Intensification of Polychaete Worm Culture in Engineered Growth Systems

Mohammad Lafi Mohammad Kaba'ah

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Environmental Engineering group

School of Civil Engineering and Geosciences

Abstract

The effect of sand substrate depth on culturing and growth of Polychaeta *Nereis virens* (ragworm), and the impact of using artificial substrate materials for culturing and growth, were investigated in three experiments. In the first experiment, a recirculating water system was designed with eight identical rectangular (6L) polyethylene culture tanks (0.05 m²) arranged in one level, all of which were connected to a single water recirculation system. After harvesting, all worms were weighed and stocking density, specific growth rate, food conversion ratio, feeding efficiency and total biomass yield (g/tank) were calculated for each tank. The differences in the stocking density between white polyethylene beads and recycled polyethylene beads culture were not statistically significant. The weight-specific growth rate in sand bed culture was higher than the white polyethylene beads, the recycled polyethylene beads and the corrugated polypropylene sheet culture. In the second experiment, a batch of *N. virens* averaging a total weight of 52 g was stocked in 0.1 m² polyethylene tanks with different bed depths of sand: 7, 10 and 15cm. All cultures were run in triplicate and 450 individuals were needed, 50 individuals in each tank. Growth was assessed from the average wet weights recorded on day 0, 90 and 180. For the period (0 to 90 days), Fisher's test showed that differences in worm average weight between 7 cm and 10 cm beds at Harvest 1 (day 90) was not statistically significant, but average weight in 15 cm bed was significantly different from the 7 cm and 10 cm beds (one way-ANOVA-Fisher's test, $p = 0.002 < 0.05$). The average weight of worms in the 7 cm and 10 cm beds at Harvest 2 (day 180) were not significantly different, but average weight in 15 cm bed was significantly higher than the 7 cm and 10 cm beds (one way-ANOVA-Fisher's test, $p = 0.028$). In the third experiment, the batches of *N. virens* with an average weight of 14.74 ± 1.01 g were stocked into 0.05 m^{-2} polyethylene tanks with bed depths of 1, 3, 5 and 7 cm. The experiment ran for 90 days, during which time mean temperature was 18 ± 1ºC, salinity varied between 31‰ and 35‰, and pH was 8.0 ± 0.2. Differences between the average worm weight in the 1 cm and 3 cm beds at harvest (day 90) were not statistically significant (one way-ANOVA-Fisher's test, $p = 0.061 < 0.05$), but average weight in 5 cm bed and 7 cm beds were

statistically different from the 1 cm and 3 cm beds. The study concludes that the artificial substrate materials did not enhance culturing and growth of *N. virens*, compared to sand, and had a significant negative effect on the productivity of ragworm culture. In summary, a 7 cm bed depth of natural sand is proposed as the ideal substrate for culturing *Nereis virens,* particularly if a vertically stacked, multi-layer culture system were to be developed.

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Table of Contents

List of Tables

List of Figures

Chapter 1. Introduction

1.1 Overview

The Polychaeta play a significant role in the ecology of marine communities, and a small number of species are also of commercial interest due to their use as live bait in the sea angling industry. It is likely that polychaetes will become an increasingly valuable resource in relation to the development of world aquaculture for Crustacea (Penaeidae), since they can provide a nutritionally balanced source of polyunsaturated fatty acids which are important for egg maturation in cultured prawns (Lytle *et al.*, 1990; Maneerat Limsuwatthanathamrong, 2012; Leelatanawit *et al.*, 2014). They can also provide other growth factors which are essential for egg maturation (Olive, 1994). Interestingly, a study of the contribution of polychaetes to the food of some Brazilian fish revealed that, amongst ten benthic feeding fish species examined, polychaetes were present in 38% to 88% of the stomachs analysed (Amaral, 1994), indicating their importance as a natural food source.

Many polychaetes are not regarded as microphagous. Indeed, this accounts for the important role they play in the processes of organic matter recycling (Olive, 1994). By consuming microparticulate organic matter and growing on this resource, they convert low value microparticulate organic matter into an aggregated and concentrated form that provides a high value food resource for larger organisms such as fish and crustaceans.

The markets for Polychaete, particularly for live polychaete biomass, have high demand and consequently this commodity has a particularly high monetary value which is considerably higher, for example, than that of human food. However, the value of Polychaetes varies between different markets. The price of ragworms from the USA

fisheries in Maine rose sharply from the mid-1950s and then remained relatively high. The value of worms to the Maine fisheries increased from about $15 per thousand in 1960 to $38 per thousand in 1980. Creaser and Sampson (1983) and Creaser and Clifford (1986) give values for the Maine (USA) bait worm fishery. In general, a wholesale value of $US 0.1 per specimen or 15-25 US$ per kg was sustained over a number of years in the USA and Japanese markets, while values in the European markets appear to be even higher. For example, in the 1970s wholesale values in the latter region were $70 - 90 per thousand worms according to size (cf. Olive, 1994).

In many parts of Europe, the total demand for bait has been found to exceed local supplies, which has driven the market for bait. This, in turn, has resulted in the growth of the large scale distribution of bait to retail outlets. In Europe, the bait market includes supplies made available from sources such as Korea, China, and the USA.

Owing to this high demand for bait, it became economically feasible to culture polychaetes specifically for supply to the bait market (Olive 1994). The high price of the worms which are supplied to the sea angling leisure industry reflects both their relative shortage and the labour intensive nature of current supply routes. Therefore, systems which could provide more intensive culture of these animals are now needed, particularly since they can also provide a means of alleviating the effect of the unsustainable collection of bait from the natural environment (digging), and reducing the need for bait importation (Olive, 1999a).

1.2 Aim and objectives

The original aim of the present research was to investigate and explore the potential of developing an engineered growth system for the intensification of marine ragworm production, and to optimise growth rates. In addition, the project aimed to investigate the possibility of using ragworms in the treatment of aquaculture wastewater.

The current technology which is commonly used for the production of marine ragworms depends on culturing them using a horizontal pond. Such a technology needs a large amount of space, which is the most important factor affecting the expansion of the ragworm production industry. Therefore, other alternatives were considered to enhance growth and minimize space. In this regard, two possibilities were identified. The first possibility was to use a vertical multi-stack engineered system which would reduce the footprint area and be efficient in minimizing space. Secondly, it may be the possible to use an indoor recirculating aquaculture system (RAS), which could be optimized to enhance worm growth rates by controlling the environmental and physical conditions of the worms reared. The second option was chosen because it allows important factors such as the ideal depth of the substrate, and their effect on growth to be identified. Furthermore, the outcomes of such an indoor RAS need to be known as a starting point in designing a vertical multi-stack engineered system. In other words, the indoor system was considered to be a piloting process for the development of an outdoor culture system.

The study was originally planned to address the following objectives:

1. To investigate the effect of the depth of sand substrate on the culture and growth of marine ragworms.
2. To investigate the effect of different artificial substrate materials on enhancing the growth of the cultured worms.
3. To investigate the effect of different levels of dissolved Oxygen on the growth of cultured worms.
4. To design a model for the growth rate of marine ragworms.

However, these original objectives were reconsidered soon after the start of the project, because of many methodological and practical problems. First, the original agreement with Sea Bait Ltd. was to run the experiment at the company site because they had the required facilities, such as space and systems, but the company faced financial difficulties

soon after the start of the project. Furthermore, at this early stage of the project, company ownership was transferred to another investor who was much less interested in supporting the project, and access to the pilot-scale culture facility on-site was withdrawn. Therefore, the second option was chosen, which was to run the experimental investigation in the laboratory of the School of Civil Engineering and Geosciences (CEG) at Newcastle University. However, the School did not have any pre-existing system in which to run the planned experiments, and the present author did not have any previous experience in marine recirculation systems. Therefore, a pilot experiment was needed to develop the necessary skills and test the feasibility of setting up such a system. Although this work confirmed the feasibility of providing a RAS facility within the CEG laboratory, there was limited space available to run parallel experiments simultaneously. Therefore only one experiment could be run at a time, and since these generally lasted for about 6 - 9 months, the number of experimental runs possible within the project was quite limited.

Based on the above issues, the initial objectives were readjusted to address the following:

1. To investigate the effect of the depth of sand substrate on the culture and growth of marine ragworms.
2. To investigate the effect of different artificial substrate materials on enhancing the growth of the cultured worms.

As can be seen from these revised objectives, the third and fourth original objectives could not be addressed without more extensive facilities and space, or a greater amount of time being available.

1.3 Outline of the thesis and acknowledgement of other contributors

This thesis is organised into six chapters. An overview of the research motivation, aims, objectives, research hypotheses, research questions and an outline of thesis are provided in **Chapter 1**.

Chapter 2 provides an introduction and a detailed literature review covering the history of the Polychaete, their economic significance and position in the angling bait market, aquaculture industry, and in other markets. In addition, their physiological characteristics, the use of sandworms or king ragworms in the bait industry, and the physiology and life history and factors affecting the growth of *Nereis virens* are also discussed. Also described are recirculating aquaculture systems, large scale food production applications, and previous research on substrate depth, the weight of worm production, stocking density and surface area.

Chapter 3 describes the materials and methods adopted in this research. These methods focus on the current technology used in the production of cultured worms.

Chapter 4 presents data for the system design of the first experiment. The source and feeding regime of the ragworms, system performance, harvesting methods, and a statistical analysis of the results of the first experiment are described.

Chapter 5 presents data from Experiments 2 and 3, investigating the effect of culture bed depth on the production of *Nereis virens.* In addition, a comparison is conducted on the numbers, total mass (weight), average mass, feeding and mortality of ragworms in the culture beds.

Chapter 6 presents the conclusion of the study, answers to the research questions, and makes recommendations for further work.

Chapter 2. Literature Review

2.1 Introduction

The Polychaeta family (or polychaetes) are a polyphyletic class of annelid worm. Polychaetes are also known as bristle worms. The class contains more than 10,000 species that can tolerate a range of environmental growth temperatures (Struck *et al.*, 2011). Polychaetes are found at all depths in the ocean, from some forms that can live close to the surface, to a 2 – 3 cm specimen (still unclassified) that can live in the deepest seabeds of the Challenger Deep. Glasby and Timm (2008) state that less than 2% of all known polychaetes (no more than 168 species) are from freshwater.

2.2 The benefits of the culturing of Polychaeta

Polychaeta play a vital part in the food chain which generates food resources from the oceans. They are significant globally because they are an important ecosystem component affecting biogeochemical cycles. By consuming micro-particulate organic matter, polychaetes convert low value dispersed organic matter into an aggregated and concentrated food source for large fish and crustaceans. Because of their economic significance, researchers are interested in these organisms and have attempted to produce large quantities by commercial production (Olive, 1994).

Polychaetes are cultured for their economic significance, predominantly in the following areas:

a. They are used for bait in sea angling sport and leisure fisheries.
b. They represent a high value food source that can be used in other aquaculture industries, such as fish and crustacean farming.

c. They can be used for toxicity testing, or for the production of other novel biological products such as nutrient supplements (omega-3 fatty acids), or haemoglobin.

An introduction to each of these uses is provided below.

2.2.1 Polychaeta in the angling bait market

The use of polychaete worms for bait in rod or hook angling has a long history, and Polychaeta are famous for being the preferred bait in the sea angling sport and leisure industry (M.C. Gambi, 1994; Olive, 1994). The European bait worm market has been predicted to have a value of about 200 million Euros (Olive, 1999a). In the UK, ragworms (*Nereis* spp.) and lugworms (*Arenicola* spp.) are the two main polychaete groups which are used for bait, and *Nereis virens* (Sars) is the most important species (Olive, 1994). It has been estimated that at least 1000 tonnes of bait worms are collected every year from the natural environment, but it is difficult to estimate the volume of this trade accurately because there is insufficient recorded information (Fowler, 1999).

In comparison, the collection of polychaetes in Portugal exceeds 45 tonnes per year (Cunha *et al.*, 2005), while the Maine (USA) bait fishery reached a peak at 180 tonnes of combined *Glycera dibranchiate* (Ehlers) and *N. virens* (Creaser and Sampson, 1983). Estimates provided by the National Federation of Sea Anglers indicate that, within the Solent region of the UK in 1997, there were about 40,000 active sea anglers (Fowler, 1999).

To be useful as bait, worms should be relatively large, strong enough to remain on a hook, available in reasonable quantities and accessible in tidal water at times of low tide. Only a few species of polychaetes meet these criteria. In Northern Europe and the USA only three species are used in large quantities: *Nereis virens, Glycera dibraniata* and *Arenicola marina.*

2.2.2 Polychaeta in the aquaculture industry

One of the possible limitations in the expansion of aquaculture is the availability of food. In this industry, the main source of food is fish meal and other concentrated foods. Many marine species cannot synthesise specific unsaturated fatty acids, such as linoleic and arachidonic acids, and these essential fatty acids must come from the diet. The availability of fish meal for aquaculture is reduced by the high demand for fish meal and other concentrated feeds by livestock agriculture. As a result, the aquaculture industry faces a considerable problem in the production of Crustacea and finfish, and producers have recognized a drop in the production of juveniles in comparison with needs (Olive, 1994). Thus, the aquaculture industry is seeking alternative feed sources which contain substantial amounts of protein and fat, with a balanced quantity of essential unsaturated fatty acids.

It has been argued that this problem can be solved by supplying polychaetes as food for aquaculture brood stocks (Olive, 1994). In India, the increased intensification of shrimp farming, hatchery production was 1.5 billion, whereas the requirement had reached 6.5 billion (MPEDA, 1994). Broodstock nutrition could be investigated to supply the best nutrients through combining marine polychaete worms, which are rich in 20:4 n-6 arachidonic acid. There is now an increasing awareness of the potential role that polychaete biomass might play in improving the output of broodstock in the aquaculture industry. However, little consideration has been given to where or how this polychaete biomass could be procured (Olive, 1999b).

2.2.3 Polychaeta in toxicity testing

Polychaetes such as *N. virens* are widely used in sediment toxicity bioassays, and more recently members of the Nereididae family have been favoured for cytogenetic toxicity bioassays (Olive and Wang, 1997). The environmental fate of contaminants such as polychlorinated biphenyls (PCB) is a global eco-toxicological problem, and

bioaccumulation is an important issue. When aquatic organisms ingest food, they take up PCBs and other lipophilic substances directly from water through passive diffusion, primarily via the respiratory surfaces.

Various models have been used to categorize such processes, and much of the research focuses on how these routes are related to bioaccumulation, and how they might vary between organisms with different modes of living (Secco *et al.*, 2005). Using bioaccumulation results obtained from measurements of uptake and elimination, it is claimed that elimination is an insignificant loss mechanism and the ability of fish to metabolically transform PCBs is limited. Models of mechanistic mass balance exist where different processes of uptake and elimination have been quantified. The advantage of these models is that they might be able to take into consideration the effects of phenomena like growth dilution and compound-specific biotransformation rates (Ruus *et al.*, 2012).

2.3 The problems of economics

World aquaculture production has experienced a significant rate of growth over the past fifty years, from a level below 1 million tonnes per year in the 1950s to 90.4 million tonnes in 2012. This includes 66.6 million tonnes of food fish, 23.8 million tonnes of aquatic algae, and 22,400 tonnes of non-food products (FAO, 2014). The sector has grown faster than any other field of food production from animal origin (Jia, 2009). The demand for fish and fish products continues to grow, and consumption has more than doubled since 1973, resulting in consequential growth of aquaculture production. The contribution of aquaculture production to the supply of fish has increased significantly, reaching the historical record of 47% of total fish food supply in 2006, as compared to 8% in 1970. This trend is projected to continue, and is expected to reach a proportion of 60% in 2020 (FAO, 2010).

From 2006, the population of sandworms has declined noticeably compared to previous years because the worms have been overharvested faster than they can reproduce. The bait industry is naturally interested in increasing the supplies of farmed bait worms. For the time being, supply is greatly below retail demand for bait, specifically at difficult times of the year, due to tide and weather conditions (Olive, 1994). Wild stocks are currently at very low levels, and there is a high demand for certain target angling species. The population decline has been attributed to many reasons, such as pollution, coastal development and deposition (Watson *et al.*, 2007). The demand for bait has become such that intensive polychaete farming is now economically viable (Olive, 1994).

As a result of the high demand, and to alleviate the pressure of fishing on fish marine stocks, it is necessary that fish production should be accelerated through aquaculture (Tal *et al.*, 2009). This production, in addition to being economically viable, takes into account the impact that it has on resources including the environment, water availability, fish feed and location on land (Zohar *et al.*, 2005; Schneider *et al.*, 2007). Among the many existing aquaculture systems, the recirculating aquaculture system (RAS) appears able to overcome these limitations and could provide a form of sustainable farming for both marine and freshwater species (Schreier *et al.*, 2010).

Seabait Ltd failed as a business venture because although, there was a high demand in the market for its worm products, the production level of the company was limited. This was attributed to the limited space available for culturing the worms. This problem is addressed in detail later in this thesis.

2.4 The cost of running recycling systems

In designing a recirculation aquaculture system, one should consider all of the relevant factors related to cost and price. Such a step is very important to give the producer a clear idea of the likely profitability of the RAS.

O'Rourke (1996) points out that in designing a RAS there are two types of costs involved: initial investment expenses and other operating expenses. Initial investment expenses refer to the capital invested in the operation of the RAS. Such expenses are fixed because they can be estimated before commencing the RAS project, and they do not change too much with variation in the volume of production. O'Rourke mentions that there are three main expenses involved in fixed expenses: depreciation, interest on the capital invested and maintenance and repair expenses.

Depreciation and interest can be estimated by using the expenses of actual interest and the allowable depreciation rules which are used by the Internal Revenue Service. Another option is to use a straight-line depreciation over the estimated economic life of assets, and estimated opportunity costs for interest on invested capital. For the first estimates of production expenses, O'Rourke (1996) states that the second option is preferred because it involves fewer calculations. Annual depreciation on assets that have expected life of more than one year can be calculated by dividing the initial investment price by the number of years of expected life. Expenses of annual interest, on the other hand, can be calculated by multiplying one half of the total initial investment by the opportunity costs of the invested funds (O'Rourke, 1996).

Maintenance and repair expenses are usually not related to the quantity of the product from the RAS. The best way to estimate such expenses is to use historical records. However, if historical records are not available, the producer should consult the manufacturer's guidelines. O'Rourke (1996) points outs that the annual expenses for

maintenance and repairs can be estimated as a percentage of the initial investment in every asset. In this regard, assets with moving parts usually have high annual rate of 5%, while assets with non-moving parts usually have low annual rates of 1%. Table 2.1 shows the initial investment expenses for the Tilapia system.

In addition to the initial investment expenses, O'Rourke (1996) mentions that there are other operating expenses. Such expenses include all inputs used to operate the RAS, such as labour, feeding, insurance, taxes, and water use.

Table 2.1 Facilities and equipment for intensive Tilapia production prototype system (O'Rourke, 1996: 4)

Item Description	Initial Investment ($)	Estimated Life (Yr)	Annual Depreciation (SL)($)	Repair and Maintenance ($)	SalvageValue (5 yr)
Land	$3,000		$0	$0	
Building 40'X80' @ $19.50	$62,400	20	$3,120	$3,120	$15,000
SUBTOTALS	$65,400		$3,120	$3,120	$15,000
EQUIPMENT					
Indoor drain plumbing	$1,900	15	$127	$100	$0
Bldg. plumbing	$4,000	10	$400	$200	$0
Effluent system	$3,000	10	$400	$200	$0
Heater	$3,465	15	$231	$100	$300
Pumps	$2,150	5	$430	$200	$200
Blower	$1,048	7	$150	$50	$100
Growout tank	$19,482	20	$974	$100	$5,000
Divider screen	$1,500	20	$75	$100	$0
Reservoir tank	$1,500	20	$75	$0	$100
Purge tank filter	$500	15	$33	$100	$0
Drum particle filter	$11,500	10	$1,150	$400	$1,500
Biofilter tank and media	$8,600	20	$430	$0	$800
Oxygenation piping	$1,000	15	$67	$0	$0
Oxygen cones	$2,000	15	$133	$0	$200
Feed storage bin	$4,500	15	$300	$25	$400
Feeding system	$2,700	10	$270	$200	$500
catwalk	$700	10	$70	$0	$50
Feed carts	$298	10	$30	$50	$50
Harvesting equipment	$2,000	10	$200	$200	$200
Water testing equipment	$4,600	10	$460	$100	$1,500
Monitor and alarm	$6,000	5	$1,200	$400	$1,000
Backup generator	$4,500	15	$300	$300	$500
Emergency O2 system	$500	15	$300	$300	$500
SUBTOTALS	$88,443		$7,638	$3,210	$12,650
TOTALS	**$153,843**		**$10,758**	**$6,330**	**$27,650**

2.5 Ecology of the polychaete *Nereis Virens*

The Nereididae family of polychaete worms contains five hundred species which are grouped into forty-two genera. The majority of these species are marine dwelling, such as the common clam worm (*Nereis succinea*) and the sandworm (*Nereis virens*). *Nereis virens* is the old scientific name, and it has recently been reclassified as *Alitta virens* (Fauchald, 2012). *N. virens* is also commonly known as the king ragworm (Figure 2.1).

Figure 2.1. King ragworm

2.5.1 Body characteristics

Sandworms have an elongated body, which thickened anteriorly and slightly flattened dorsoventrally (Pettibone, 1963). They are large polychaetes, growing up to 90 cm long, and can have over 200 segments. The first segment is small with two pairs of eyes, one pair of small antennae, and two stout fleshy palps which are organized distally (see Figure 2.2). They have blue heads with two large pincer teeth, and have numerous,

highly vascularized parapodia. The parapodia function both as external gills (the primary respiratory surfaces of the animal), and as means of locomotion similar to short legs.

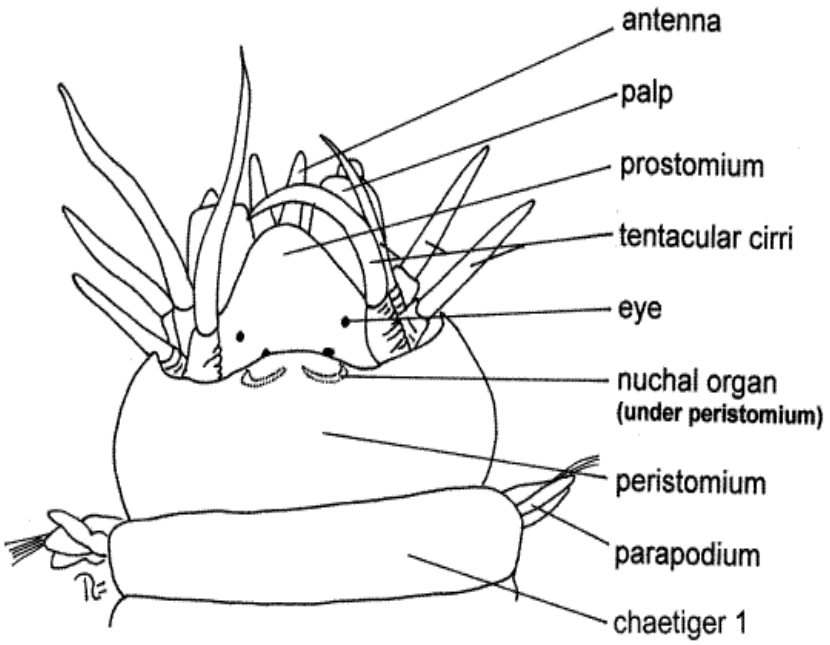


Figure 2.2 Dorsal view of the head of *Nereis virens* (Fox, 2001)

2.5.2 Habitat

Sandworms can be found in the Pacific and Atlantic Oceans (Pettibone, 1963; Hartmann-Schröder, 1971), particularly along east coast of the USA. They live from a depth of 150 m to the high water mark, especially in sheltered flats bordering the mouths of rivers, estuaries and sounds (Pettibone, 1963). The highest densities can be found near the low water mark of sandy and muddy shores (Pettibone, 1963; Wilson, 1988). Sandworms exist in a vast range of sediments, but muddier sediments seem to be preferred. In addition, they are found under cobbles and boulders on rocky shores, and under attached algae where they are very common (Pettibone, 1963). *N. virens* normally burrows down to 45 cm, with larger individuals going even deeper (Pettibone, 1963). The worms feed with their anterior end protruding out of the burrow, allowing them to crawl towards their food. They also have an ability to detect waterborne odours.

2.5.3 Feeding habits of N. virens

N. virens is mainly omnivorous, and uses its two jaws and proboscis to collect food (Fauchald, 1979). The teeth of Ragworms are made of a very strong, but lightweight material. As opposed to bone and tooth enamel, the teeth of Ragworms are not mineralised with calcium, but consist of a histidine-rich protein, with bound zinc ions. Primarily, they eat seaweed and microorganisms, but also consume dead organic matter of animal and plant origin. *N. virens* is categorized as an opportunistic and omnivorous species, which uses various feeding modes such as predation, deposit feeding and scavenging (Goerke, 1976).

The adults are primarily predators, but the young or juveniles feed largely on organic matter. *N. virens* feeds by either attacking prey with their pharynx and large jaws, or by using the pharynx to sweep and indiscriminately swallow the surface deposits around their burrows. In addition, Goerke (1976) found that *N. virens* were deposit-feeders, whilst Fauchald (1979) defined *N. virens* as a herbivore in the intertidal zone near Woods Hole Oceanographic Institute (Massachusetts, USA). Sandworms are considered to be a natural prey for many species of fish and seabirds (Wilson, 1988).

2.5.4 Migration

Sandworms can migrate, and they have been seen moving over the surface of sand flats and along drainage channels. When sandworms migrate, they use the water column at specific times of the year and in accordance with the lunar cycle (Dean, 1978). Non-reproductive worms have been collected from the water column at night swimming at the surface (Dean, 1978).

2.5.5 Temperature and reproductive behaviour

The growth of *Nereis virens* can be influenced by many environmental factors, especially temperature. *N. virens* is a semelparous species, which means that it reproduces only once in a lifetime (Wilson, 1988). Prior to spawning, mature males transform into epitokes, which can swarm in the water column. The fertilization success of *N. virens* has been shown to be significantly affected by temperature, the best success being achieved at 15-18°C (Lewis, 2002). Deschenes *et al.* (2005) observed that the highest relative activity was at 18°C.

Figure 2.3 Different life stages of Polychaete worm.

(A) Trocophore larvae, at 24 hours post-fertilization, with a ciliated equatorial ring. (B) Nectochaeta larvae, at 72 hours post-fertilization, with elongated shape and developing head and segments. (C) and (D) juvenile and non-mature adult (atokous) worms grazing on food surrounding their burrows. (E) Mature (epitokous) female worm. (F) Mature (epitokous) male. (García-Alonso *et al*., 2013: 144)

Swarming requires a temperature above 7°C (Creaser and Clifford, 1982), and happens between March and June in New England, USA. Females neither change into swarming epitokes, nor do they swim. Fecundity ranges from 0.5 to 1.3 million eggs for each female (Creaser and Clifford, 1982), and fertilization is likely to happen in the burrows of the females. Development happens largely at the sediment surface, although some authors have shown that small larvae exist as plankton (Wilson, 1988). Despite the ambiguity in the demography of such populations, it has been observed that maturation is usually correlated with the size of the worm (Creaser and Clifford, 1982).

In spring (April/May), adults naturally reach sexual maturity, at which point the coelomic fluid of the different sexes is filled with sperm or maturing oocytes (Bass and Brafield, 1972). Although the age at spawning varies between 1 and ≥8 year, the process of maturation itself takes place in spring (Oliver, 1995). Age at maturity is partly dependent on initial growth rate, such that at high growth rates the onset of sexual maturation happens early; but size at maturity is smaller when the growth rate is lower and age at maturity greater (Desrosiers *et al.*, 1994; Olive *et al.*, 1997).

Depending on local conditions, the average age at reproduction in natural populations of *N. virens* is normally between 2 and 7 years (Olive, 1993; Desrosiers *et al.*, 1994). Mature individuals of *N. virens* usually breed in the spring despite variations in age at reproduction between and within populations. Such patterns of synchronised spring breeding have been preserved in artificially cultured populations over a period of 12 years, even though the time of maturation was decreased and could be as little as only one year (Olive *et al.*, 1998).

Seabait Ltd set up populations of *N. virens* which were brought to a stage of sexual maturity from birth under conditions which were different from those in any natural environment, but which were planned to maximise the rate of growth. The company had

the capability of controlling temperature close to ambient levels, or at controlled temperatures above or below this, providing an opportunity to vary and optimise growth rates (Olive *et al.*, 1997).

2.5.6 Availability of feed

Adults of *Nereis virens* are predators but juveniles feed largely on organic matter (Desrosiers *et al.*, 1994). However, food is imported from the shores into the subtidal zone by flood tides, and exported by ebb tides (Roman, 1989). Consequently, food is found for only short periods of time each day. To overcome food shortages, *N. virens* might store organic matter in their burrows (Olivier *et al.*, 1995). Deschenes *et al.* (2005) noticed relative activity from 43 to 79% in conditions of low food availability and from 50 to 88% at high food availability.

2.5.7 Feeding efficiency

Food conversion efficiency (FCE), food conversion ratio (FCR), and food conversion rate are measures of the efficiency of an animal in converting food mass into increased body mass. The FCR is the mass of food eaten divided by body mass gain over a specified period (Section 5.2.1.4). For example, Safarik *et al*. (2006) investigated the daily growth of *Diopatra aciculata* after 14 weeks of incubation, and it was found that mean daily growth was higher during weeks 1 - 7 than during weeks 7 - 14 for all growth variables at each density level (for more details, see Section 2.7.1).

2.5.8 Photoperiod

Photoperiod is linked directly to day length, and *N. virens* is normally active at night (Miron *et al.*, 1992). It has been reported to exhibit high activity when the lights were turned off in its culture tanks (Last, 2003). *N. virens* has been claimed to be strongly

nocturnal, and this was proven by the observation that emergence events were not recorded on video during the photophase, and that foraging behaviour occurred only in the absence of light (Last, 2003).

2.5.9 Density

In an investigation of the spatio-temporal evolution of *N. virens* burrows, Miron *et al.*, (1991b) revealed experimentally that density is an essential in space-sharing modalities within the sediments. They suggested that strong territorial behaviour prevented connections between burrows, even under high-density conditions. In high density conditions, *N. virens* competed for burrow space more than for food (Miron *et al.*, 1991b).

2.6 Recirculating aquaculture systems

The recirculating aquaculture systems (RAS) is one of the platforms which can offer a continuous method for the intensive rearing of marine and freshwater fish. The RAS is one of the most recent forms of fish farming production systems. These are typically indoor systems that allow farmers to have control over environmental conditions throughout the year. They use a closed (or recycled) farming system which permits the reuse of farm water through of a series of filtration steps. This limits not only the removal of water from natural water resources, but the impact of the system on the environment. This process in turn reduces the amount of waste which is generated from its treatment in the RAS (Lahav *et al.*, 2009). Although the costs of constructing a RAS are typically higher than either cage or pond culture, if the system is properly managed to produce fish all year round, there can be increased economic returns (Schneider, 2006).

The RAS is considered to be one of the most complicated aquaculture systems, and so new users of the technology should learn how to operate and control the system. The capability of these systems to handle, store and treat waste products accumulated during the growth of farmed fish represents a key factor in the development of environmentally friendly aquaculture production systems (Van Rijn *et al.*, 1996; Piedrahita, 2003). Efficient RAS management allows the effective control and treatment of soluble and particulate waste coming from the system, and minimal inputs of water are needed except to make up for losses due to evaporation (Zohar *et al.*, 2005; Michaud, 2007; Tal *et al.*, 2009). The RAS also provides the ability to monitor key environmental parameters associated with the rearing and life cycle of farmed fish, thus maximizing production yields, and the occurrence of infections caused by pathogenic bacteria or parasites can be reduced (Michaud, 2007).

The treatment of wastewater within a RAS is undertaken using different filtration steps, which are primarily divided into mechanical and biological filtration. The former uses physical agents such as oxygen, temperature, ozone, UV, pH and salinity for the removal of waste substances in the water outlet from the rearing tanks, and for its disinfection. Meanwhile the latter uses biological oxidation and redox reactions mediated by micro-organisms to reduce the accumulation of nitrogen species. The microbial compartment plays a major role in wastewater treatment, and the activities of the bacterial communities in an RAS are comparable to those of the fish in terms of biomass and metabolic processes (Michaud, 2007), and oxygen consumption (Blancheton, 2000).

2.6.1 Large scale RAS food production applications

There are many inland aquaculture businesses in the north east of the United States striving to achieve good reputations and high profits (Andrew M. Lazur, 2003). Importantly, such inland aquaculture businesses have witnessed rapid development in terms of technology systems and efficiencies in production, which have led to decreased

operating costs. However, these businesses still have some unresolved issues affecting economic viability. Because developments in technology take a long time, and may have minor effects on profitability, the best option for many operations is to expand into higher value products, and to target higher value market niches. Inland-based systems have gained a reputation for flexibility and capability in producing marine and freshwater animals and plants, for use as bait, food, recreation, ornamentals purposes, and laboratory research. Current research in the northeast of the United States focuses on the identification and demonstration of those market strategies and products that have the best returns on investment. Table 2.2 summarises the high priority research needs for pond, flow-through, and recirculating aquaculture production systems.

Table 2.2 Highest priority research needs for land-based aquaculture systems in the NE region of the USA (not in ranked order). [Source: (Andrew M. Lazur, 2003)]

Research Need
Evaluation of market characteristics and economic opportunities of high value bait, biotechnology, food, ornamental, recreation and restoration species and products including value added products.
Evaluation of cost effective effluent treatment and waste utilization options for all culture technologies.
Investigation of genetic improvements in fish and plant species
Assessment of incidence and volume of fish mortality following delivery of live fish in recreational markets. The development of protocols/methods and educational materials to minimize stress and subsequent mortality during and immediately after distribution.
Evaluation of alternative disease management treatments or practices.
Identification of lower capital cost technology and cost reduction of operational practices for recirculating system technology.
Development of feeds and feeding practices which reduce amount of phosphorus and nitrogen in waste.
Evaluation of integrated production systems for multiple species (plants, shellfish or fish) to reduce the nutrient concentrations of point and non-point source discharges.
Evaluation of the use of alternative water sources for aquaculture production.
Development of feeds that are suited for RAS conditions for specifically identified species, e.g. tilapia.

Singh *et al.* (1999) investigated four circular, 2000 L fiberglass tanks in four independent, indoor recirculating aquaculture systems which were stocked with hybrid striped bass fingerlings (60g average weight) with identical densities (320 fish per tank at the begining of the study). The four systems in the study corresponded to four different recirculating system configurations formed by combining two types of biofilters (bead and trickling) and two types of solids removal filters (multi-tube settling basin and rotating screen filter). Commercial fish food was used during the experimental trials, and supplied at two different feeding rates, 600g (two trials) and 800g (two trials) per system per day. The characteristics of water quality were observed in all four trials in the study, including biochemical oxygen demand (BOD) and total organic carbon (TOC). The effects of the biofilter, particulate filter, feeding rate, and interactions among them on water quality characteristics and system water exchange rate, were also investigated. The systems with trickling biofilters, compared to those of the systems with bead biofilters, were found to have lower concentrations of nitrite–nitrogen (NO_2–N), lower total ammonia nitrogen (TAN), and lower TOC in the tank water. It was also noted that the systems with a screen-type particulate filter needed lower amounts of make-up water.

Consequently, it was concluded that indoor fish production using recirculating aquaculture systems could be compatible with the environment, highly expandable and sustainable. In addition, such systems could guarantee the quality and safety of the fish produced throughout the year. Dramatic developments in RAS technology have been made over the last two decades (Andrew M. Lazur, 2003), but further developments in cost efficiency are still being sought so that food fish can be produced on a competitive basis compared to traditional large-scale outdoor systems.

The RAS offers many advantages when compared to traditional technologies, such as the possibility to be placed near the fish markets, high product quality, shorter production cycles as a result of high food conversion factors, and a constant monitoring of the farm environment in order to improve rearing conditions (Singh *et al.*, 1999).

2.7 Previous research on the effect of bed depth on worm production

There has been no specific research carried out on the effect of depth of the culture bed on the production of *Nereis virens*. However, some relevant studies have been conducted for other factors, such as food and stocking density, which have employed different depths of culture bed substrate.

Brown *et al.* (2011) carried out two experiments to examine the effect of feeding the polychaete worm *Nereis virens* with solid wastes which were collected from a marine recirculating system. The water depth was set at 4 cm above the substrate, which consisted of a layer of sand/fine gravel approximately 8 cm deep throughout the tank. In the first experiment, worms with an initial mean weight of 0.37 g were fed for 80 days with halibut faecal waste, commercial worm food, uneaten halibut feed pellets, or a 1:1 mixture of feed pellet waste and faecal waste. It was found that the average weight of harvested worms and the resulting biomass were significantly higher in the group fed on uneaten halibut feed pellets than in the other three groups. In the second experiment, worms with an initial mean weight of 0.18 g were fed various proportions of waste mixed with commercial worm food. As for the other groups, the commercial worm food group was fed with just commercial food, the W100 group was fed with just waste, and two intermediate treatments was fed 50% of each (W50) or 75% waste (W75). Interestingly, no significant differences were noticed in terms of average resulting weight or biomass.

Last (2003) constructed artificial burrows at the bottom of an acrylic aquarium (width-height-depth: 37, 35, 21 cm) to culture *N. virens.* Sieved (0.5 mm), sterilised and washed sand was strongly attached to the bottom of the tank, and clear silicone gel was applied to make it easy for the animals to move over a textured surface. To hold the artificial burrows, a series of acrylic stubs were inserted into the base of each aquarium. Each stub was 15 mm in length, with an internal diameter of 5 mm and a wall thickness of 1 mm.

Lengths (350 mm) of nontoxic PVC hosing with an internal diameter of 7 mm were attached to the stubs providing artificial burrows about 15 cm deep, which equates to the mean burrow depth of *N. virens*. The between-burrow spacing was considered a significant factor in the design of the actograph, since emergence rates decreased with density. Each burrow entrance was 70 mm from its exit and from its nearest burrow neighbour, simulating a relatively low density population. Such measurements were used to minimize competition in food prospecting among individuals, because the rates of population prospecting activity can be affected by high density. Last (2003) found that the design of the actograph was successful and the worms survived in the artificial burrows. However, he did not address the effect of using artificial burrows on the growth rates or productivity of the worms.

Miron *et al.* (1991) carried out experiments with *N. virens* within two 1 m^2 aquaria filled with a 30 cm-deep layer of homogeneous sandy sediments brought from the sampling site. Sediments were defaunated but not sterilized, and were sieved through a 1mm mesh sieve. In order to avoid excessive worm activity, no complementary food was provided. This investigation aimed at describing and quantifying the prospecting activity displayed at various density conditions within a twenty-four hour period. It defined the undefended part of the *N. virens* home range. Furthermore, the study attempted to find out some of the expected functions of undefended feeding area in the biology of *N. virens*. The results showed that prospecting time was ≈ 2.5 - 3 min at low density and ≈ 2 min at high density. Where density was high, the number of out-of-burrow segments rose, while there was no significant difference in values between day and night. Behavioural investigation of *N. virens* showed an antipredator strategy. Therefore, it was claimed that space is more important than food for culturing worms.

Deschenes *et al.* (2003) set up experiments to examine carbon sequestration and food utilisation by *Nereis virens*. The aquaria used in their study combined two glass plates

which measured 30 cm (height) by 30 cm (length) and set 0.5 cm apart, filled with sifted sediments which were left to settle in water for a week before introducing the polychaetes. The animals used in the study were captured from a small bay near the research centre, and were introduced randomly in groups of three in every aquarium. They were not fed for a week and were left in the dark to avoid exhausting activities and allow them to build their burrows. It was found that 43% of the worms died; 93% of these before treatment, and 7% died between treatments. During the treatments, worms were not replaced and no mortality was recorded. Overall, the study found that instant storage was better than feeding worms when they were capable of capturing extra rations. Such a strategy, the researchers proposed, prevails when food is available because there are more advantages than disadvantages. The study concluded that it was not easy to evaluate the importance of storage on the carbon cycle. However, the densities of *Nereis virens* and its bio-geographical distribution indicate an important effect on the growth of *Nereis*.

2.7.1 Stocking density and surface area

Density-dependent factors, including competition for space and food, are integral structuring agents for populations of a number of sediment dwelling polychaete species (Beukema *et al.*, 2000; Omena and Amaral, 2000; Reise *et al.*, 2001). An increase in density has been linked to reduced reproduction and juvenile recruitment in the polychaetes *Ceratonereis pseudoerythraeensis* (Kent and Day, 1983) and *Polydora ligni* (Zajac, 1986).

Safarik *et al.* (2006) assessed the daily growth of *Diopatra aciculata* after 14 weeks of incubation, which was lower at high density (2.3 mm/day) when compared to medium density (2.8 mm/day) and low-density (3.0 mm/day) conditions. In their study, the negative effects of *D. aciculata* density on growth were due to intraspecific interaction, including competition for resources other than food. Three polychaete densities (500, 1000 and 2000 worms/m^2) were represented within triplicate 0.30 m^2 boxes which

contained late juvenile *D. aciculata*, sandy sediment and recirculating seawater. The daily food ration per worm was held constant across all density levels. Total length, weight and number of segments were recorded for 20 polychaetes which were randomly removed from each of nine treatment boxes at weeks 1, 7 and 14. Mean daily growth was higher during weeks 1 - 7 than during weeks 7 - 14 for all growth variables at each density level. Polychaetes at the highest density level exhibited lower rates of growth and more broken and/or regenerating posterior segments than those at low density. High *D. aciculata* density (2000 worms/m^2) was also related to lower dissolved oxygen concentrations and higher polychaete mortality (20%). At medium polychaete density (1000/m^2), *D. aciculata* exhibited low levels of apparent stress and high biomass return per unit area, both of which are important considerations in the aquaculture rearing of this species.

Scaps *et al.* (1993) investigated the impact of food intake, intraspecific density, and interactions between individuals on the growth of laboratory-raised juveniles of *Nereis diversicolor* which were fed different quantities of Tetramin®, an aquarium fish food containing a selected mixture of highly nutritious and functional ingredients with vitamins, minerals and trace elements. It was found that a daily intake of 3.33 mg of Tetramin per individual resulted in an optimal mean mass gain. The findings suggested that the growth rate decreased at densities above 3000 individuals/m^2. Thermo-differential shock and frequent handling created stress with the same negative effects. `Variations in responses to isolation or aggregation were found to be high, as shown by groups of juveniles which were captured in winter or in spring reacting differently. The study concluded that the breeding conditions in the winter-caught worms did not have an effect on their growth. However, growth was faster in spring-caught individuals kept in isolation.

Batista *et al.* (2003) carried out research where a 65-day experiment was carried out to determine the growth, survival, oogenesis and feed utilization of *N. diversicolor* (Müller, 1776) fed with a commercial dry food that was developed for gilthead seabream (*Sparus*

auratus L. 1,758) (S. auratus dry feed , SBDF). Worms were reared in three replicate tanks with 50 worms per tank (255 individuals per m²). Tetramin® ornamental fish dry feed (OFDF) was used as control feed. The results showed high survival rates for both treatments (>95.3 %) which were not significantly affected by food type. Specific growth rates and feed efficiency ratios were not significantly different between worms fed with either food. Also, there were no significant differences in the proportion of individuals in the oocyte size classes. The results shown in Table 2.3 indicate that specific growth rate, survival and food conversion ratios did not change significantly ($P > 0.5$).

Table 2.3 Survival, specific growth rate and food conversion ratio of *N. diversicolor* fed with S. auratus dry feed (SBDF) and ornamental fish dry feed (OFDF) for 65 days. Data represent mean of three tanks (SE). [Source: (Batista et al., 2003)]

Foods	Survival (%)	Specific growth rate (% d^{-1})	Feed efficiency ratio
OFDF	99.3 (0.7)	7.78 (0.03)	1.62 (0.04)
SBDF	95.3 (1.3)	7.88 (0.17)	1.89 (0.14)

Based on the studies discussed above, it can be concluded that marine aquaculture, and especially RAS based aquaculture, for specific species and phyla of marine invertebrates is now a manageable and feasible technology, and such a technology could be scaled to satisfy the requirements of commercial supplies. Furthermore, the cost effectiveness of the technologies involved should be determined in order to lay a solid foundation for competitiveness among companies which are involved in the fish and aquaculture industry. In addition, further work is required to optimize controlled environment tank culture systems, because land-based systems ultimately have the lowest risk, a high possibility for controlled production, and minor environmental effects, especially when compared to open-sea systems which are subject to the ever changing conditions of nature.

2.7.2 Different bedding materials

To provide low-cost aquaculture technologies, a range of substrate-based aquaculture systems have been studied and improved for both shellfish and fish. Researchers have tried to increase periphyton production as food source, and to provide shelter, thereby increasing the efficiency of aquaculture production (van Dam *et al.*, 2002).

Wahab *et al.* (1999) carried out a project to evaluate the impact of installing scrap bamboo (`kanchi') as a substrate for periphyton on the production and growth of the indigenous major carp calbaush, *Labeo calbasu* (Hamilton). The impact of fish grazing on the periphyton community were also investigated. The researchers used six ponds, three of which were provided with kanchi poles (700 per pond, spaced 30 cm apart). Ponds were limed and fertilized and stocked with *L. calbasu* fingerlings (mean total length =5.16 cm; mean weight = 2.10 g) at a rate of 10,000 fingerlings per ha ±1 (75 fish per pond). No significant differences in water quality between treatments were observed, although differences in phytoplankton community composition were observed. The same number of zooplankton were observed in both treatments. Although there was evidence that the periphyton was exploited by the fish, the Chlorophycae being most affected, grazing was not sufficient to cause significant reductions in total periphyton densities. Fish survival and specific growth rates (SGR) were significantly higher in ponds with substrates, where production in treatments with and without scrap bamboo substrate were 712.90 and 399.11 kg ha^{-1} respectively, over the 120-day period. In comparison with other studies, production in both treatments was low, as water temperatures (23.6 ± 32.7 °C) were less than the optimum for growth. It was concluded that it is feasible to use kanchi and other locally available materials to increase the production of some species of fish. However, there should be further evaluation of the production economics.

Keshavanath (2001) studied the effects of sugarcane bagasse as an artificial substrate for periphyton in fish ponds. An on-farm polyculture experiment was carried out, stocking catla (*Catla catla*), rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*) in an 800 m^2 earthen pond partitioned into eight 100 m^2 compartments with fine meshed nylon netting. Fifty catla and 25 each of common carp and rohu were stocked per compartment and grown for 180 days. Sugarcane bagasse bundles (length 80 cm, diameter 3.3 cm) at densities of 0, 39, 78 and 156 (or 0, 7.0, 13.7 and 28.2 kg 100 m^{-2}) were hung in two compartments each as a substrate. Supplemental feed (a 1:1mixture of groundnut cake and rice bran) was provided in one replicate of each substrate density at a rate of 5% body weight during the first half, and 3% throughout the second half of the experiment. Periphyton biomass and water quality were monitored at fortnightly and monthly intervals, respectively. There was no adverse effect of bagasse substrate on water quality, since dissolved oxygen levels were between 4 and 13 $mg.l^{-1}$ during the experiment. Minor differences in plankton density and periphyton were observed between treatments, but marked differences in fish production were recorded. Without feed and periphyton (control), total fish production of 8076 $g \cdot 100\ m^{-2}$ was obtained. Feeding alone increased yield compared to controls by 20%. Bagasse substrate alone increased yields by 38, 61 and 62% in the 39, 78 and 156 bagasse bundles per 100 m^{-2} treatments, respectively, while the combination of feeding and periphyton resulted in 45, 67 and 84% increases in yield in the 39, 78 and 156 bagasse bundles per 100 m^{-2} treatments, respectively. From regression analysis it was estimated that maximum total fish production would be achieved at bagasse densities of 117 bundles (21 kg) per 100 m^2 without feeding and 156 bundles (28 kg) per 100 m^2 with feeding. The results show that sugarcane bagasse can be effectively used as a substrate for periphyton.

These trials showed that fish production in ponds with periphyton substrates was higher than that from substrate-free controls, and the technology appears to be promising (Wahab *et al.*, 1999; Keshavanath, 2001). In addition, it can be stated that through a series of experiments, a periphyton-based polyculture technology, further referred to as "pre-

optimized periphyton-based production system", has been developed with three key Indian carp species in ponds in which the submerged substrate area and the pond surface area were the same (with a substrate/pond ratio equal to 1) (Azim *et al.*, 2001; Azim *et al.*, 2002).

2.8 Cost reduction and the enhancement of productivity

Reducing the costs of production is an essential aim for any RAS technology. The equipment which is used to carry out the individual unit processes contributes to the overall capital costs. In practice, competitive food fish production should aim to decrease capital costs in order to achieve efficiency level similar to conventional systems. Therefore, innovations are needed to combine unit operations or decrease the costs of current technologies (Andrew M. Lazur, 2003).

From the limited amount of published literature available, it is clear that the issue of worm culturing, and its relationship to production space (area), and the type of culture substrate material used has received a minimal amount of research interest. In the case of Seabait Ltd, for example, the main problem in increasing production was the limited space available. In other words, previous research has focused on horizontal ponds to culture worms and has not explored the potential benefits from intensifying the culture tanks in three dimensions; that is, as vertical multi-stack culture units. If the optimal depth for culturing worms could be defined, along with the feasibility of stacking shallow depths of growth media one above the other, then the design of culturing units could be improved and made more efficient. Also, the issue of bedding material and the possibility of using different technologies and materials other than sand to enhance growth could be an option to increase the production. To bridge this gap in the literature of polychaete worm aquaculture, this thesis reports new research that has focussed on these two main issues.

In particular, this study addresses the following research questions:

1) What is the impact of artificial substrate materials on the growth and production of cultured worms?

2) What are the effects of different sand bed depths on the growth and production of cultured worms?

This research poses two hypotheses with regards to use of artificial materials as a substrate for culturing ragworms, and the use of sand at different depths.

- Use of artificial materials

  Hypothesis one: artificial materials enhance the growth of ragworms.

- Depth

  Hypothesis two: sand depth affects the growth and production of ragworms.

The following chapter presents the methodology used to answer these research questions and test the proposed hypotheses.

Chapter 3. Materials and Methods

This chapter describes the basic materials and methods used for growth experiments with the polychaete *Nereis virens*. Specific details of the methodology of the growth experiments for artificial substrate materials and recirculating aquaculture system design can be found in Chapter 4, and specific methods used in the growth experiments on sand and the recirculating aquaculture system design are given in Chapter 5.

3.1 Source of experimental animals and husbandry

All juvenile animals used in this research had been reared under natural daylight conditions at the aquaculture farm of Seabait Ltd, Northumberland, prior to the experiments being conducted in the laboratory. Animals from single spawning events in the springs of 2007, 2008, 2009 were transferred to the laboratory 4-5 months after hatching. Details of the dates of fertilization and photoperiodic history of all animals are given in the description of each experimental method.

The culture method at the farm followed a standard protocol (pers. comm., Craig, 2007). After fertilization, the developing larvae were maintained in a hatchery for two weeks in trays of 1μm filtered seawater under controlled optimum temperature conditions (≈16°C), and under continuous illumination. The nectochaete larvae were then stocked into larger, sand-filled ponds (100m^2 with 15 cm depth), located at an open outdoor location on the site, and these were supplied with warm (16-18°C) sea water, as indicated in Figure 3.1. They were fed twice daily, morning and afternoon, to their maximum intake, with commercial aquaculture pelleted tout feed, and kept at high stocking density (3000/m^2) for two months. Then, they were thinned into growing beds at a lower density (500/m^2). They were then harvested for commercial sale between 6-12 months old (Figure 3.2)

based on size-related demand, which was generally when the animals had reached a mass of 1 - 4g. At this stage, they were collected for the majority of the experiments detailed in this thesis.

Figure 3.1. Sand filled pond (100m2) at Seabait Ltd. The ponds are supplied with warm (ca. 18°C) power station coolant seawater for the culture of the polychaaete *Nereis virens* (Photo: Seabait Ltd.)

Figure 3.2. Worms at the point of normal harvesting, between 6-12 months. Animals were then graded, sorted, packaged and sold commercially as fishing bait (Photo: Seabait Ltd.)

Once transferred to the experimental laboratory at the School of Civil Engineering and Geosciences, the animals were stored in a cold room at 5°C in order to minimize their activity. The next day, they were weighed (wet weight) and sorted into different groups according to their weight.

For the experiments investigating sand as a growth substrate, all culture tanks were filled with (1 mm) sieved sand that had been collected from Seabait Ltd., being originally from the local shore. These tanks were operated in conjunction with a biological sand filter recirculating seawater system, at a salinity of 34 to 36 ‰, and the temperature was maintained at ≈18°C. This protocol was adopted for the growth experiments described in Chapters 5 and 6.

N. virens has been described as a broad-spectrum omnivore (Fauchald, 1979). So, all animals were fed a proprietary pelleted feed of various grain sizes formulated for Seabait Ltd. Animals for all experiments detailed in Chapter 4 and 5 were fed daily in the morning with a ration of three to twelve per cent body weight per day, always up to or just above the maximum intake, and the daily rations were increased as the worms increased in weight.

3.2 Evolution of RAS with novel substrates

3.2.1 Initial batch work

Initial batch work was carried out as the first step to investigate the feasibility of culturing worms in novel types of artificial substrate packing materials by using a constructed recirculation aquaculture system (RAS).

The RAS (Figure 3.3) consisted of five polyethylene tanks measuring 0.1 m², which had different substrates as culture media; namely, white engineered polyethylene beads, black

plastic beads, engineered corrugated plastic sheets, random plastic media, floating shredded recycled plastic and sand (as control).

Figure 3.3 Initial recirculation aquaculture system

Each of these containers was connected to a single shared water sump through connecting inlet and outlet pipes as shown Figure 3.3. The water sump was used to provide aerated recycled water continuously to the culture beds. An aerator was immersed within the sump to maintain a high dissolved oxygen concentration (7 mg/L). This sump was filled initially with seawater which was then pumped to the culture containers through an aquarium pump.

The sump water was changed on a daily basis to avoid ammonia and hydrogen sulphide accumulation in the system, and to provide adequate environmental conditions for worm growth. Each of the tanks was stocked with 90 juvenile worms of the same age group prepared as described in Section 4.2.2. These worms had been fed with Coarse 23 fish feed (Skretting, Wincham Lane, Wincham, Northwich, CW9 6DF, UK) once a day at

5pm for one month. This experiment was carried out at the natural ambient temperature of the laboratory for that month (July, 2007), and the mean temperature was 18 ± 2ºC.

The outcomes which were recorded for the initial batch work were as follows:

1. Structured plastic materials and floating shredded recycled materials could not be used as culture media for marine worms because the worms were did not show stress-free activity in these particular media, which, in turn, significantly affected the daily activity of the worms. Most of the worms were seen to burrow vertically downwards until they reached the tank bottom, where they gathered together in a tight grouping, presumably as an innate response against potential predators. This response stopped them from their normal daily feeding activity. Consequently, the survival rate was significantly lower than in the control tank.
2. High density white and black plastic beads (sinking beads) can be used as culture media for marine worms because the survival rate was significantly higher, which indicates that the worms were relatively stress-free in these substrate media. Thus, it was worth investigating the potential impacts of using these two materials as substrates for culturing marine worms in future experimental work.
3. Because of the above issues, there was a significant amount of uneaten food and organic material (dead worms) in some of the culture tanks, which caused the degradation of water quality in the sump. As a result, all tanks connected to the sump were adversely affected. Thus, the basic RAS system was not effective in treating the water, which indicated the need to design a new modified system to overcome these problems in future experiments.

All of these observed outcomes were taken into account in the final batch system design.

3.2.2 Final batch work

Based on the initial observations discussed above, the recirculation aquaculture system was modified to include a protein skimmer and a large biological sand filter in order to provide recycled water of an improved quality. Also, structured plastic materials and floating shredded recycled materials were disregarded as potential culture substrates, while further investigation was required for the white and black plastic beads. For full details of the final batch work, see Section 4.2.1

Chapter 4. The Impact of artificial substrate packing materials on the growth and production of cultured worms

4.1 Introduction

The large quantities of polychaete worms harvested for commercial purposes from natural environments is regarded as being environmentally unsustainable because it depletes natural resources (Olive, 1999a; Pires *et al.*, 2012), and morphologically and ecologically changes the natural habitats (Beukema, 1995; Olive, 1999a). Over the last 15 - 20 years, the supply of wild polychaete worms appears to have been insufficient to satisfy the market needs of bait for European recreational fishing, because the European countries import most living baits from Eastern Asia (Olive, 1994; Costa *et al.*, 2006).

However, the whole expenditure for the activity of recreational fishing in the European countries has been estimated to be above € 25 billion per year (Dillon, 2004). The European Angler Alliance has determined that 8 – 10 million recreational anglers support an industry with an approximate value of € 8–10 billion (Pawson *et al.*, 2008). In Italy, for example, the number of recreational anglers is above 1 million and the recreational fishing market has been assessed in 2004 to have a turnover of € 350 million a year (FAO, 2014). Up-to-date evaluations demonstrate such an activity is expanding, for instance, in Venice Province the number of recreational fishing licences increased by 31.9% during the period 2000–2006.

Some new projects have been established as a result of the growing interest in the indoor production of fishing baits, and a profit-bearing collaboration between scientific and production sectors has developed; some of these projects have become successful commercial ventures. For example, a leading European company for the commercial

production of *Nereis virens* is Topsy Baits, with an annual production over 100 tonnes (Topsybaits, 2014). Applied research has led to the formation several commercial producer companies in different non-EU countries, e.g. China, Australia, and Korea. In Australia, for example, the main farm is Aquabait, which was set up in 1996 at Dora Creek (NSW). It chiefly produces *Diopatra aciculata*. In the Chinese province of Qidong, Jiangsu, the newly founded Qidong King Power Polychaete Aquaculture Co., Ltd., produces mainly *N. virens*. Using aquaculture techniques for the production of polychaetes as fishing baits can give many associated benefits, e.g. it reduces indiscriminate environmental damage, helps to reduce imports of foreign species, and develops new aquaculture products (Olive, 1999a).

The production of living polychaetes in controlled conditions can also help to produce healthy strains, which can be used as broodstock feed in shrimp hatcheries where the diseases caused by farmed species are prevented (Vijayan *et al.*, 2005). In Italy, rearing trials were conducted by several research groups who work with different species, such as the eunicid *Marphysa sanguinea,* the nereidids *Perinereis rullieri* and *P. cultrifera* (Prevedelli, 1994; Prevedelli and Vandini, 1997; Prevedelli and Cassai, 2001; Prevedelli and Simonini, 2003), the sabellid *Sabella spallanzanii* (Giangrande *et al.*, 2000; Pierri *et al.*, 2006), and the lumbrinerid *Lumbrineris impatiens* (Messina *et al.*, 2005). In addition to these species, worm producing companies have also concentrated on the onuphid *Diopatra neapolitana* (De Murtas *et al.*, 2003) and the common ragworm, the nereidid *Hediste diversicolor* (Tola Masala and Piergallini, 2007). Regarding *H. diversicolor*, it is extremely popular in Italy as a sea angling bait (Gambi, 1994), in France (Scaps, 2002) and along the Portuguese coasts (Costa *et al.*, 2006). The length of largest individuals can be 20 cm, despite the fact that their commercial size is around 10 cm, which corresponds to a mean fresh weigh of about 0.5 g (Gambi, 1994). In the lagoon of Venice, *H. diversicolor* is highly distributed in the salt marsh areas and specific areas along the edges of channels, where it can reach densities up to 2000 m^{-2} (Maggiore and Keppel, 2007). In the past, harvesting of “tremoline” (the local commercial name) was a promising

seasonal activity which included the activities of controlled groups of professional fishermen. Some sources have commented on the failure of this commercial activity and attributed the observed decrease of the resources to its overexploitation, and to many anthropogenic and environmental changes occurring in the lagoon since 1980 (Secco *et al.*, 2005; Bernardello *et al.*, 2006).

Consequently, many of the worms which are sold locally as baits are imported. World Trading Ltd, an important company for live bait import–export in Venice, pointed out that the majority of their products (95%) which are sold throughout Italy are imported from America Asia, Spain and France. The majority of imported products are living organisms, belonging to foreign species (i.e. *N. virens*, *Glycera dibranchiata*, *Perinereis aibuhitensis*), resulting in potential risks as invasive species and pest vectors. Consequently, there has been a review of the existing knowledge on the ecology, biology and probable use of the common ragworm (Scaps, 2002).

H. diversicolor is a semelparous and cosmopolitan species, which can play an important part in the functioning of estuarine and coastal ecosystems: it is a key food resource for many bird species and estuarine fish, and it largely influences the biogeochemical cycles of contaminants and nutrients through its bioturbation activity. *H. diversicolor* is generally considered to be a deposit-feeder that consumes organic matter and plant detritus from the sediment (Olivier *et al.*, 1995; Reise, 1979), but filter-feeding and carnivorous behaviours have also been documented (Vedel, 1994). The common ragworm shows a "bentho-pelagic life cycle" (Scaps, 2002): the pelagic larval short period is completely spent within the mother burrow, which, in turn, delimits the larval dispersion (Bartels-Hardege and Zeeck, 1990).

The major physiological features, such as capability to cope with broad environmental fluctuations, e.g. salinity, water temperature, dissolved oxygen and sediment grain-size

(Neuhoff, 1979; Kristensen, 1983a; Kristensen, 1983b), keeps growth rates at high values (Fidalgo e Costa *et al.*, 2000), and indicates that *H. diversicolor* would be a suitable species for farming. An intensive worm aquaculture system must be efficient to ensure that the costs of production are low, so that it will be lower than the market price. In this regard, if an active breeding protocol is to be implemented, the first step is to determine the suitable types of stocking densities and food, as these are among the most influencing exogenous parameters that must be controlled for the optimization of biomass production (Prevedelli, 1994; Prevedelli and Vandini, 1997; Olive, 1999a; Safarik *et al.*, 2006).

Some studies have determined the negative impacts of high intraspecific density on nereidid polychaetes, that often demonstrate an aggressive behaviour related to the defence of their burrows (Scaps, 1995; Bridges *et al.*, 1996). Changes in the gallery shape can be caused by high density (i.e. fewer sub-surface connections). It also changes the time the time spent in various activities such as locomotion and feeding (Miron *et al.*, 1991a; Miron *et al.*, 1991b). Commonly, high intraspecific density causes a reduction in growth, depletion in feeding and reproduction rates and increase of migration and mortality (Wilson Jr, 1983; Miller and Jumars, 1986; Zajac, 1986; Bridges *et al.*, 1996; Safarik *et al.*, 2006). However, the impacts of different kinds of food and of intraspecific stocking density on growth and survival performances in European populations of *H. diversicolor* have been under-researched (Nielsen *et al.*, 1995; Oliver *et al.*, 1996; Fidalgo e Costa *et al.*, 2000; Batista *et al.*, 2003).

It is well known that the quality and quantity of available food can affect reproduction and growth in marine invertebrates (Minor and Scheibling, 1997; Prevedelli and Vandini, 1997; Prevedelli and Simonini, 2000). The available data for *H. diversicolor* shows that the optimization of feeding rate would yield an early sexual maturation (Batista *et al.*, 2003), causing the production of fast growing organisms breeding at smaller size. Such a balance between size and initial growth rate at maturity can be found in many organisms

(Sibly and Atkinson, 1994; Atkinson and Sibly, 1997), including nereidid polychaetes with mixed or variable age at reproduction (Desrosiers *et al.*, 1994; Olive *et al.*, 1997; Last and Olive, 1999; Prevedelli and Cassai, 2001). High intraspecific density might also influence the feeding ability of these benthic organisms, and negative effects have been observed on their reproductive capacity in relation to this condition (Wilson Jr, 1983; Miller and Jumars, 1986; Zajac, 1986).

The objective of this experiment is to investigate the effects of different artificial substrates on the growth, stocking densities, survival and reproductive performance of *Nereis virens* in order to identify the best laboratory conditions for the production of market-size worms. The results will be helpful to estimate the potential for commercial worm biomass production, and evaluate the physiological suitability of this species in intensified commercial production systems.

4.2 Materials and methods

4.2.1 Culture system design

A recirculating culture system was designed with eight identical rectangular (6L) polyethylene, juvenile tanks (0.05 m^2) arranged in one level, all of which were connected to a single recirculation system (Figure 4.1).

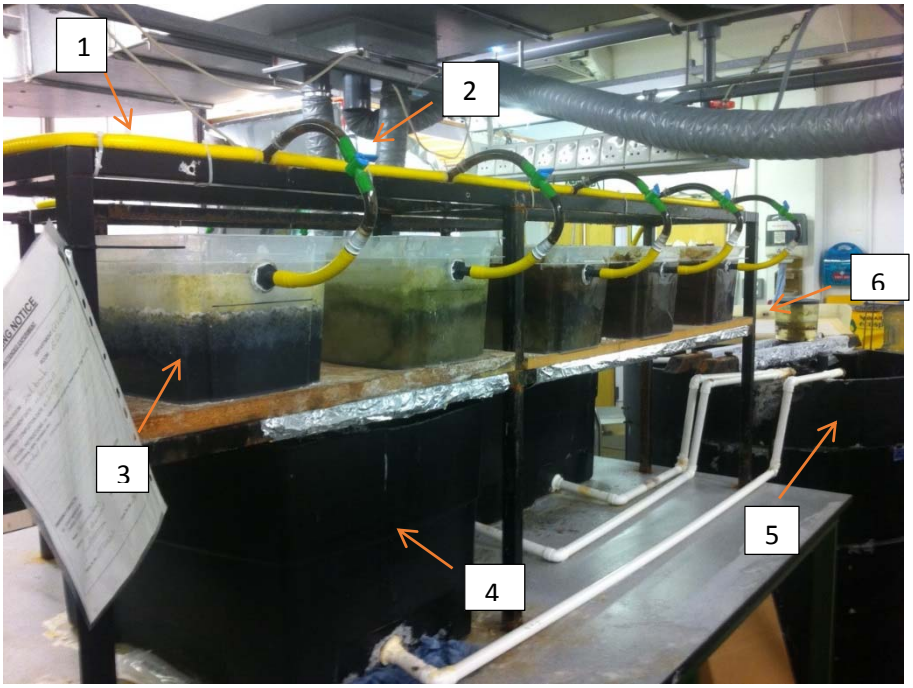


Figure 4.1 Recirculating aquaculture system used for culturing worms.

1) Inflow PVC hose pipe 2) Valve 3) Culture tank 4) Sand filter 5) Shared sump 6) Protein skimmer

Worms were reared under natural photo period (February to June in 2011; 54º 98' N, 1º 62' W) at ambient temperature, with a mean ± SD temperature of 17.5 ± 3.2 ºC. Salinity was maintained at 33‰. Water entered each tank through a PVC hosepipe mounted at one end, and exited the tank through an outlet pipe at the other end. The opening of the outlet pipe of each tank was covered with a 300 µm mesh screen to prevent the escape of worms to the sand filter, and reduce feed losses. The water depth was set at 2 cm above the bed substrate, which was placed at 10 cm deep throughout the tank. Three different artificial packing materials, white polyethylene beads, recycled black polyethylene beads, corrugated polypropylene sheets (Correx), and sand were used as the substrates. The water flow was regulated through manual valves, fitted to the PVC hose at the inlet of each tank, and maintained at approximately 27 L/hour to each tank. Water leaving the tanks was passed through a 100 L sand filter, before flowing back to the shared sump (500 L) by gravity, from where it was pumped (Iwaki MX 70 VM 13 Polypropylene headed magnetically coupled pump, Unit 2 Monkmoor Industrial Estate, Monkmoor Road, Shrewsbury, SY2 5TX, UK) to the protein skimmer (TMC V2 Skim Protein

Skimmer 1500, Seapets Online Centre, 9 Westside, Colchester, Essex, CO3 8PH, UK) and distributed back to the tanks. Fresh tap water was added to the system at a rate of approximately 6% of the total system volume per day, to compensate for evaporation.

4.2.2 Ragworm and feeding

Juveniles of *Neries Virens* (wet weight 0.53 ± 0.2 g, mean ± SD) were used and a total biomass of 17.63 ± 0.5 g was stocked into each tank (33 worms per bed), four treatment groups were allocated randomly to the tanks, with two duplicate tanks per treatment. These juveniles were collected on 22nd of January 2011 from Seabait aquaculture facility located in Lynemouth, Northumberland, Newcastle upon Tyne.

After a starvation period of 24 h, all worms were fed a 34% protein food (Coarse Fish 23 from Skretting, Wincham Lane, Wincham, Northwich, CW9 6DF, UK) by hand once daily at 17:00 h, and the amount of feed added to each tank was 3% of initial wet weight and adjusted as necessary in an attempt to keep a slight excess of feed on the top of the substrate layer.

4.2.3 System performance parameters

Dissolved oxygen (DO) concentration, pH, water temperature and salinity were measured daily between 9:00 and 10:00 h from the shared sump using a handheld (Multi-Probe System) unite YSI 556 MPS (Yellow Springs Instrument Company, Yellow Springs, Ohio, USA).

4.2.4 Harvest

Ragworms were cultured for 107 days. On the day of harvest, 14 June 2011, the water levels in each culture tank were reduced to approximately 0.5 cm above the top of

substrate layer. Then the substrate was removed from each tank and placed in a PVC or stainless steel tray for hand sorting, however, the control media (sand) was sieved with a 0.6 mm mesh sieve to remove worms. The collected worms were then placed in clean seawater and batch weighed after wiping on tissue paper to remove most of the surface retained water Figure (4.2).

After harvesting and weighing, the stocking density, specific growth rate, food conversion ratio, feeding efficiency and total biomass gain (g/ tank) were calculated for each tank.

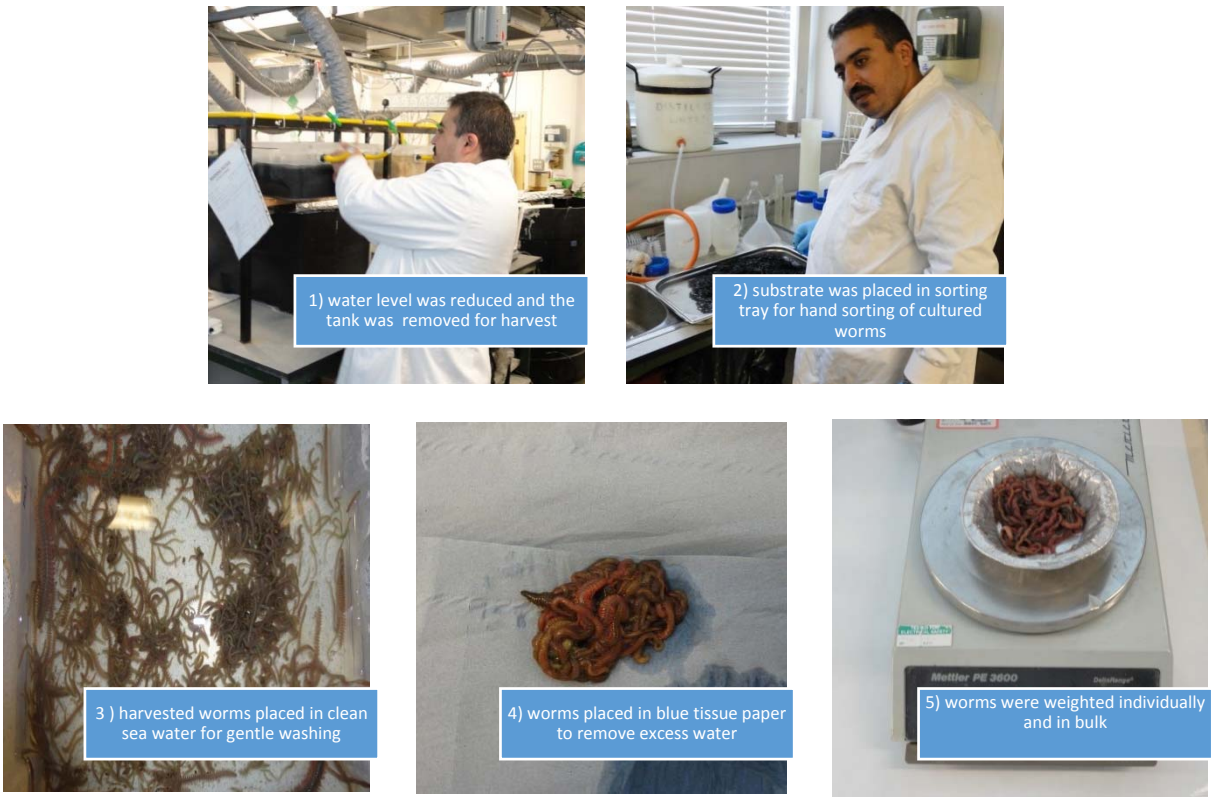


Figure 4.2 Flow chart for worms harvesting process

4.2.5 Statistical analyses

Mean values of final total biomass weight, specific growth rate, feeding efficiency and stocking density were compared by one-way ANOVA, with type of substrate used as the variable factor. Whenever a main effect was significant, a Fisher's multiple-comparisons

test for means ($p < 0.05$) was used to assess whether significant differences had occurred. All statistical analyses were carried out using the ANOVA procedure of Minitab for windows version (16). Microsoft Excel 2010 was also used to plot the graphs and to perform mass and productivity calculations.

4.3 Results

The effects of different types of bed substrate material on the numbers, total and average biomass weight, feeding and mortality rate of ragworm are presented in Table 4.1. Initial numbers of ragworm in each treatment was 33 worms, their average worm weight ranged between 0.53 ± 0.01 to 0.54 ± 0.04 g, and total initial worm biomass was 17.6 ± 0.33 g, 17.4 ± 1.1 g, 17.7 ± 0.54 g and 17.8 ± 0.16 g in sand, white polyethylene beads, recycled black polyethylene beads and corrugated polypropylene sheet, respectively. During the experiment (107 day), as a result of feeding worms, total weight of worms increased in sand, white polyethylene beads and recycled black polyethylene beads, whereas, in the corrugated polypropylene sheets, all worms died within the first two weeks.

For example, the total weight of worms after 107 day were 86.5 ± 3.5 g , 22.7 ± 1.4 g and 24.8 ± 3.7 g in sand, white polyethylene beads and recycled black polyethylene beads cultures, respectively. The differences in the total weight of worms between the start of experiment and Harvest 1 were only statistically significant in the sand bed culture (one way-ANOVA-Fisher's test, $p < 0.001$), and the Fisher's test shows that differences in the total weight of worms between other type materials at the start and Harvest 1 was not statistically significant (Table 4.1).

The stocking density of ragworm on the stocking day 1 ranged between 0.352 ± 0.007 kg biomass m^{-2} to 0.356 ± 0.003 kg biomass m^{-2} (Figure 4.3), at the end of the experiment, the stocking density of ragworm had increased sharply in the sand bed culture to 1.73 ±

0.07 kg biomass.m^{-2}, while, the stocking density increased only slightly in the white polyethylene bead and recycled black polyethylene bead cultures to 0.454 ± 0.03 kg biomass.m^{-2} and 0.50 ± 0.08 kg biomass.m^{-2}, respectively (Figure 4.3).

The differences in the stocking density between white polyethylene beads culture and recycled black polyethylene beads culture are not statistically significant (Table 4.1). However, differences in ragworm weight between sand bed culture and other beds culture is statistically significant, this also being the case for the difference between ragworm weight at start and end of experiment in the sand bed culture (one way-ANOVA-Fisher's test, $p < 0.001$).

Table 4.1 Comparison of the numbers, total weight, average weight, feeding and mortality of ragworm in different bed materials related to harvesting day. Error bars: ± 1 standard deviation (n=2).

Bed material type	At stocking (1day)			At Harvest (107day)		
	Numbers	Total biomass weight (g)	Average weight (g)	Total biomass weight (g)	Feeding (g/day)	Mortality (%)
Sand	33	17.6±0.33 a	0.53±0.01 a	86.5±3.5 a	0.88±0.02 a	Not measured [1]
White Polyethylene beads	33	17.4±1.1 a	0.53±0.03 a	22.7±1.4 b	0.87±0.05 a	Not measured [1]
Recycled black polyethylene beads	33	17.7±0.54 a	0.54±0.02 a	24.8±3.7 b	0.89±0.03 a	Not measured [1]
Corrugated polypropylene sheets	33	17.8±0.16 a	0.54±0.04 a	0.00±0.00	0.89±0.01 a	100%[2]

[1] as survival was extremely high, mortality was not measured.

[2] in this treatment after two weeks period, all of worms were died.

Note: Values (mean ± S.D) in a column with the same code letter are not significantly different ($p > 0.05$)

Table 4.2 The ANOVA table for the main factor (experiment duration and bed depth) on experimental results.

Treatment	Source of variation	Degrees of freedom	Sum of squares	Mean squares	P value
Stocking density	**Between groups: Duration**	2	0.605	0.605	0.12
	Bed material type	3	4.948	1.649	0.001
Specific growth rate	**Bed material type**	3	3.984	1.328	0.001
Feeding efficiency	**Bed material type**	3	10800.20	3600.07	0.001
Weight gain	**Bed material type**	3	3176.6	1009.51	0.001
Total biomass weight	**Between groups: Duration**	2	1513	1513	0.12
	Bed material type	3	12369.89	4123	0.001

The effect of culture bed substrate material type on the specific growth rate of ragworm is shown in Figure 4.4. It can be seen that the specific growth rate in sand bed culture was higher compared to the white polyethylene bead, the recycled black polyethylene bead and the corrugated polypropylene sheet cultures. For example the rate was 1.49 ± 0.06 % day^{-1} in sand bed culture, whereas these rates were 0.25 ± 0.001 % day^{-1} and 0.31 ± 0.11 % day^{-1} in the white polyethylene bead, the recycled black polyethylene bead cultures, respectively. The differences in specific growth rate between the white polyethylene bead and the recycled black polyethylene bead cultures are not a statistically significant (Figure 4.4). However, the greater specific growth rate in the sand bed culture, compared to the other cultures, is statistically significant (one way-ANOVA-Fisher's test, $p < 0.001$).

The daily increase in average total biomass gain of ragworm in sand bed culture was 0.64 ± 0.04 g /day (Figure 4.5). As a result of feeding, the total biomass gain increased through the experiment reaching a maximum value of up 68.9 ± 0.39 g /day at the end of the experiment. However, the average total biomass gain in the white polyethylene beads and the recycled black polyethylene beads was very small. The difference between average biomass gain in the sand bed and other bed material types is statistically significant (one way-ANOVA-Fisher's test, $p < 0.001$), but the difference between the average biomass gain in the white polyethylene bead, and the recycled black polyethylene bead culture beds, is not a statistically significant (Table 4.1).

The effects of culture bed packing materials type on the feed efficiency of ragworm are illustrated in Figure 4.6. It can be seen that the feed efficiency in sand bed culture was much higher than the white polyethylene bead, the recycled black polyethylene bead and the corrugated polypropylene sheet cultures. For example this rate was 73.36 ± 5.5 % in sand bed culture, whereas these percentages were 5.66 ± 0.03 % and 7.43 ± 0.3 % in the white polyethylene bead and recycled black polyethylene bead cultures, respectively. The differences in feed efficiency between the white polyethylene bead, the recycled black polyethylene bead and the corrugated polypropylene sheet cultures were not a statistically significant (Table 4.1). However, the feed efficiency in the sand bed culture is significantly different from that in the other beds (one way-ANOVA-Fisher's test, $\boldsymbol{p} <0.001$).

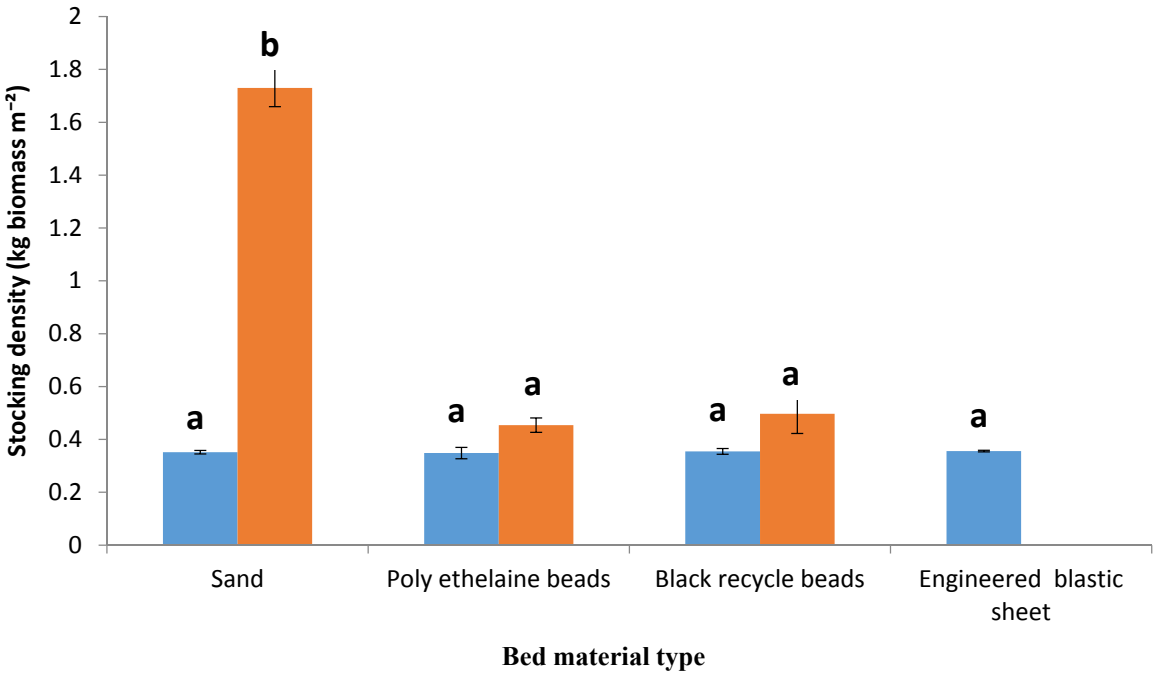


Figure 4.3 Average stocking density of ragworm at day 1 (blue) compared to stocking density at Harvest 1, day 107 (red). Error bars: ± 1 standard deviation (n=2).

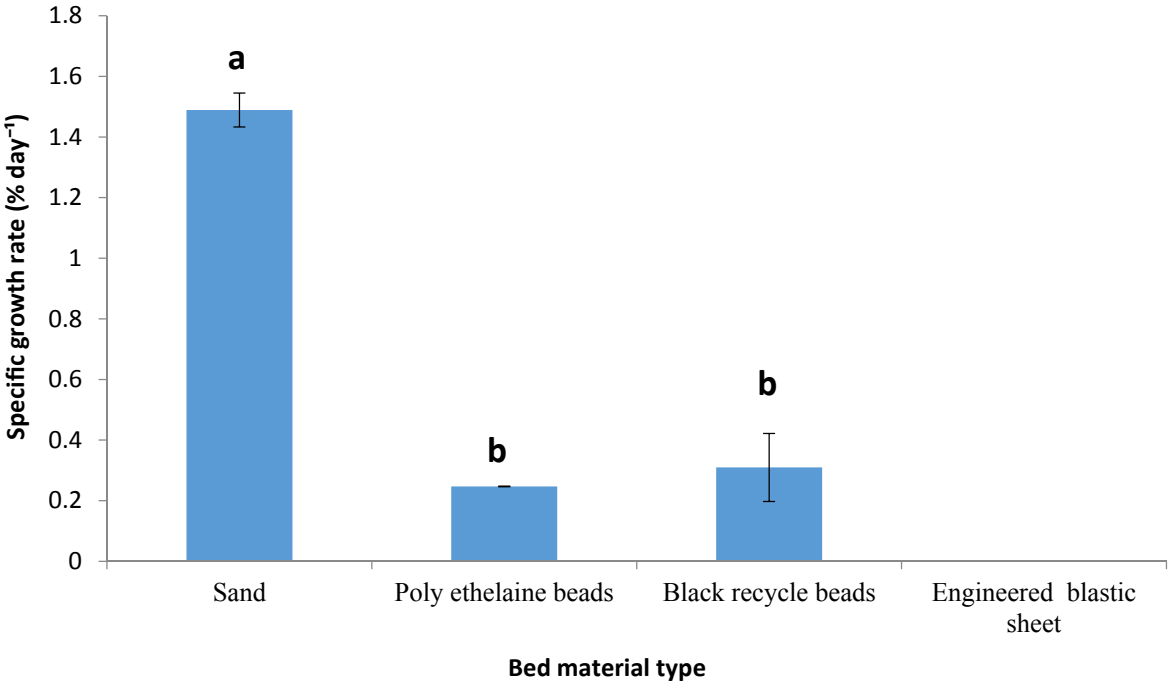


Figure 4.4 Average specific growth rate of ragworm over 107 days of growth. Error bars: ± 1 standard deviation (n=2).

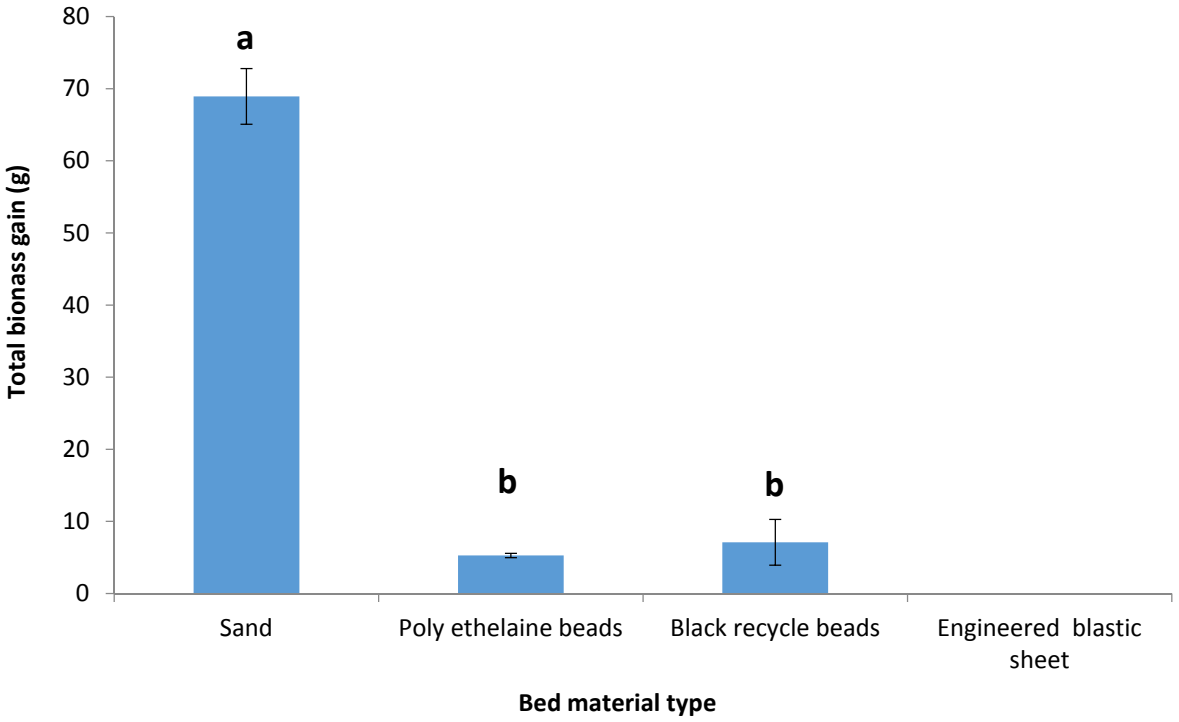


Figure 4.5 Average total biomass gain of ragworm in culture beds over 107 days of growth. Error bars: ± 1 standard deviation (n=2).

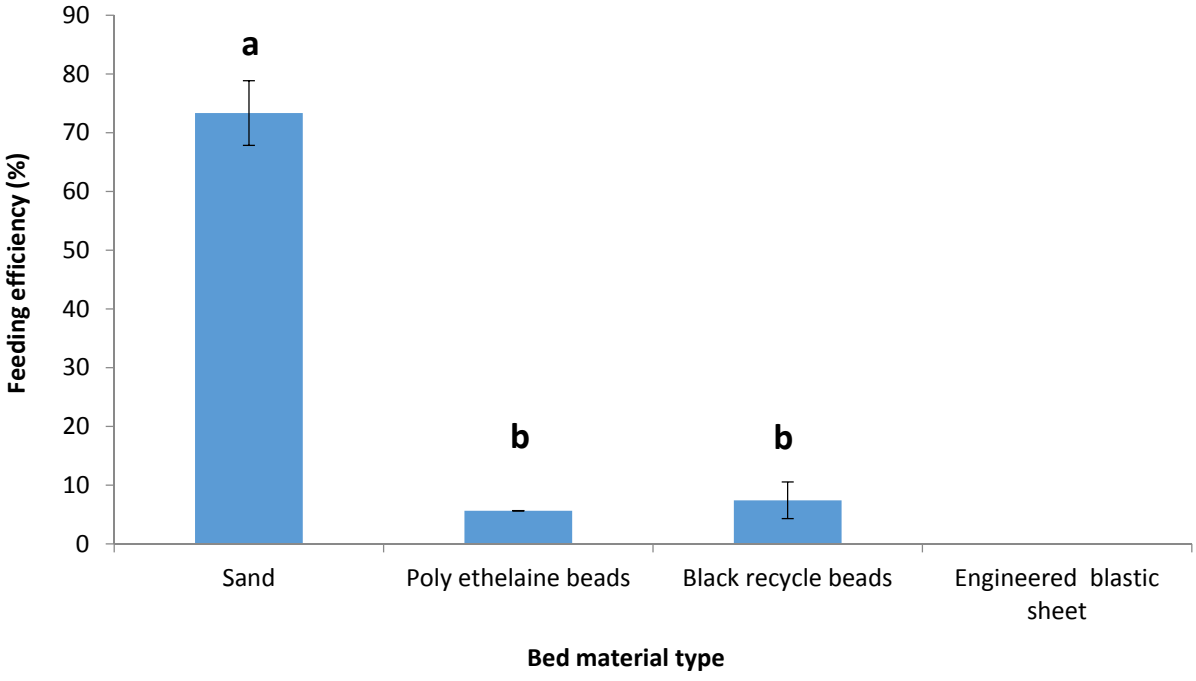


Figure 4.6 Average feed efficiency of ragworm. Error bars: ± 1 standard deviation (n=2).

4.4 Discussion

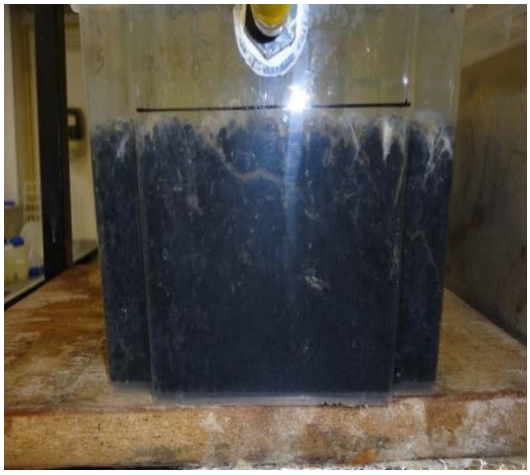
In this experiment, artificial materials, such as the white polyethylene beads, recycled black polyethylene beads and corrugated polypropylene sheets, were used in an attempt to intensify the production of ragworm, while sand was used as a control to compare with the ragworm productivity in the artificial substrate materials. It is clear that the culture bed substrate material type had a great influence on productivity of ragworm. This could be due to a change in the daily growth behaviour of the ragworm, or possibly unidentified toxic chemicals within the plastic materials. The productivity of ragworm in the sand bed culture was high, with high total biomass gain, stocking density, total biomass weight and feed efficiency, because the sand bed culture provides better conditions for normal function and behaviour, such as building the burrow, food searching outside the burrow, seizing of food particles, and maintenance scraping of burrow wall by coating it with a layer of mucus.

The ragworm productivity in the white polyethylene beads culture and recycled black polyethylene beads culture was very low compared to sand bed culture. From this experiment, it is clear that the artificial materials selected in this investigation not suitable for ragworm productivity because they gave only low productivity of ragworm in the white polyethylene bead and recycled black polyethylene bead cultures, and gave no productivity at all in the corrugated polypropylene sheet culture. The particle diameter and shape of the plastic beads may have been the main negative effect influencing productivity. The particle diameters of white polyethylene beads and recycled black polyethylene beads were very large compared to the sand particles (Figure 4.7). This prevented the worm from constructing burrows, and caused them to expend substantial energy and time coating and binding the large particles together in attempt to maintain the structure of a burrow. Moreover, the daily behaviour of the ragworm changed considerably, worms staying in the burrow for longer periods, reducing the search for food outside the burrow, and showing less seizing of food particles from the surface of culture bed, due to the collapse and closing of the burrow after the worm had left the

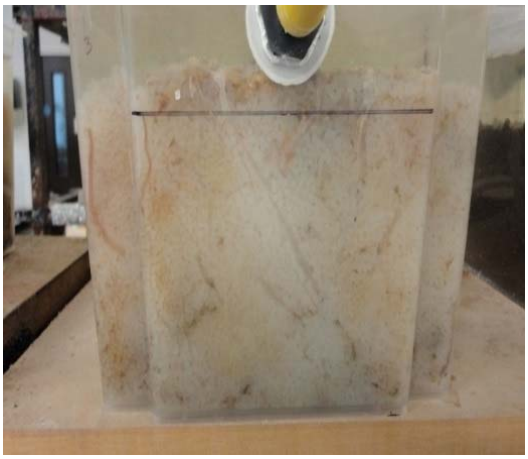
burrow entrance. Furthermore, the shape and size of particles did not help the ragworm to seize food particles from the surface of culture bed because food could slide down between particles and disappear from the surface layer.

In the corrugated polypropylene sheet culture, all ragworm died within the first two weeks. This could be attributed to the shape and structure of the corrugated polypropylene sheets which did not support the ragworm internally within the corrugation, consequently, their growth and daily activity was adversely affected to the extent that they all died (Figure 4.7).

Based on this experiment, it was concluded that the artificial plastic bead and sheet materials tested in this study did not provide an acceptable substrate (alternative to sand) for the growth of ragworm.



Black recycled polyethylene bead culture



White polyethylene bead culture

Corrugated polypropylene sheet culture

Figure 4.7 Different artificial plastic materials used in the culture bed experiment

Chapter 5. The Effect of Substrate Depth on Culturing and Growth of Polychaeta (*Nereis virens*)

5.1 Introduction:

Based on the previous chapters, it is clear that Polychaeta have an important economic value. There is, naturally, a considerable commercial interest in increasing supplies of farmed bait worms for the retail trade. Currently farmed bait mainly comprise the king ragworm *Nereis virens*. The environmental benefits that may be gained from increased bait farming and a reduction in bait digging activity are considerable. Many anglers state that they would prefer to purchase cultured bait rather than dig their own, if supplies were of high quality, reliably available, and reduced the environmental impact of angling activity. For that reason worm farmers are trying to produce *Nereis virens* more intensively. However, when worm farms such as Seabait ltd and Topsy Baits have reached maximum ragworm production with their existing ponds, the only option is to source additional space to build new ponds for the culture of ragworms by traditional methods. As a possible solution to this issue, a multi-stack culture system has been proposed whereby a vertical multi-layer system of stacked beds could be employed for culturing ragworms with a much lower land requirement.

Recirculating Aquaculture Systems (RAS) are one of the sustainable methods for the intensive rearing of *Nereis virens*. While the costs associated with constructing a RAS are typically higher than either pond or cage culture, it is important to investigate new developments which may make RAS more economically feasible.

For large scale production of *Nereis virens,* sand is normally used as the substrate material. But there has been no clear information about the minimum depth required to optimize productivity and make greater profit. *Nereis virens* normally burrows down to 45 cm, with larger individuals burrowing deeper (Pettibone, 1963; Last, 2003) and cultured *Nereis virens* can grow artificially using 15 cm depth without causing any

detrimental effects on growth. In different laboratories, researchers have used different depths of substrate by chance, rather than intentionally, e.g. Miron *et al*. (1991) and Deschenes *et al.* (2003) used 30 cm depth, Brown *et al*. (2011) used 29 cm, whilst Last (2003) used 21 cm, all these systems worked without any adverse effects being reported. However to date no systematic investigation has been conducted on sand depth as the major variable.

Stocking density and surface area are two other factors of importance for the production of *Nereis virens* in Recirculating Aquaculture Systems. Cultures that have the same depth and higher surface area of culture for the same number of worms will reduce the stocking density by increasing available bed area for each worm. Furthermore, increases in bed culture depth resulted in increased available space for each worm, which led to an increase in the size of each worm. The present study was carried out to investigate the effect of culture bed depth on the production of *Nereise virens.*

5.2 Experiment 1: Effect of substrate depth on the culture and growth of *Nereis virens*

5.2.1 Materials and Methods

5.2.1.1 Collection of worms

Four months old, hatchery-reared juveniles *Nereis virens* were collected on the 20th of May 2008 from Seabait Ltd, Lynemouth, Northumberland, Newcastle upon Tyne.

5.2.1.2 Ragworm and rearing conditions

A group of ragworm (*Nereis virens*) averaging a weight of 56 g was stocked into 0.1 m² polyethylene tanks with different bed depth: 7, 10 and 15cm. All cultures were run in triplicate and 450 individuals were needed, 50 individuals in each tank. The experiment lasted for 180 days during which time mean temperature was 18 ± 2ºC, salinity varied between 31‰ and 35‰, and pH was 8.0 ± 0.2. A closed flow through recirculation system

was used (Figure 1). The flow rate through each tank was 54 l/h to achieve 432 exchanges per day. This water exchange was in excess of that needed to maintain the dissolved oxygen level at 7 ± 0.3 mg/l under all tested condition. The worms were fed with dry commercial feed (Coarse Fish 23 from Skretting, Wincham Lane, Wincham, Northwich, CW9 6DF) 6 days per week. Pellets were supplied in the evening by manual feeding. Feed ration was changed with time starting from 3% of initial weight of worm per day and finishing with 7% of the initial weight of total biomass in each tank (Table 5.1).

Table 5.1 The feed percentage in different bed depths related to culture periods

Bed depth (cm)	1st week	2ed week	3rd - 6th week	7th - 9th week	10th-13th week
7	3%	4%	5%	6%	7%
10	3%	4%	5%	6%	7%
15	3%	4%	5%	6%	7%

Figure 5.1 Recirculation aquaculture system used for culturing worms

5.2.1.3 Sampling

Worms were measured for growth (wet weight) at day 0, 90 and 180 of culture. Total initial (day 1) sample size for each stocked population was 150,150 and 150 individual for bed depth of 7, 10 and 15 cm.

5.2.1.4 Definition of growth Parameters

Growth was assessed from the average wet weights recorded on day 0, 90 and 180. The corresponding stocking densities (A) were also obtained. Worm biomass produced per unit time, and specific growth rate (B and C), were also calculated at the end of each culture period.

$$\mathrm{SD} = \frac{(N * W_t)}{S} \qquad \text{(A)}$$

Where, SD = stocking density (kg biomass/m²); *N* = number of individuals; W_t = average weight at sampling day (g); *S* = surface area (m^2).

$$\mathrm{AWG} = \frac{W_t - W_0}{(N * T)} \qquad \text{(B)}$$

Where, AWG = absolute weight gain (g $worm^{-1}$ day^{-1}); W_t = worm biomass at final day (day 180) (g); W_0 = worm biomass at initial sampling day (day 0) (g); *N* = number of individuals; T = duration of the experiment (days).The specific growth rate (μ, d^{-1}) of *Neries Vireins* was calculated according to Jørgensen (1990) by using the equation:

$$\mu = \ln\left(\frac{W_t}{W_0}\right) t^{-1} \qquad \mathbf{(C)}$$

Where W_0 and W_t, are mean body mass (wet weight) of the polychaetes on Day 0 and Day t respectively.

The feed efficiency was also calculated (D):

$$\text{FE} = {}^{1}/_{FCR} \qquad \text{(D)}$$

Where:

$$FCR = \frac{Total\ feed\ given(g)}{Biomass\ gain\ (g)}$$

$$\text{Biomass gain (g)} = \text{Final biomass (g)} - \text{Initial biomass(g)}$$

Where: FE = feed efficiency; FCR = Feed conversion ratio

5.2.1.5 Statistical analysis

The data was tested for normality of homogeneity variance to confirm compliance with the assumptions of analysis of variance (ANOVA). The data were statistically analysed using Minitab for Windows (Version 16). Significant effects of the three tested bed depths on the mean worm weight, and worm weight gain were evaluated through the use of one-way ANOVA. The Fisher's multiple-comparisons test for means ($p < 0.05$) was used to assess were significant differences occurred. Microsoft Excel 2010 was also used to plot the graphs and to obtain the required calculations.

5.2.2 Results

Comparison of the numbers, total and average biomass weight, feeding and mortality rate of ragworm in culture beds, in relation to harvesting day (Experiment 1) are presented in Table 5.2. Initial numbers of ragworm in each culture bed was 50 worms, the average worm weight within different culture beds ranged between 1.13 ± 0.03 to 1.15 ± 0.01g, and total worm biomass in each culture bed was 56.49 g, 56.89g and 56.48g in 7 cm, 10 and 15 cm beds, respectively. Within the first two days of the experiment, 5, 9, and 16 worms died in the 7 cm, 10 cm and 15 cm beds, respectively. This represents 10.7 ± 4.6%, 17.3 ± 5% and 31.3 ± 1.2% of initial total worm number.

During the first part of experiment (first 90 days) as a result of feeding at 3% mass feed per mass worm per day (Table 5.1), average weight of worms increased substantially in all beds, with differences in average weight of worms between the start of the experiment (day one) and Harvest1 (day 90), being statistically significant (one way-ANOVA-Fisher's test, $p < 0.001$). Moreover, Fisher's test showed that differences in worm average weight between 7 cm and 10 cm beds at Harvest 1 (day 90) was not statistically significant, but average weight in 15 cm bed was significantly different from the 7 cm and 10 cm beds (one way-ANOVA-Fisher's test, $p = 0.002 < 0.05$).

The total weights of worm in each bed depth at Harvest 1 (day 90) were higher in comparison with initial value at day 0 (one way-ANOVA-Fisher's test, $p < 0.001$). For example, at day 90 the total weight of worms were 291.66 ± 10.2g, 282.5 ± 13.6 g and 286.79 ± 47 g for 7 cm, 10 cm and 15 cm bed treatments, respectively, but the differences in total worm weights between beds at day 90 was not statistically significant (one way-ANOVA -Fisher's test, $p = 0.983 > 0.05$).

The second part of experiment was run for a further 90 days until Harvest 2; the total experimental duration was 180 days. 2 worms died in the 7 cm bed and 1 in 10 cm bed. The average weight of worms increased in three different beds, compared to Harvest 1, and the differences in average worm weight between Harvest 1 and Harvest 2 were found to be statistically significant (one way-ANOVA-Fisher's test, $p < 0.001$). Moreover, the ANOVA test (Table 5.3) shows that the average weight of worms in the 7 cm and 10 cm beds at Harvest 2 (day 180) were not significantly different, but average weight in 15 cm bed was significantly higher than the 7 cm and 10 cm beds (one way-ANOVA-Fisher's test, $p = 0.028$). The total weights of worm in each bed depth at Harvest 2 were all significantly higher than at Harvest 1 (Table 5.2) (one way-ANOVA-Fisher's test, $p < 0.001$).

The average gain in ragworm biomass in the different culture beds was related to harvesting day (Figure 5.2). Specifically, it can be seen that the first period of experiment had a greater influence on biomass gains, which were 234 ± 10g, 240.5 ± 16 g and 231 ± 50 g, compared to gains of 66.8 ± 17 g, 69.3 ± 17g and 89.7 ± 53 g in the second period of experiment (Harvest 2).

Table 5.2 Comparison of the numbers, total mass weight, average mass weight, feeding and mortality of ragworm in culture beds related to harvest day (Experiment 1). Error bars: ± 1 standard deviation (n = 3).

Bed depth (cm)	At Stocking (1 days)			At Harvest 1 (90 days)					At Harvest 2 (180 days)				
	Numbers	Total biomass weight (g)	Average weight (g)	Numbers	Total biomass weight (g)	Average weight (g)	Feeding (g/day)	Mortality (%)	Numbers	Total biomass weight (g)	Average weight (g)	Feeding (g/day)	Mortality (%)
7	50	56.49±1.74^{a}	1.13±0.03^{a}	45±2	291.66±10.2^{b}	6.55±0.51^{b}	2.83±0.03^{a}	10.7±4.6	43±1	360.98±11^{cd}	8.46±0.3 ^{c}	20.42±0.7^{b}	4.3±3.8
10	50	56.89±3.25^{a}	1.14±0.07^{a}	41±3	282.50±13.6^{b}	6.84±0.18^{b}	2.67±0.1 ^{a}	17.3±5	40±3	349.30±6 ^{c}	8.70±0.8 ^{c}	19.78±1 ^{b}	2.5±2.6
15	50	56.48±3.01^{a}	1.15±0.01^{a}	34±1	286.79±47 ^{b}	8.37±1.5 ^{c}	2.99±0.08^{a}	31.3±1.2	34±1	376.50±12 ^{d}	10.97±0.4^{d}	20.08±3.3^{b}	0.0±0

Note: Values (mean ± S.D) in column with the same superscripts are not significantly different (P>0.05)

The difference in average biomass gain between culture beds is not statistically significant (one way-ANOVA-Fisher's test, $p = 0.983$). However, weight gains for the two experimental periods are statistically significant (one way-ANOVA-Fisher's test, $p < 0.001$). Although the average total mass of feed given to the ragworms, in the second period of the experiment was roughly 10 times higher than during the first part of experiment (Table 5.2), the production of ragworm biomass in first 90 days was 4 times greater than the second period of 90 days.

Table 5.3 ANOVA for the main factors (experiment duration and bed depth) for Experiment 1.

Treatment	Source of variation	Degrees of freedom	Sum of squares	Mean squares	P value
Gain weight	Between groups: Duration	1	115001	115001	0.000
	Bed depth	2	298	149	0.983
Feeding efficiency	Between groups: Duration	1	0.002	0.002	0.000
	Bed depth	2	35	18	0.992
Specific growth rate	Between groups: Duration	1	0.88	0.88	0.000
	Bed depth	2	0.0011	0.0005	0.991
Absolute worm gain	Between groups: Duration	1	0.009	0.009	0.000
	Bed depth	2	0.0006	0.0003	0.651
Stocking density	Between groups: Duration	1	47.2	23.6	0.000
	Bed depth	2	0.08	0.04	0.835
Total biomass weight	Between groups: Duration	1	447341	218671	0.000
	Bed depth	2	769	385	0.835
Average Individual weight	Between groups: Duration	1	293	147	0.000
	Bed depth	2	16.72	8.36	0.028
feeding	Between groups: Duration	1	1334	1334	0.000
	Bed depth	2	0.5	0.3	0.997

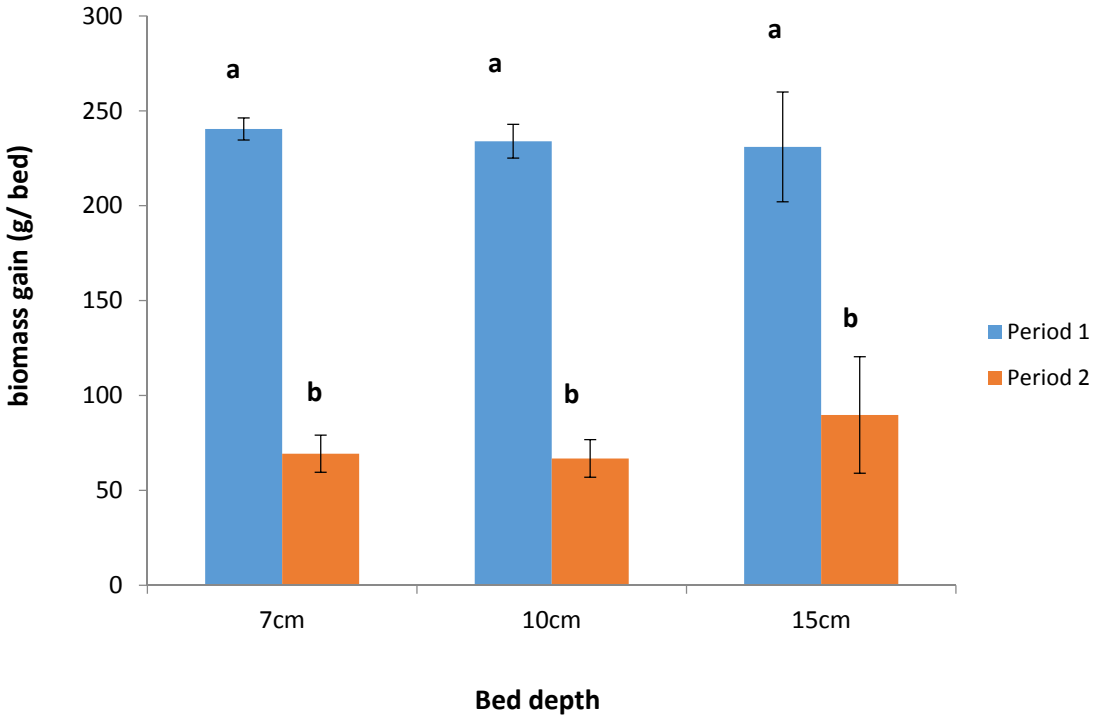


Figure 5.2 The average gain (± S.E) of ragworm biomass in culture beds related to culture period (n=3).Period1 end at day 90, Period 2 end at day 180.

The effects of culture bed depth and experiment duration on the feeding efficiency of ragworm are illustrated in Figure 5.3. It can be seen that the first period of the experiment produced higher feeding efficiency compared to second period, and the differences in feeding efficiency between culture beds for the same period are not statistically significant (Table 5.3). However, these differences between two experimental periods are statistically significant (one way-ANOVA-Fisher's test, $p < 0.001$).

The daily increase in ragworm weight was approximately 2% per day in the first growth period, while in the second growth period weight increase was only 0.25 % per day (Figure 5.4). Furthermore, the absolute ragworm gain showed the same pattern.

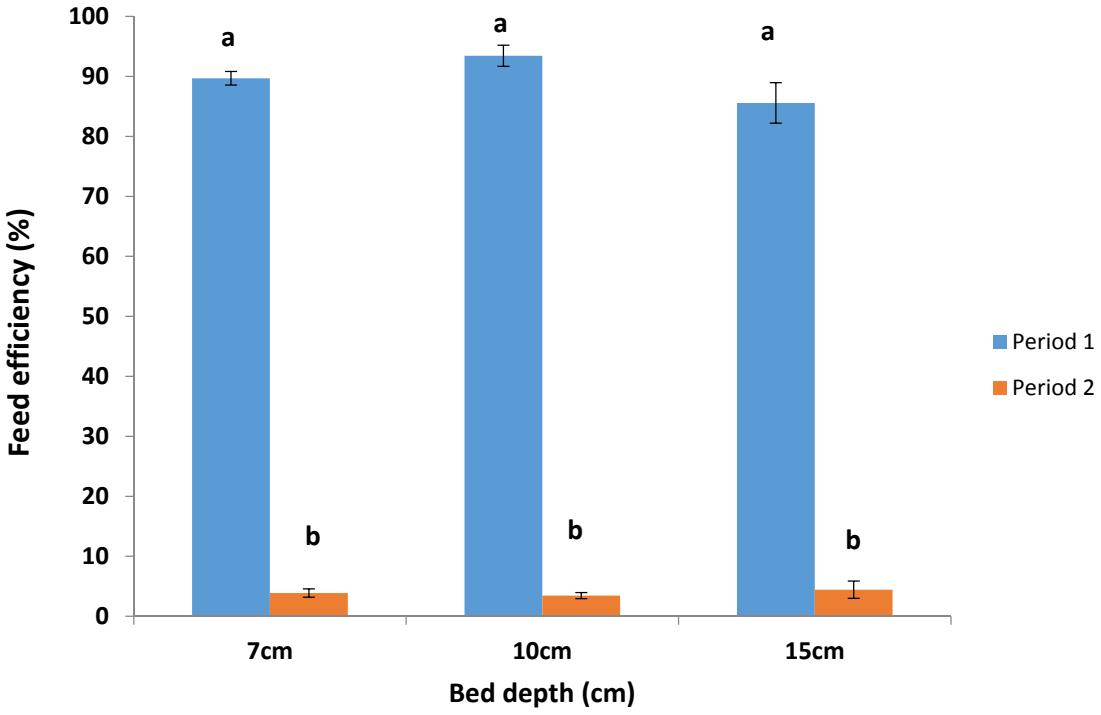


Figure 5.3 Average feed efficiency (± S.E.) of ragworm related to culture period (n=3). Period 1 (0-90) day, Period 2 (91- 180) day.

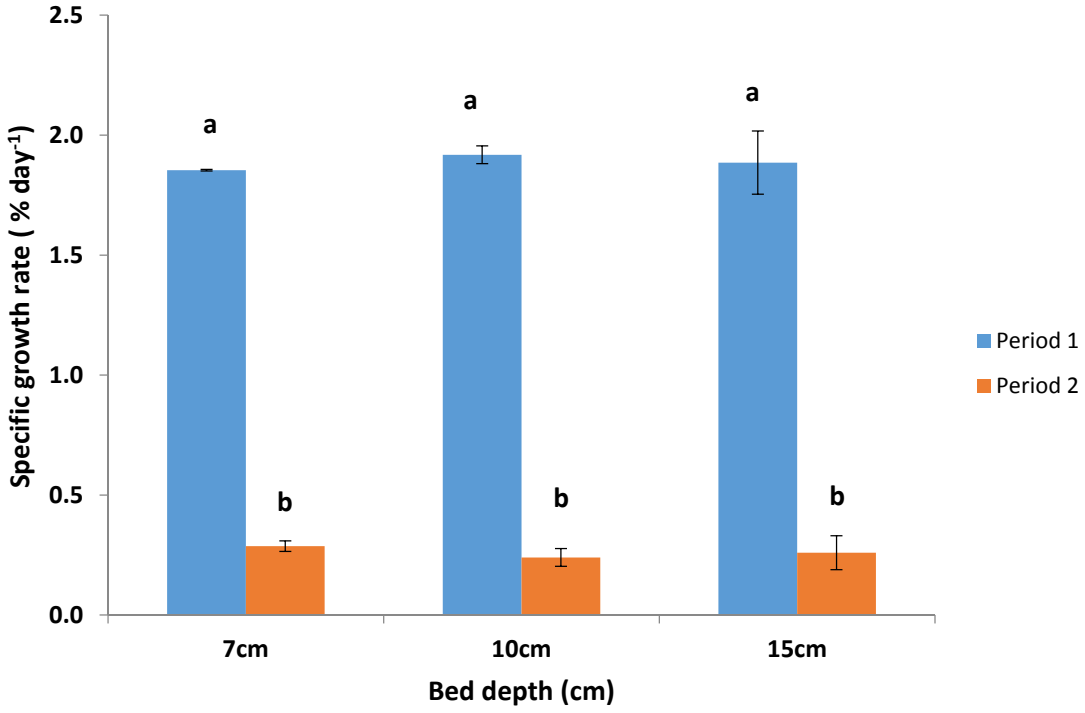


Figure 5.4 Specific growth rate (± S.E.) of ragworm related to culture period. (n=3).

For example, the absolute ragworm gain in first 90 day period were 0.057 ± 0.003 g day^{-1}, 0.061 ± 0.003g day^{-1} and 0.075 ± 0.003 $g.day^{-1}$ for 7 cm, 10 cm and 15 cm culture beds, respectively (Figure 5.5), whereas, they were 0.017 ± 0.004 $g.day^{-1}$, 0.017 ± 0.005$g.day^{-1}$ and 0.024 ± 0.01 $g.day^{-1}$ for 7 cm, 10 cm and 15 cm culture beds, respectively in the second 90 day period. The differences between culture beds are not statistically significant (one way-ANOVA-Fisher's test, $p = 0.651$). However, the differences between two experimental periods are statistically significant (one way-ANOVA-Fisher's test, $p < 0.001$).

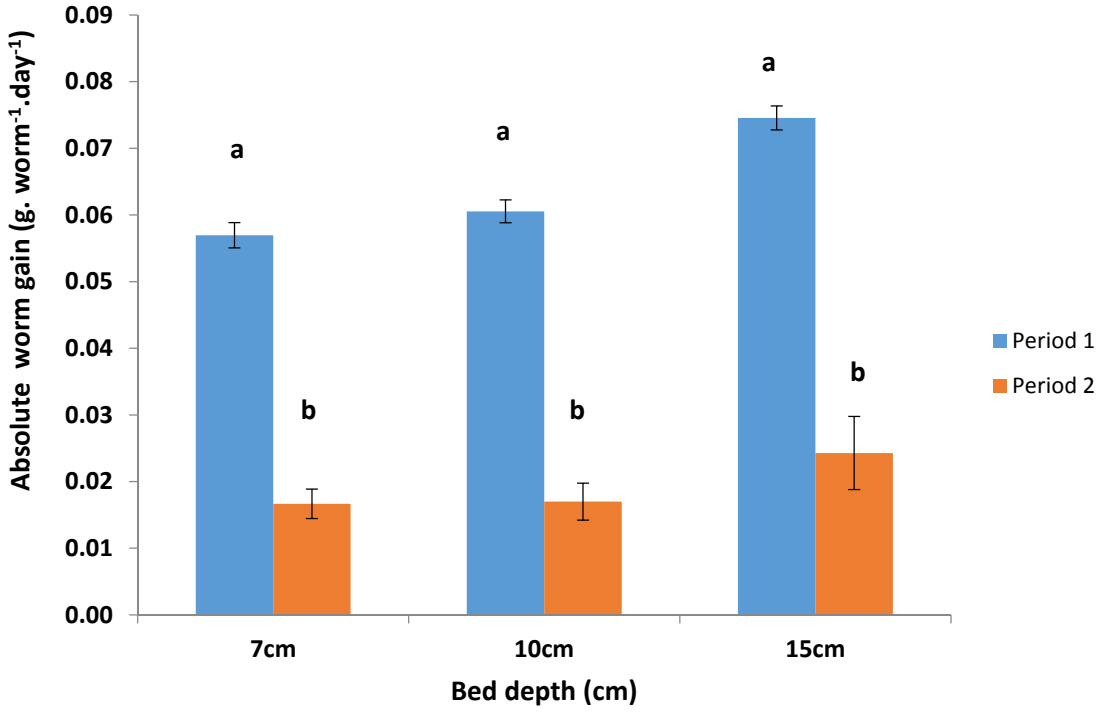


Figure 5.5 Average absolute weight gain (± S.E.) of ragworm related to culture period, (n=3).

The stocking density of ragworm on initial stocking (day 1) was 0.51 ± 0.003 kg $worm.m^{-2}$, 0.49 ± 0.03 kg $worm.m^{-2}$ and 0.56 ± 0.03 kg $worm.m^{-2}$ for 7 cm, 10 cm and 15 cm culture beds, respectively (Figure 6), but at the end of first 90 day period, the stocking density of ragworm had increased sharply to 2.92 ± 0.1 kg $worm.m^{-2}$, 2.83 ± 0.14 kg $worm.m^{-2}$ and 2.87±0.47 kg $worm.m^{-2}$ for 7 cm, 10 cm and 15 cm culture beds, respectively. Moreover, incubation for a

further 90 days resulted in only small increases in stocking density (Figure 5.6). The differences between culture beds of different depth at each time point are not statistically significant. However, differences between productivity on different dates are statistically significant (one way-ANOVA-Fisher's test, $p < 0.001$).

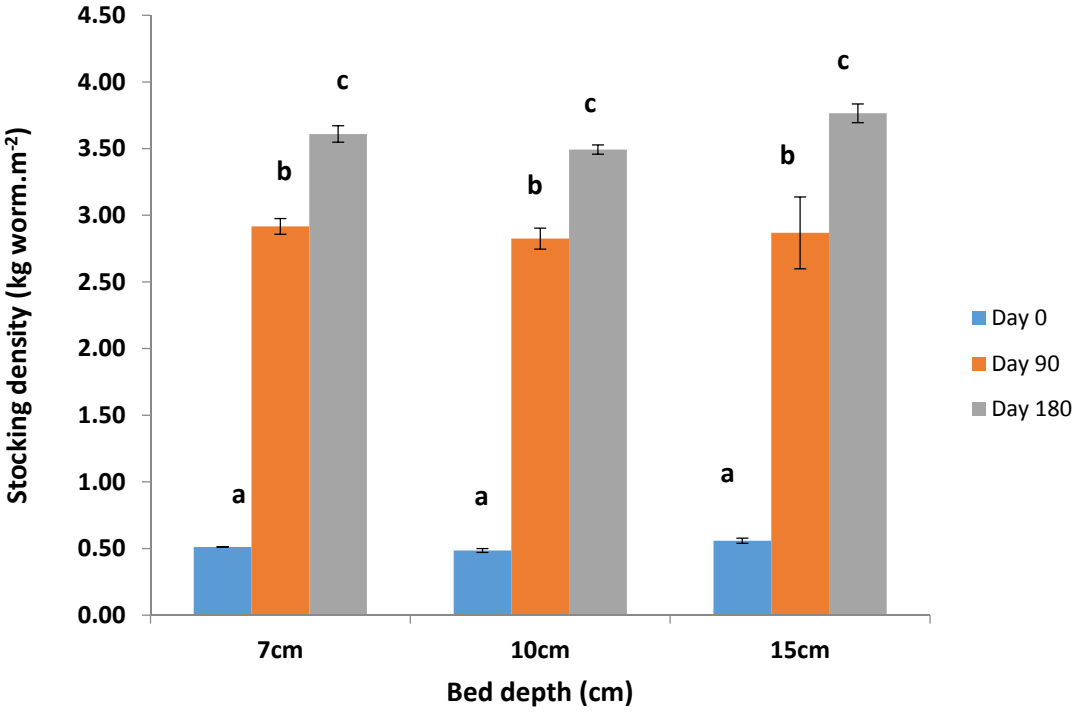


Figure 5.6 Average stocking density (± S.E.) of ragworm related to harvesting day. (n=3).

5.3 Experiment 2: Effect of reduced substrate depth on the culture and growth of *Nereis virens*

5.3.1 Materials and methods

5.3.1.1 Collection of worms:

Four months old**,** Hatchery-reared juvenile *Nereis virens* was collected on the 5th of March 2009 from Seabait Ltd, Lynemouth, Northumberland, Newcastle upon Tyne.

5.3.1.2 Ragworm and rearing conditions:

The group of ragworm (*Nereis virens*) collected for this experiment had an average weight of 14.74 ± 1.01 g. Worms were stocked in 0.05 m^{-2} polyethylene tanks with sand bed depths of 1, 3, 5 and 7 cm. Sand was prepared as described in Section 3.1. Initial numbers of ragworm in each culture tank was 20 for 1cm bed depth and 25 for 3cm, 5cm and 7cm beds depth, respectively. The experiment lasted for 90 days, during which time mean temperature was 18 ± 1ºC, salinity varied between 31‰ and 35‰, and pH was 8.0 ± 0.2. A closed recirculation system (Figure 5.1) was used; the flow rate through each tank was 54 l/h to achieve 432 exchanges per day.

This water exchange was in excess of that needed to maintain dissolved oxygen level at 7.3 ± 0.2 mg/l under all tested conditions. The worms were fed with dry commercial feed (Coarse Fish 23 from Skretting, Wincham Lane, Wincham, Northwich, CW9 6DF) 6 days per week. Pellets were supplied in the evening by manual feeding. Feed ration was changed with time, starting at 3% of initial weight, and finishing with 12% of the initial worm weight in each tank (Table 5.4).

Table 5.4 The feed percentage in different bed depths related to culture periods

Bed depth (cm)	**1st week**	**2ed week**	**3rd - 5th week**	**6th - 7th week**	**8th week**	**9th week**	**10th - 13th week**
1	3%	4%	5%	6%	8%	10%	12%
3	3%	4%	5%	6%	8%	10%	12%
5	3%	4%	5%	6%	8%	10%	12%
7	3%	4%	5%	6%	8%	10%	12%

5.3.1.3 Sampling

Worms were measured for growth measurement (weight) at day 0 and 90 of culture. Total initial (day 1) sample size for each stocked population was 40, 50, 50 and 50 individuals per condition for bed depths of 1, 3, 5 and 7 cm, respectively.

5.3.1.4 Parameters used in the study:

The parameters used for calculating growth, stocking density, feeding efficiency, and total biomass gain are the same as those described in Section 5.2.1.4, since they are the main parameters used in all of the cited literature.

5.3.1.5 Statistical analysis:

The data was tested for normality of homogeneity variance to confirm compliance with the assumptions of analysis of variance (ANOVA). The data were analysed statistically using Minitab for Windows (Version 16). Significant effects of the four tested bed depths on the mean worm weight, and worm weight gain were evaluated through the use of one-way ANOVA. Fisher's multiple-comparisons test for means ($p < 0.05$) was used to assess whether significant differences existed. Microsoft Excel 2010 was also used to plot the graphs and to carry out the required calculations.

5.3.2 Results

Comparison of the numbers, total and average mass, and feeding and mortality rates of ragworm in the culture beds, in relation to harvesting day (Experiment 2) are presented in Table 5.5. Initial numbers of ragworm in each culture bed (tank) was 20 for 1cm bed depth and 25 for 3cm, 5cm and 7cm bed depth, respectively. The average worm weight within different culture beds was 0.66 ± 0.03g, 0.61 ± 0.01g, 0.61 ± 0.01g, and 0.61 ± 0.01g, and total worm biomass in each culture bed was 13.22 g, 15.31g, 15.20g and 15.22g in the 1 cm, 3 cm, 5 cm, and 7cm culture beds, respectively. Within the first week of experiment, a mortality of 1, 4, 2 and 1 worms was observed in the 1, 3, 5 and 7 cm beds, respectively.

During the culture period (90 days), the average mass of worms increased significantly in the four different culture bed depths. For example, the average ragworm weight was 0.66g in the 1 cm culture bed at the beginning of experiment, but after 90 days the average weight had

increased to 3.6g. The differences in average weight of worms between the start of experiment (day one) and at harvest (day 90) is statistically significant for all bed depths (one way-ANOVA-Fisher's test, $p < 0.001$). Moreover, the differences in the average of ragworm weight between 1 cm and 3 cm beds at harvested (day 90) is not statistically significant (one way-ANOVA-Fisher's test, $p = 0.061 < 0.05$), but average weight in 5 cm bed and 7 cm is statistically different from the 1 cm and 3 cm beds.

The total weights of worm in all culture beds at harvest (day 90) were higher compared to the start of the experiment (one way-ANOVA-Fisher's test, $p < 0.001$). For example, the total weight of worms was 68.58 ± 10.5g, 89.84 ± 1.6 g, 110.07 ± 2 g and 116.73 ± 1.2 for 1 cm, 3 cm, 5 cm and 7cm beds, respectively. The differences in total worm weights between 1cm and 3cm culture beds at day 90 is not statistically significant, however there is a statistically significant difference (one way-ANOVA-Fisher's test, $p < 0.003$) between 1 cm, 5cm and 7 cm culture beds.

Table 5.5 Comparison of the numbers, total biomass weight, average weight, feeding and mortality rates of ragworm in culture beds related to harvesting day (Experiment 2). Error bars: ± 1 standard deviation (n=2).

Bed depth (cm)	At stocking (1 days)			At harvest (90 days)				
	Numbers	Total biomass weight (g)	Average weight (g)	Numbers	Total biomass weight (g)	Average weight (g)	Feeding (g/day)	Mortality (%)
1	20	13.22 ± 0.1[a]	0.66 ± 0.01[a]	19 ± 1	68.58 ± 10.5[a]	3.60 ± 0.3 [a]	1.00 [a]	5.0 ± 7.1
3	25	15.31 ± 0.1[b]	0.61 ± 0.0[a]	21 ± 1	89.84 ± 1.6 [a]	4.38 ± 0.08 [ab]	1.16 [a]	18.0 ± 2.8
5	25	15.20 ± 0.1[b]	0.61 ± 0.00[a]	23 ± 4	110.07 ± 2[b]	4.95 ± 0.7 [b]	1.16 [a]	10.0 ± 14.1
7	25	15.22 ± 0.1[b]	0.61 ± 0.01[a]	24 ± 1	116.73 ± 1.2 [c]	4.97 ± 0.1 [b]	1.16 [a]	6.0 ± 2.8

*Values, (mean ± S.D), in column with the same superscripts are not significantly different ($P > 0.05$)

The average gain of ragworm biomass for the different culture beds at harvest day is presented in Figure 5.7 and shows that as the depth of culture bed increases, the average biomass gains increase. The average biomass gains were 55.4 ± 10.5g, 74.6 ± 1.6g, 94.9 ± 2g and 101 ± 1.2g for 1cm, 3cm, 5cm and 7cm culture bed depths, respectively. The effect of culture bed depth on the average of total biomass gain was tested by one way-ANOVA-Fisher's test (Table 5.6), and the result showed no statistically significant difference between 5 cm and 7 cm culture bed depth. However, the differences between the 1cm, 3cm , 5cm and 7cm culture bed depths are statistically significant $P = 0.003 (< 0.05)$.

Table 5.6 ANOVA for the main factors (experiment duration and bed depth) for Experiment 2.

Treatment	Source of variation	Degrees of freedom	Sum of squares	Mean squares	P value
Weight gain	**Bed depth**	3	2611	870	0.003
Feeding efficiency	**Bed depth**	3	1386	465	0.018
Specific growth rate	**Bed depth**	3	0.28346	0.09449	0.023
Absolute worm gain	**Bed depth**	3	0.0004	0.00012	0.039
Stocking density	**Between groups: Duration**	1	10.66	10.66	0.000
	Bed depth	3	1.13	0.38	0.003
Total biomass weight	**Between groups: Duration**	1	26643	26643	0.000
	Bed depth	3	2833	945	0.003
Average weight	**Between groups: Duration**	1	59.4	59.4	0.000
	Bed depth	3	2.48	0.83	0.061
Feeding	**Bed depth**	3	0.0371	0.013	0.725

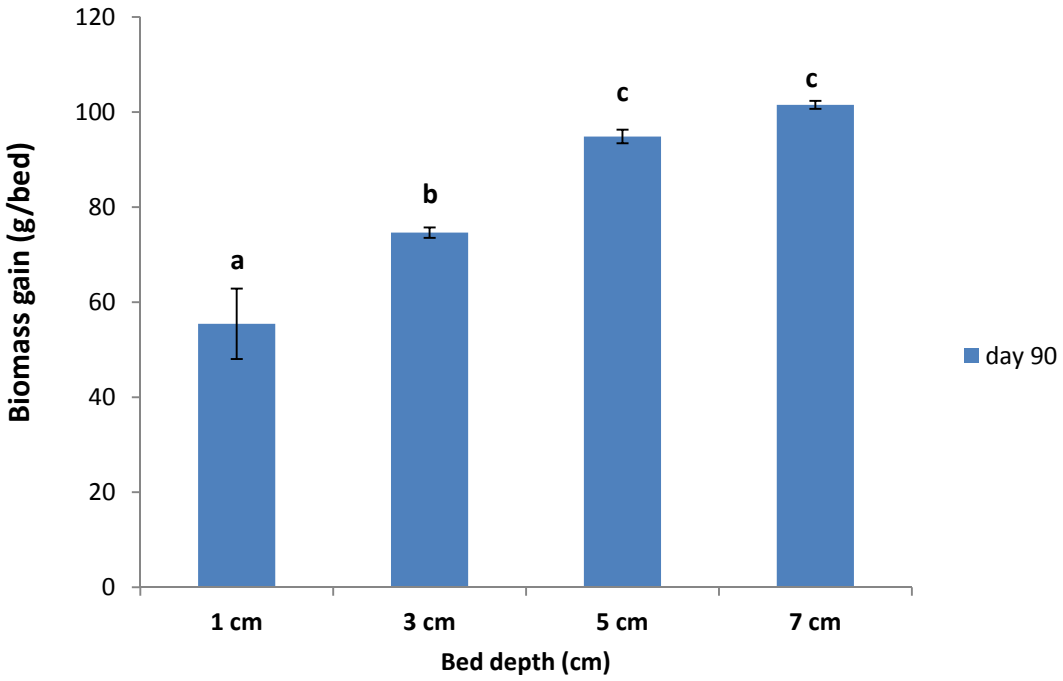


Figure 5.7 The average biomass gain (±SE) of ragworm in each culture bed at harvest day. (n = 2).

The feeding efficiency of worms in the different culture beds ranged between 64% for 1cm culture bed depth to 95% for the 5cm culture bed depth (Figure 5.8). The effects of culture bed depth on the feeding efficiency was tested by one way-ANOVA-Fisher's test and the results showed that there is no statistically significant difference between 5cm and 7cm culture bed depth, however, the differences between the 1cm, 3cm , 5 cm and 7 cm culture beds depth are statistically significant P = 0.018 (<0.05).

The weight of ragworm increased daily by approximately 1.98 ± 0.09%, 2.3 ± 0.02%, 2.4 ± 0.16% and 2.4 ± 0.02% in the 1cm, 3cm, 5cm and 7cm culture beds, respectively (Figure 5. 9). Furthermore, the absolute ragworm weight gain shows the same trend. For example, the absolute ragworm weight gains were 0.034 ± 0.0004 $g.day^{-1}$, 0.042 ± 0.0006$g.day^{-1}$, 0.05 ± 0.007 $g.day^{-1}$ and 0.05 ± 0.001 $g.day^{-1}$ for 1cm, 3cm, 5cm, and 7cm culture bed depths, respectively (Figure 5.10).

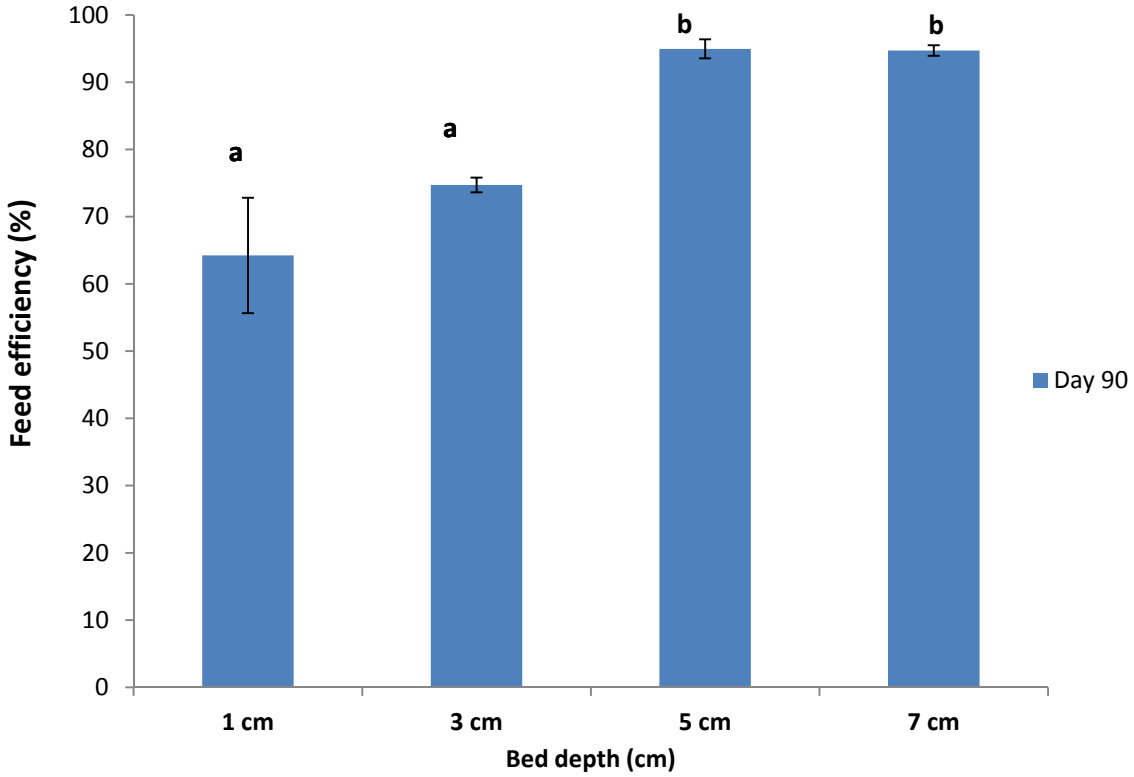


Figure 5.8 Average feed efficiency (± S.E.) of ragworm over the growth period. Beds with the same letter code are not statistically different.

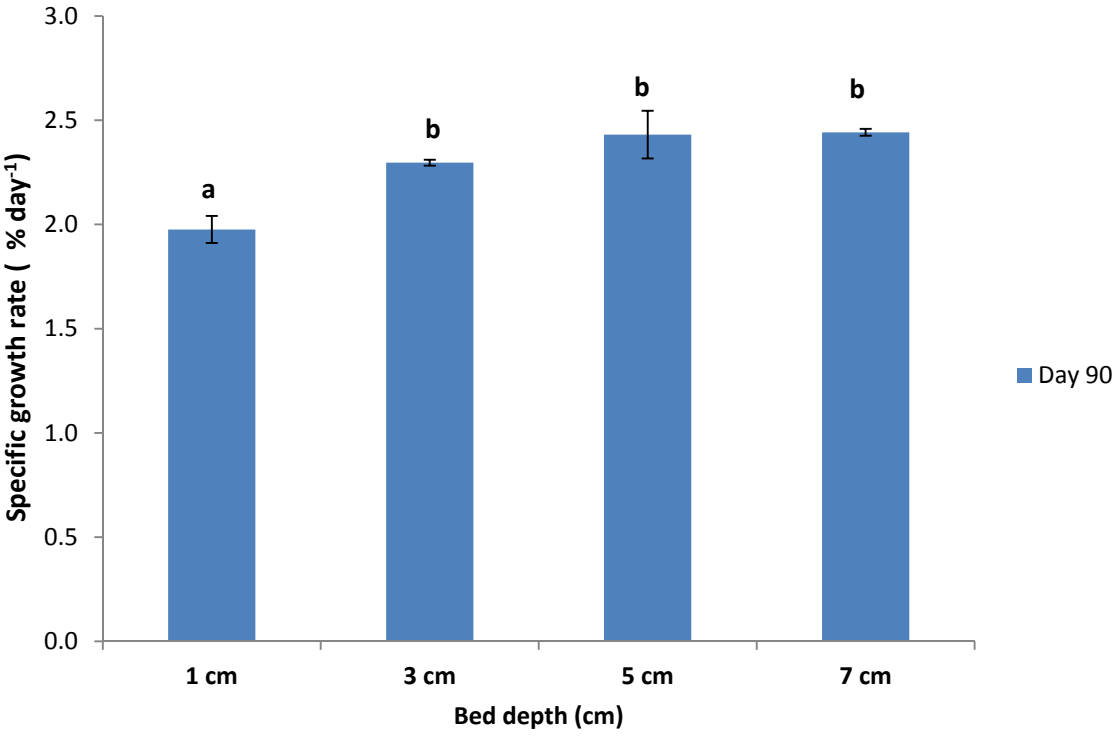


Figure 5.9 Specific growth rate (± S.E.) of ragworm over the growth period. Beds with the same letter code are not statistically different.

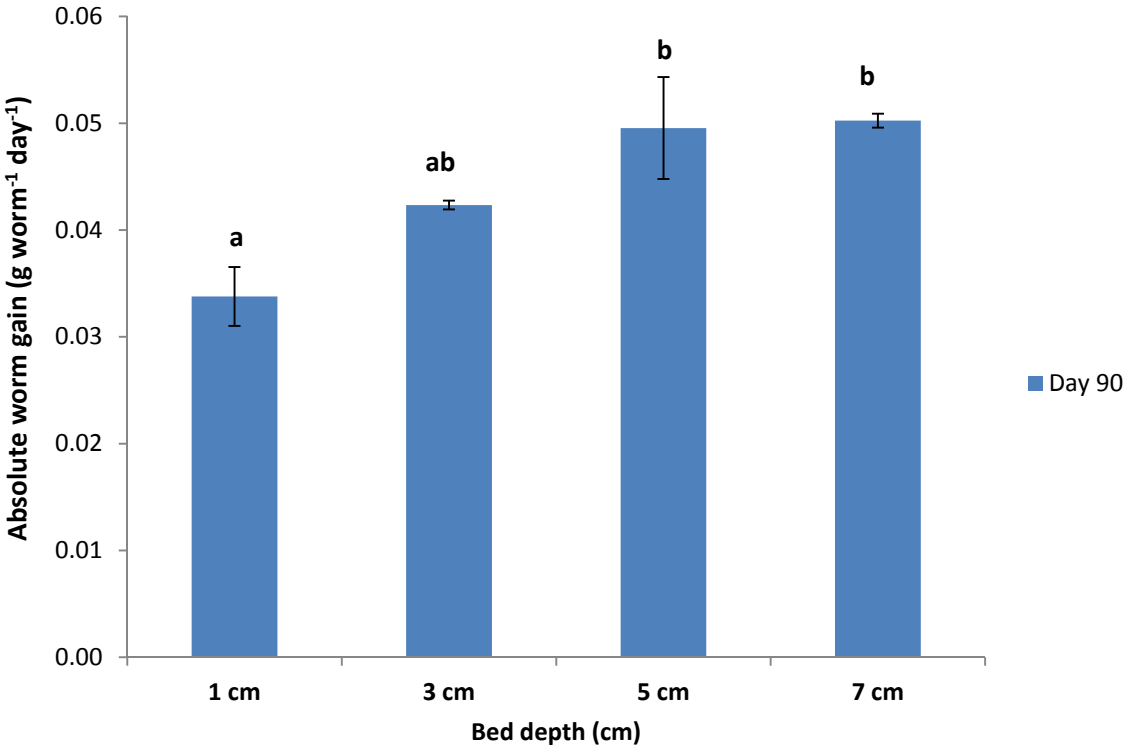


Figure 5.10 Average absolute weight gain (± S.E.) of ragworm over the growth period. Beds with the same letter code are not statistically different.

The differences in average absolute weight gain (Figure 5.10), between 3cm, 5cm and 7cm culture bed depth are not statistically significant. However, the difference between the 1 cm culture bed and other beds is statistically significant (one way-ANOVA-Fisher's test, $p = 0.039 < 0.05$). The stocking density of ragworm was very low at the start of the experiment with 0.26 kg worm.m^{-2}, 0.30 kg worm.m$^{-2,}$ 0.30 kg worm.m^{-2} and 0.30 ± 0.03 kg worm.m^{-2} for 1cm, 3cm, 5cm and 7cm culture bed depth, respectively (Figure 5.11).

At the end of experiment period (90 day), the stocking density of ragworm had increased significantly to 1.37 ± 0.2 kg worm.m^{-2}, 1.8 ± 0.03 kg worm.m$^{-2,}$ 2.2 ± 0.04 kg worm.m^{-2} and 2.34 ± 0.2 kg worm.m^{-2} for 1cm, 3cm, 5cm and 7cm culture bed depths, respectively. The differences in the stocking density between beginning and the end of experiment are statistically significant (one way-ANOVA-Fisher's test, $p = 0.003 < 0.05$). Moreover, the differences between culture beds at the end of experiment are also statistically significant, with

the exception of differences between the 5 cm and 7 cm culture depth were not statistically significant (one way-ANOVA-Fisher's test, P=0.003 < 0.05).

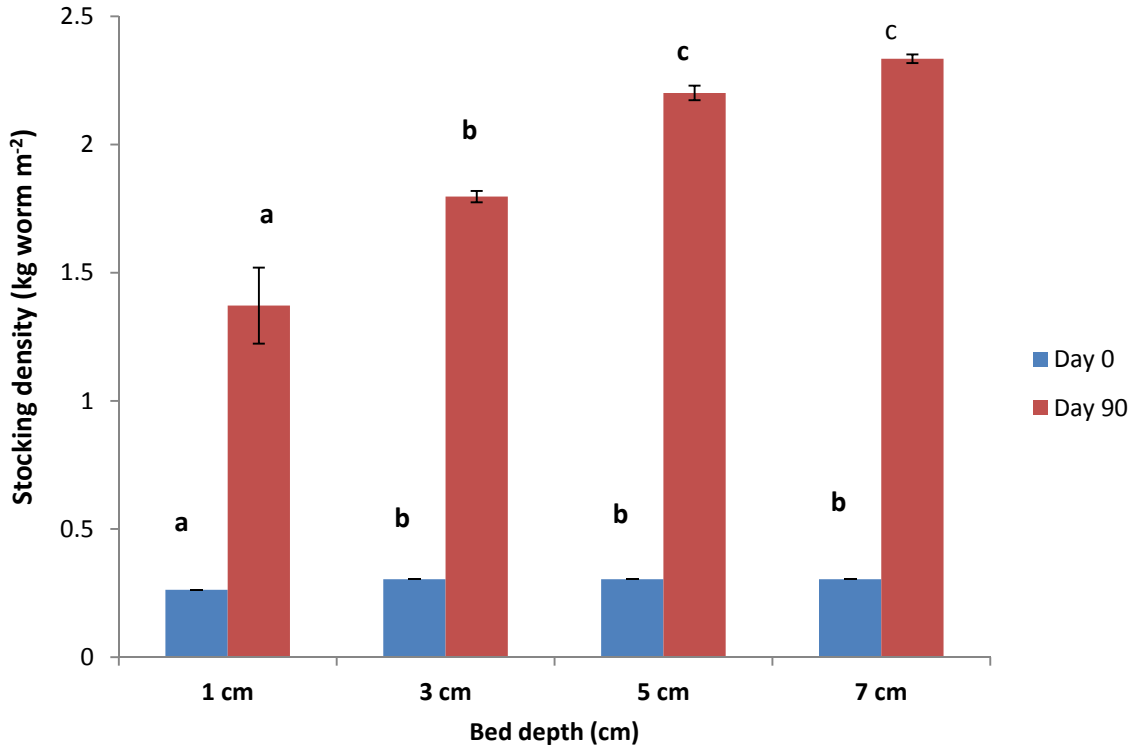


Figure 5.11 Average stocking density (± S.E.) of ragworm related to harvesting day. Beds with the same letter code are not statistically different.

5.3.3. Discussion

It is clear from Experiment 1 that total biomass did not increase significantly more in the 15 cm bed depth than in the 10 and 7 cm bed depths, though the number of worms were low at 15 cm depth. At the beginning of the experiment some worms died in all bed depths, but this death was due to transport and not due the effect of bed depth. This is why no further deaths were observed up to 90 days in any of the beds. As worms are sensitive to stress, this death was unavoidable, and it might cause partial errors in the results; for instance, Deschenes *et al.*, (2003) set-up experiments for the examination of food utilisation in which 43% of the worms died, 93% of these dying before treatment.

However in the present study, after 90 days only 4.3 ± 3.8% of the initial worm numbers died in 7 cm bed and 2.5 ± 2.6% died in 10 cm bed. This could be due to competition among the worms for space. In, the 7 cm bed, the total number of worms was higher than in the other two bed depths, so these mortality percentages would not have had a significant effect on the result. The average weight of worms were 6.55 ±0.51g, 6.84 ±0.18g and 8.37 ± 1.5g in in 7, 10 and 15 cm bed depth respectively, being clearly higher in 15 cm bed depth, however, other parameters such as total biomass, feed efficiency, stocking density and weight specific growth rate were very similar in the different bed depths (P value range 0.981-0.991). For example, the feed efficiencies were 89.7 ± 1.92 %, 86.5 ± 3.04 % and 85.6 ± 5.84% in 7, 10 and 15 cm bed depths, respectively.

Therefore, a 7 cm bed depth can be proposed as the ideal sand depth for the culture of *Nereis virens.* The bed depth chosen in practice depends on the final use of the worms. If the worms are used for angling then they need to have a larger size, and therefore a 15 cm depth bed would be more suitable. But if the worms are used for fish feeding in the aquaculture industry, then the 7 cm bed would be enough, because it gives same biomass as the 15 cm bed depth but has significant advantages over deeper beds for the potential development of a multi-layer culture system. For example, total biomass weights were 291.66 ± 10.2 and 286.79 ± 47g in 7 and 15 cm bed depths, respectively. From the first experiment it can be concluded that *Nereis virens* could be cultured at sand depths as low as 7 cm without showing any detrimental effect on the production of these worms. The daily specific growth rate was 2% which is acceptable for a laboratory-based experiment, because the weight-specific growth rate for natural *Nereis virens* is 0.8% (Desrosiers *et al*., 1991), and under experimental conditions is normally 0.4 to 1.7% (Oliver *et al*., 1996).

However, in the second stage of this experiment (90 – 180 days), the growth of the worms was very slow compared to first 90 days, and even though the feed consumption was higher, the feed conversion rate was lower. Stocking density could be one of the reasons for the reduced

growth seen in the second stage, because in the high density conditions of the second stage, *Nereis virens* competed more for burrow space rather than for food (Miron *et al.*, 1991). Studies undertaken by Esnault *et al.* (1990) and Scaps *et al.* (1993) have shown a similar reduction in growth, biomass and survival at high densities, even in the presence of abundant food.

Saarik *et al.* (2006) estimated the daily growth of *D. aciculate* after 14 weeks of incubation, and it was lower at high density (2.3 mm/day) compared to medium density (2.8 mm/day) and low density treatments (3.0 mm/day). Nesto *et al.* (2012) also observed negative effects of growth and survival rates of *Hediste diversicolor* with high stocking density, the intermediate densities showing the highest daily biomass production. The reduction in suitable area for each worm in second stage of the experiment could have forced worms to consume food energy for protection their burrow area from adjacent worms.

A full grown *Nereis virens* burrows down to 45 cm, with larger individuals burrowing deeper (Pettibone, 1963). Last, (2003) constructed artificial burrows of 15 cm in the bottom of each acrylic aquarium of 21 cm depth to culture *Nereis virens,* and found a density more than 3 $kg.m^{-2}$ would make it difficult for *Nereis virens* to grow. However, the daily specific growth rate was 0.25%, which was less than for *Nereis virens* growing in a natural environment (0.8%, Desrosiers *et al*., 1991). From the above discussion, it can be concluded that bed depths of 7 cm will be enough to produce *Nereis virens* efficiently in cultures running up to 90 days. If worms need to be cultured longer then a greater bed depth would be necessary to achieve efficient growth.

In the first experiment, as 7 cm was found to provide enough depth to produce *Nereis virens* up to 90 days, and that was the minimum bed depth, a second experiment was carried out to identify whether a bed depth below 7 cm might be sufficient for the production of *Nereis virens.* The results showed that the total biomass weight was significantly higher in the 7 cm bed depth

than in the shallower bed depths. Specifically, in the 7 cm bed depth the biomass increase was almost double that found in the 1 cm bed depth. Not only biomass, the average weight was also higher in the 7 cm bed depth where individual worms had enough space to make their burrow so their growth was satisfactory. However, in bed depths less than 7 cm, it is likely that the worms did not have enough space, and growth suffered as a consequence. Furthermore, the lowest mortality was found in 7 cm bed depth, which also agrees with the above observations. Although in a 7 cm bed depth the stocking density reached levels greater than that of the lower bed depths, it was still below 3 kg m^{-2}, the density known to have adverse effects on growth. The feed efficiency was also higher in 7 and 5 cm beds compared to the two shallower bed depths. The feed efficiency in both experiments was more than 90% for 7 cm bed depth, confirming that the worms grew very efficiently in those conditions.

5.3.4. Conclusion

From these two experiments, it appears that bed depth would have a considerable influence on production of *Nereis virens,* and could be optimized depending on the final commercial use of the worm biomass. To make a profitable production of *Nereis virens* biomass in a multi-layer aquaculture system, a 7 cm bed depth would give the optimum conditions, particularly if the worms were to be used in the aquaculture industry. Whereas, to produce larger size or higher weight worms for bait, a 15 cm bed depth would be preferable.

Chapter 6. Conclusions and future work

6.1 The impact of different artificial substrate materials on growth and production of *Nereis virens*

An experiment was carried out to investigate the effect of three different artificial substrate materials (White polyethylene beads, recycled black polyethylene beads, corrugated polypropylene sheets) on the culture and growth of *Nereis virens.* Sand was used the control substrate for these three novel materials. The results showed that when using the artificial substrates, there was a significant negative effect on the growth and stocking density of the cultured ragworms, when compared to the natural culture substrate media (sand). The grain size of the sand particles used was much smaller than the particle size of the white polyethylene beads and the recycled black polyethylene beads. It was observed that the ragworms had more difficulty forming and maintaining burrows within the relatively large particles of white polyethylene beads and recycled black polyethylene beads, and were more restricted in these synthetic media in carrying out normal activities like feeding. Essentially, the physical shape and size of these engineered plastic beads made it difficult to construct and stabilize their burrows in comparison with sand. Importantly, these two materials led the ragworms to spend more time and resources stabilizing the structure of their burrows, most notably, they needed to produce much more mucus to create a continuous lining to their burrows. Consequently, a substantial part of the food consumed by these ragworms was not devoted to growth, but to the production of mucus instead. Furthermore, the ragworms spent less time searching for food around the entrance of their poorly stabilized burrows. As a result, can be concluded that the ragworms invested relatively more time and resource to maintaining their burrows than to the activity of feeding, when compared to worms growing in a sand substrate. In summary, the ragworms preferred the physical characteristics of the finer material (sand) than the coarse granular white polyethylene beads and recycled black polyethylene beads. It was observed that

none of the ragworms adapted to the corrugated polypropylene sheets with ready-built burrows provided by the plastic corrugations, and their rejection of this material left them exposed at the bottom of the tank, causing stress which finally resulted in death.

6.2 Effect of substrate depth on the culture and growth of *Nereis virens*

Two experiments were conducted in order to investigate the effect of substrate depth on the culture and growth of *Nereis virens*. The first experiment was carried out to check the effect of three different depths of sand (7cm, 10cm, and 15, cm) on the culture and growth. The results of statistical analysis show that sand depth had no significant effect on the culture and growth of *Nereis virens*. Consequently, it can be stated that a bed depth of 7 cm would be an appropriate depth to culture and grow *Nereis virens*, since there was no adverse effect of reducing the substrate depth to that level when culturing the worms. This finding has an economic impact on companies which deal with the commercial culture of these worms. Harvesting time of the cultured worms, which are mainly reared to use for the aquaculture industry as food source of juvenile fish and Crustacea, would be reduced in comparison to the sand depth of 15 cm, which used by Seabait as their preferred standard substrate depth.

In addition, the multi-layer aquaculture system proposed as a novel culture method to intensify the production of *Nereis virens*, a sand depth of 7 cm would make a considerable impact on the costs of the construction materials used to build such vertical multi-layer system, and these would be markedly reduced in comparison with a multi-layer aquaculture system utilising 15 cm bed depths.

As shown in Chapter 5, the first experiment consisted of two culture periods: Period 1, 0 - 90 days; and Period 2, 90 -180 days. It was shown that these two different periods resulted in very different growth rates, with the growth rate in the first period being four times higher than that of the second period. In the second period, the feeding efficiency was 90 per cent lower than

that of the first period. Furthermore, the stocking density of the ragworms increased only by 0.5 kg per m^2 in the second period as compared to the first period in which the stocking density was 3 kg per m^2. Thus it is concluded again that the second period (90-180 days) does not provide any significant economic benefit. This significant difference in growth rate indicates that culturing ragworms for long periods (180 days) provides no economic benefits, since long culture periods would generate lower total annual productivity of worm biomass from a culture bed than shorter periods (90 days). It was therefore concluded that a stocking density of 3 kg worm.m^{-2} should be the maximum carrying capacity for the cultured bed.

To test the effect of further reductions in substrate depth on worm growth, a second experiment was carried out and showed that any further reduction in bed depth below 7 cm did cause an adverse effect on the growth and culture of the ragworms. This means that extremely shallow sand bed depths would have a significant impact on culturing these ragworms. These findings can be accounted for by assuming that further reductions in bed depth reduced the daily activity of these ragworms as they concentrated more on maintaining and protecting their burrows due to their territorial behaviour. This territorial behaviour was most probably caused by the limited space available to these ragworms which lead them to protect their burrows from other ragworms and seek refuge in their burrows for longer periods rather than search for food.

Based on observations made during the daily monitoring of worm activity, especially after feeding, it can be stated that if food supplies are not sufficient, the worms concentrate on feeding rather than storing food. Conversely, if there is an excess of feed, the worms concentrate on storage activity rather than only feeding. This difference in ragworm behaviour can be accounted for by the hypothesis that there would be less competition between worms when there was an excess of food. By nature, the worms tend to store food in their burrows if there is sufficient available.

In addition, when the culture tanks reach their maximum capacity, the ragworms show very strong territorial behaviour, concentrating on burrow protection rather than searching for food. Many broken tails also appeared on the surface of the substrate used, because the ragworms lost their tails due to both limited space availability and the ability to go further in their burrows when the burrow length was restricted by the depth of the sand. It is concluded that the presence of tail regenerating segments in the ragworms is evidence for this process of tail loss. Furthermore, when the physical dimensions of the bed are limited by extremely shallow depths of sand, rates of immigration among the ragworms is increased because they leave their burrows more frequently to look for better places to dig new burrows.

6.3 Future work

Although the current work has provided important findings related to the intensification of ragworm culturing in engineered growth systems, there are some aspects worthy of further research. Firstly, the effect of change in oxygen concentration on the growth rate of the ragworms should be investigated in order to determine if it affects growth. Secondly, the effect of the timing of specified periods on the growth of groups of ragworms of different sizes should be investigated in order to examine the relationship between timing and growth. Thirdly, the same technique used in current study can be applied to other types of worms in order to provide more comprehensive account on the culturing of commercially relevant species of worm.

Since this study investigates the effect of three different packing materials to ease harvesting, other softer and finer artificial materials should be investigated, including smaller particle sizes of the polyethylene beads, and compared with sand and other different artificial materials in order to determine most effective method of harvesting, and minimum mass and volume of the worm culture bed. A study investigating vertical multi-layer systems should be conducted to assess the capability of such systems to overcome the space limitation problems which face all

commercial producers of ragworm. Experiments could also be designed to investigate the potential of using ragworms to recycle wastewater from other aquaculture facilities.

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