg/culture area) versus Culture Period (d)

A. Morning; D = 5 shrimp/m²
(a) Fm = 1; Tf = 1,472 kg
(b) Fm = 2; Tf = 1,164 kg

B. Morning; D = 20 shrimp/m²
(a) Fm = 1; Tf = 5,886 kg
(b) Fm = 2; Tf = 4,657 kg

C. Mid-day; D = 5 shrimp/m²
(a) Fm = 1; Tf = 1,575 kg
(b) Fm = 2; Tf = 1,089 kg

D. Mid-day; D = 20 shrimp/m²
(a) Fm = 1; Tf = 6,300 kg
(b) Fm = 2; Tf = 4,355 kg

E. Afternoon; D = 5 shrimp/m²
(a) Fm = 1; Tf = 1,859 kg
(b) Fm = 2; Tf = 1,216 kg

F. Afternoon; D = 20 shrimp/m²
(a) Fm = 1; Tf = 7,435 kg
(b) Fm = 2; Tf = 4,863 kg

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Table 6.6: Sensitivity analysis of the total amount of feed consumed (Tt) to model parameter variability. The sensitivity index (Equation 6.1) was calculated for the entire parameter variability range.

Parameter[a]	Variability	Sensitivity Index (SI)
		Tf
D	1 - 20	0.95
Fm	1- 2	0.21

[a] D = shrimp stocking density (number/m^2) and Fm = feeding method (dimensionless).

6.4 Discussion

6.4.1 Simulations of Shrimp Feed Ingestion

In shrimp aquaculture systems, feed loss has been estimated based on results of diet digestibility, food conversion ratios (Primavera, 1994) or by direct observations of uneaten feed from feeding trays (Rosenberry, 1994; Jory, 1995, 1996; Goddard and Nunes, 1997). In these systems, excess feeding has been attributed to the inability of feed management techniques to predict accurately penaeid feeding responses to temporal variability, a product of environmental (Sastrakusumah, 1971; Brisson, 1977; Marte, 1980; Hill and Wassenberg, 1987; Wassenberg and Hill, 1987; Reymond and Lagardère, 1990; Nunes *et al.*, 1996, 1997b) and endogenous cues (Bursey and Lane, 1971; Huner and Colvin, 1979; Reymond and Lagardère, 1990; Hill and Wassenberg, 1992; Nunes *et al.*, 1996).

In the present study, although assumptions employed during model development offered a simplistic view to shrimp feeding, simulations produced expected forecasts of shrimp hourly feed ingestion [commensurate to the data used from Nunes and Parsons (in review); Chapter 4], total feed consumption and feed waste. Such laboratory-derived data can be combined with regular feed management practices to provide more precise assessments of food consumption and feed loss in shrimp ponds, anticipating undesirable conditions arising from excess feeding. The model may also assist in defining feeding rates for cultured *Penaeus subtilis*. At present, these rates are defined based on

observations of feed consumption from feeding trays or derived from feeding tables used for other penaeids.

In a more complex situation, environmental effects in shrimp culture systems would have to be considered and interrelated to generate the model outputs. While shrimp feeding responses to environmental variability have been described and the variables identified to some extent, the existing relationships have not yet been established. Under culture conditions, the isolation and quantification of these processes appear to be complicated. Nunes (1998) working in a semi-intesive culture system, measured bi-hourly variations of *Penaeus subtilis* food intake and monitored water quality (temperature, salinity, pH and dissolved oxygen) fluctuations at 10-min intervals over 24-h periods. Multiple regression analysis indicated that while water quality parameters significantly influenced *P. subtilis* feeding patterns, only 26% of the variations on shrimp food intake could be explained by fluctuations in water quality. Other environmental cues, such as light intensity and natural food availability, are also thought to generate significant effects on penaeid feeding (Hill and Wassenberg, 1987; Wassenberg and Hill, 1987; Nunes *et al.*, 1996, 1997b). In the presence of a constant food supply (naturally occurring prey items), shrimp ingestion and evacuation may display an irregular pattern and occur in a continuous fashion, contrasting with the present model assumptions and simulations. Also, light may suppress or regulate some *Penaeus* spp. feeding activity (Hughes, 1969; Brisson, 1977; Reymond and Lagardère, 1990).

In the present study, simulations of shrimp mortality appeared to have been underestimated (30% in 80 d). Data used to establish the relationship between culture period and shrimp survival were derived from empirical survival tables (Jory, 1995).

Estimations of shrimp survival under pond culture conditions is highly subjective, and although attempts to design more accurate sampling techniques have long been made (Hutchins *et al.*, 1980), reliable methods are still not available.

The increased trend in population stomach weight (*PSW*) was a reflection of the continuous shrimp body weight gains predicted by the model. These increments contributed to an increase in stomach weight, and thus in *PSW*. In general, the model was able to integrate and simulate the effects of feed dispersal method and time of day on shrimp feeding responses. Since these simulations employed data derived from field studies, this model should allow more direct comparisons to commercial situations if realistic estimates can be produced for shrimp survival.

6.4.2 Simulations on Polychaete Population Dynamics

Polychaete population dynamics in shrimp ponds is monitored by random substrate sampling, followed by separation and counting of animals. This method requires periodical collection to obtain reasonable estimates of the changes of polychaete abundance over shrimp rearing cycles. Sampling techniques however, are impaired by their inability to predict the responses of polychaete populations to alterations in management strategies, such as shrimp stocking densities and use of supplemental feeding. Thus, forecasts are generally based on intuition and practice, rather than on scientific grounds.

In the present study, simulations were able to predict polychaete numerical responses to different culture management conditions and anticipate critical levels based on data collected from previous studies. The model revealed that polychaete abundance was highly affected by both initial shrimp (D) and polychaete densities (Dpi). A relationship between these parameters is also suggested by observations and common practice employed by commercial shrimp culture operations. Feed supply played a role in sustaining polychaete growth, particularly at higher densities of shrimp. At increased Dpi, simulations showed that external feed supply was not required to maintain polychaete abundance, even at more intensive culture conditions (i.e., $D = 20$ shrimp/m^2), but was necessary at lower Dpi. Therefore, the initial state of polychaete populations (i.e, before shrimp stocking) is important prior to establishing shrimp stocking densities and feed management regimes. Model simulations could also preclude uneconomical conditions during a growth cycle, as when Dpi was depleted in early stages of the culture period. Under these circumstances, an increase in feeding rates is necessary to support most or all of shrimp survival and growth.

Simulations of polychaete growth presented in this study reflect specific environmental settings for a mixed, but limited number of polychaete families. Under different environmental conditions or polychaete species, reproductive rhythm is expected to differ, possibly altering the regeneration factors used in this model. Also, the population dynamics of polychaetes is controlled by not only predation or external food supply, but also by a number of other external and endogenous conditions not considered in the present study. Despite these limitations, the model may still serve as a valuable and

insightful tool for forecasting or at least appreciating changes in polychaete abundance in shrimp ponds under different management conditions.

6.5 Conclusions

In the present investigation dynamic modelling integrated previously collected data and produced expected forecasts of food intake by *Penaeus subtilis* and its predatory impact on the abundance of pond polychaete populations. *P. subtilis* hourly food intake reacted directly to feeding frequency and shrimp body weight, but was mostly affected by changes in the amount of feed ration. As feeding frequency decreased, a lower number of feeding sequences occurred over the simulation period, while at higher rations the total amount of feed consumed increased. Levels of food intake progressively increased as shrimp body weight increased, but declined after reaching a peak at 11 g shrimp. Maximisation of shrimp feed consumption was achieved when total ration administered was equivalent to ingestion rate per hour, and feeding frequency was set at four times/d. At higher shrimp stocking densities, feed consumption by the total shrimp population increased, but it also changed according to time of the day and feeding method. More feed was consumed in the afternoon, particularly when the broadcast method was used. The abundance of polychaetes was highly affected by both initial shrimp and polychaete densities. At an increased shrimp density or under a low initial polychaete abundance, the polychaete population was either depleted or reached critical levels during the simulation period. Feed supply played a role in sustaining polychaete growth, mainly at higher

shrimp stocking densities, but was not required after an increase in the initial polychaete abundance. Shrimp stocking density produced greater and inverse effects in the model outputs when compared to the initial polychaete density. A 50% increase in shrimp density was able to reduce the final polychaete population by 85.5%.

CHAPTER 7

GENERAL DISCUSSION

7.1 Significance and Implications of Results to *Penaeus subtilis* Culture

7.1.1 Feeding Rates and Feeding Frequency

The present research has shown that *Penaeus subtilis* feeding parameters (maximum meal, ingestion rate and return of appetite) were all statistically correlated with its body weight. Lowest gastric evacuation measures for *P. subtilis* were observed within 3 h, while complete emptying was predicted to occur at 4.26 h.

Studies on the feeding patterns of *Penaeus subtilis* conducted under semi-intensive culture conditions have found no direct relationship between the amount of food contained in shrimp stomachs and body size (Nunes, 1995; Nunes *et al.*, 1996, 1997b). As observed in the present investigation (Chapter 3), and in the work by Nunes (1995) and Nunes *et al.* (1996), there was an almost constant pattern in total food intake as body sizes of *P. subtilis* were increased. These investigations however, were able to detect a progressive increase of feed occurrence in the diet of *P. subtilis*. Ontogenetic-related changes in the type of food consumed by *Penaeus* spp., including *P. subtilis*, have been reported under cultured and natural conditions (Leber, 1983; Wassenberg and Hill, 1987; Stoner and Zimmerman, 1988; Reymond and Lagardère, 1990; Nunes *et al.*, 1997b). In

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these studies, such dietary shifts may have produced the observed overall declining pattern in food intake, biasing possible relationships between food consumption and shrimp body size.

In general, the present study indicated that *Penaeus subtilis* body weight is a reliable indicator of its quantitative feeding parameters. These relationships are likely to be a reflection of internal morphological and physiological aspects of its digestive apparatus. Both *P. subtilis* stomach volume and the weight of its digestive gland increase in proportion to body weight gains (Nunes, 1997), suggesting that this species may also increase its capacity to ingest and process food during its growth cycle. These observations are commensurate with the results obtained in the present study which indicated progressive increments in shrimp food intake until a certain body weight, when reducing tendencies started to occur.

In the present study, *Penaeus subtilis* feeding intensity and appetite revival were not markedly influenced by the degree of stomach fullness or by longer food administration intervals. As opposed to displaying a periodical feeding pattern, *P. subtilis* appeared to feed continuously. At present, in commercial shrimp farming operations, daily feeding is performed at fixed frequencies, which generally do not exceed three times/d. Results from the present study suggest that the daily administration of food in shorter feeding intervals, but at continually reduced amounts may be more advantageous in the culture of *P. subtilis* than current methods in use. The overall results and the corresponding computer model developed in this study should assist in the design of feed management programmes for this species. Currently, specific feeding schemes for *P. subtilis* are unavailable. Feeding rates and feeding frequencies are determined empirically based on estimations used in the culture

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of other penaeids. This often leads to inaccurate assessments of food consumption, which ultimately results in feed waste and organic pollution problems.

7.1.2 Food Particle Size and Feeding Methods

In the present work, the food manipulation efficiency of *Penaeus subtilis* was inversely related to feed particle size, with selectivity favouring crumbles and broken pellets. Under culture conditions, *P. subtilis* natural food selectivity has been suggested to be a result of its apparent increasing ability to capture larger food particle sizes as greater body sizes are attained (Nunes, 1995). These observations have been largely based on the examination and identification of food items in shrimp stomach contents. Although the present study did not support completely these findings, results confirmed that food size selectivity occurs in *P. subtilis*. Overall, food handling capacity for juvenile *P. subtilis* was as efficient as that for adults within the feed size range examined. In aquaculture ponds however, size and shape of natural food biota are diverse, and thus must be considered when comparisons are made.

In this study, feed distribution at 1430 h and at 0930 h produced statistically higher shrimp stomach content weights when compared to 0600 h. Higher food consumption by *Penaeus subtilis* at later times of the day has also been previously detected in another investigation with this species (Nunes *et al.*, 1997b; Nunes, 1998). The authors found that under semi-intensive culture conditions, shrimp food intake was more pronounced just before dusk (*i.e.*, 1730 h) when dissolved oxygen and temperature reached the highest

levels. In the present work, the lower shrimp food ingestion observed in the early morning may in fact, also be the result of less favourable water quality conditions.

Penaeus subtilis food intake was also more pronounced when feed was broadcast over the culture area. The effects of feed dispersal methods on *P. subtilis* food consumption appeared to have been mostly the result of inter-animal behavioural associations. Nowadays, commercial operations use feeding devices to concentrate formulated food in various, but limited locations of culture ponds. Alternatives to these current feeding procedures must be considered and evaluated against the present results, to achieve more effective and economical forms of food distribution.

7.1.3 Patterns in the Availability of Natural Food

In the present study, *Penaeus subtilis* grazing pressure caused a decrease in the density of polychaetes. Numerical abundance and biomass of polychaetes were greatly reduced at higher shrimp stocking densities. Their population patterns appeared to be the result of the interactive influence of several factors (*e.g.*, shrimp feeding activity, changes in environmental parameters and feed inputs). However, the fluctuations observed in the present study were most likely the result of an increased recruitment of polychaetes.

The effects of *Penaeus subtilis* predation on polychaetes in culture ponds have been identified in previous studies. Nunes (1995) reported that these organisms were intensively preyed upon during a 60-d rearing cycle, accounting for 81% of all prey ingested, which was equivalent to 33% of all food consumed by *P. subtilis*. In the present investigation,

artificial feeding promoted higher polychaete levels even when shrimp were present, but was not effective in alleviating *P. subtilis* predation at increased stocking densities (15 and 20 shrimp/m^2). Therefore in semi-intensive ponds of *P. subtilis*, normal polychaete levels may not be sustained if these stocking densities are exceeded. Computer simulations revealed that the level of initial polychaete populations is a primary factor in its sustainability throughout the growth cycle. In fact, farming operations of *P. subtilis* empirically define shrimp stocking densities based on observed initial polychaete abundance. Further research should emphasise the development of methods to boost their productivity in shrimp ponds, especially during the final stages of the growth cycle, when increased amounts of formulated food are required to support shrimp growth and survival.

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APPENDICES

Appendix A: Variables, type and description of components in the feed management sector. Type refers to the following: V = variable with value set by user, C = command; I = integration.

Variable	Type	Definition
feeding frequency	V	defines the number of feedings per day
rounding	C	rounds feeding frequency values to its nearest integer value
conditions for feeding frequency	C	provides a logical expression for the daily gastric evacuation pattern for a given *feeding frequency* value
conditions for feeding intervals	C	provides a logical expression to determine pre-established hourly intervals between feeding based on a given *rounding* value
feeding interval	C	same as *conditions for feeding intervals*
feeding	C	same as *feed ration* (g)
ration in	C	gives a logical expression to define the amount of food consumed (g) by shrimp based on a pre-established *max. feeding capacity per BW* (g)

Appendix A: Cont.

Variable	Type	Definition
percentage transformation	C	calculates a fraction dividing *feeding* (g) by *maximum feeding capacity per BW* (g) to be used on calculations of *total amount of feed consumed*
feeding scheme	I	sets a value of a specific size (g) and interval (h) for *feed to shrimp* based on *ration in* and *feeding interval*
feed to shrimp	C	integrates *feeding scheme* values

Appendix B: Variables, type and description of components in the shrimp internal feeding control sector. Type refers to the following: V = variable with value set by user; C = command; I = integration; R = pre-established relationship.

Variable	Type	Definition
shrimp BW	V	defines shrimp body weight (g)
max. feeding capacity per BW	R	mean ingestion (g) value generated by Equations (6.3), (6.4) and (6.5)
timing	R	defines the time (h) required for evacuation (%) based on Equation (6.7)
effect of stomach evacuation	C	same as *timing*
total amount of feed consumed	I	adds 24-h values of feed consumption
FF1, FF2, FF3 and FF4	R	defines percentage evacuation values over a 24-h period based on Equation (6.7) for a given feeding frequency
effect of time	C	same as *conditions for feeding frequency*

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Appendix B: Cont.

Variable	Type	Definition
rate of evacuation	C	multiplies values of *feed to shrimp* by *effect of time*
start ingestion	C	same as *rate of evacuation*
amount of food in stomach	I	integrates *start ingestion* values

Appendix C: Variables, type and description of components in the feed loss sector. Type refers to the following: V = variable with value set by user; C = command; I = integration.

Variable	Type	Definition
feed ration	V	defines feed ration (g)
feeding administration	C	mutiplies *feed ration* (g) by the number of feedings per day (*rounding*)
total amount of feed ration	I	integrates values of *feeding administration*
condition for feed loss	C	provides a fraction (dimensionless) of feed loss when *feed ration* (g) is greater than *max. feeding capacity per BW* (g)
fraction of feed loss	C	fraction set by *condition for feed loss*
total amount of uneaten feed	I	adds 24-h values of feed loss (g)

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Appendix D: Variables, type and description of components in the shrimp population sector. Type refers to the following:

V = variable with value set by user; C = command; I = integration; R = pre-established relationship.

Variable	Type	Definition
initial shrimp density in No per m²	V	defines an initial density of stocked shrimp in number of animals per m²
pond area in hectares	V	sets a value for the culture area (ha)
total shrimp population	C	calculates the initial stocked shrimp population by converting *pond area in hectares* to m² and mutiplying the resulting value by *intial shrimp density in No per m²*
survival rate	R	fraction (dimensionless) value generated by Equation (6.11)
total No of shrimp remaining	C	calculates the number of shrimp alive by multiplying *total shrimp population* with *survival rate* (dimensionless)
effect of mortality	C	same as *total No of shrimp remaining*
changes in shrimp population	I	integrates values of *total No of shrimp remaining* using Equation (6.14)
harvest	C	null function

Appendix E: Variables, type and description of components in the polychaete population sector. Type refers to the following: V = variable with value set by user; C = command; I = integration; R = pre-established relationship.

Variable	Type	Definition
conditions for regeneration factor	C, R	gives a logical expression to define an applicable percentage value (Table 6.1) based on a *initial shrimp density in No per* m^2 and *round external feed supply*
regeneration fraction	C	increases *regeneration* at a constant rate (value defined by *conditions for regeneration factor*) regardless of the dt used by the model
regeneration	C	gives a growth rate defined by *regeneration fraction*
polychaete density per m^2	V, I	defines the initial number of polychaetes per m^2 prior to shrimp stocking, and integrates this value in accordance to *regeneration*

Appendix F: Variables, type and description of components in the shrimp grazing pressure sector. Type refers to the following: V = variable with value set by user; C = command; I = integration; R = pre-established relationship; Cons = constant.

Variable	Type	Definition
external feed supply	V	defines if external feed supply is used (with supplementary feed supply $\geq$ 0.5; without external feed supply < 0.5)
round external feed supply	C	rounds *external feed supply* (dimensionless) to its nearest integer value
contribution of polychaetes	C	gives a logical expression to define the percentage contribution of polychaetes to shrimp's diet (with feed = 0.33; without feed = 0.50)
shrimp growth	R	defines *shrimp growth* (g) based on a given time (d) using Equation (6.16)
maximum daily consumption per shrimp BW	R	correlates *shrimp growth* (g) with daily food consumption [*i.e.*, mean value of Equations (6.3), (6.4) and (6.5)]

Appendix F: Cont.

Variable	Type	Definition
shrimp predation factor on polychaetes	C	mutiplies the *maximum daily consumption per shrimp BW* (g) by a factor (dimensionless) of four and by the *contribution of polychaetes*
polychaete individual dry weight	Const	defines *polychaete individual dry weight* (g) as 0.7×10^{-3}
No polychaetes preyed per shrimp BW	C	uses Equation (6.18) to calculate the number of polychaetes preyed per shrimp body weight
changes in No shrimp per m²	C	converts *changes in shrimp population* to m² applying Equation (6.19)
No polychaetes grazed per m²	C	converts the resulting value of *No polychaetes preyed per shrimp BW* to m² by mutiplying it by the *changes in No of shrimp per m²*
changes in polychaete population per m² after shrimp predation	I, C	integrates the resulting subtraction of *polychaete density per m²* by *No of polychates grazed per m²*

Appendix F: Cont.

Variable	Type	Definition
condition for simulation pause	C	pauses the simulation process when *changes in polychaete population per m² after shrimp predation reaches zero*

Appendix G: Variables, type and description of components in the shrimp feeding response sector. Type refers to the following: V = variable with value set by user; C = command; I = integration; R = pre-established relationship or value.

Variable	Type	Definition
shrimp growth	R	defines *shrimp growth* (g) based on a given time (d) with Equation (6.16)
broad SW in morning	R	determines stomach weight (g) in the morning for broadcast feeding based on *shrimp growth* (g) using Equation (6.22a)
conc SW in morning	R	determines stomach weight (g) in the morning for concentrated feeding based on *shrimp growth* (g) using Equation (6.22b)
feeding method	V	defines the feeding method to be used (broadcast = 1; concentrated = 2)
round feeding method	C	rounds *feeding method* (dimensionless) to its nearest integer value

Variable	Type	Definition
conditions SW in morning	C	gives a logical expression to select from pre-established stomach weight (g) values based on *round feeding method* (broad SW in morning = 1; conc SW in morning = 2)
changes in shrimp population	I	integrates values of *total No of shrimp remaining* using Equation (6.14)
population SW in morning	C	converts shrimp stomach weight (g) to kg by mutiplying *conditions SW in morning* with *changes in shrimp population* and dividing the resulting value by 1,000
SW in morning in kg	C	determines the population stomach weight (kg) in the morning over the production cycle
broadcast	R	defines the fraction (dimensionless) relative to the amount of feed contained in the shrimp stomach for the broadcast method (Table 6.1)

Appendix G: Cont.

Variable	Type	Definition
concentrated	R	defines the fraction (dimensionless) relative to the amount of feed contained in the shrimp stomach for the concentrated method (Table 6.1)
conditions fraction feed consumed	C	gives a logical expression to define the relative occurrence of artificial food (%) in the shrimp stomach contents (*broadcast* = 1; *concentrated* = 2)
feed consumed morning	C	multiplies the fraction (dimensionless) relative to the amount of feed in the shrimp stomach contents by *SW in morning in kg*
total feed consumed morning	C	gives the cummulative amount of feed consumed (kg) in the morning over the production cycle
broad SW in mid day	R	determines stomach weight (g) in the mid-day for broadcast feeding based on *shrimp growth* (g) using Equation (6.22c)

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Variable	Type	Definition
conc SW in mid day	R	determines stomach weight (g) in the mid-day for concentrated feeding based on *shrimp growth* (g) using Equation (6.22d)
SW in mid day in kg	C	determines the population stomach weight (kg) in the mid-day over the production cycle
feed consumed in mid day	C	multiplies the fraction (dimensionless) relative to the amount of feed in the shrimp stomach contents by *SW in mid day in kg*
total feed consumed in mid day	C	gives the cummulative amount of feed consumed (kg) in the mid-day over the production cycle
broad SW in afternoon	R	determines stomach weight (g) in the afternoon for broadcast feeding based on *shrimp growth* (g) using Equation (6.22e)

Variable	Type	Definition
conc SW in afternoon	R	determines stomach weight (g) in the afternoon for concentrated feeding based on *shrimp growth* (g) using Equation (6.22f)
conditions SW in mid day	C	gives a logical expression to select from pre-established stomach weight (g) values based on *round feeding method* (*broad SW in mid day* = 1; *conc SW in mid day* = 2)
population SW in mi day	C	converts the shrimp stomach weight (g) to kg by mutiplying *conditions SW in mid day* by *changes in shrimp population* and dividing the resulting value by 1,000
conditions SW in afternoon	C	gives a logical expression to select from pre-established stomach weight (g) values based on *round feeding method* (*broad SW in afternoon* = 1; *conc SW in afternoon* = 2)

Appendix G: Cont.

Variable	Type	Definition
population SW in afternoon	C	converts the shrimp stomach weight (g) to kg by multiplying *conditions SW in afternoon* by *changes in shrimp population* and dividing the resulting value by 1,000
SW in afternoon in kg	C	determines the population stomach weight (kg) in the afternoon over the production cycle
feed consumed in afternoon	C	multiplies the fraction (dimensionless) relative to the amount of feed in the shrimp stomach contents by *SW in afternoon in kg*
total feed consumed in afternoon	C	gives the cummulative amount of feed consumed (kg) in the afternoon over the production cycle

Appendix H: Presentation of models in the STELLA® II environment, software version 3.0.7 for Windows.