UNIVERSITY OF SOUTHAMPTON
FACULTY OF SCIENCES

Department of Oceanography

GROWTH AND THE ENERGY BUDGET OF JUVENILES OF THE ABALONE *Haliotis tuberculata* (L.)

by

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ABSTRACT

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GROWTH AND THE ENERGY BUDGET OF JUVENILES OF THE ABALONE *Haliotis tuberculata* (L.)

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The spawning season of the most commercially important European abalone (*Haliotis tuberculata*) was evaluated during the summers of 1996,1997 and 1999. The results of the present study show that the best time to start with semi-artificial spawning is from mid July using cultured abalone from the open sea in Guernsey, Channel Isles.
Until 1996 *Tetraselmis sueccica* was the main source of food for juvenile *H. tuberculata* during their rearing stage (one to twelve weeks old) on glass plates. Nevertheless, this study showed that *T. sueccica*, as a sole food, is not sufficient for successful development and growth of early juvenile abalone. However, marine diatoms such as *Skeletonema costatum, Navicula ramosissima* and *Cylindrotheca closterium* were easily ingested and assimilated depending on the size of the animal and conditions of culture.

This thesis studied the effect of different diets (mix of fresh seaweed, fishmeal and an abalone commercial) and temperatures (15°, 18° and 22°C) on growth and energy budget of juvenile abalone *H. tuberculata* over a 210 day period. Energy budget was obtained by Ingestion (*I*), Egestion (*E*), Somatic growth (*Pg*), Reproductive investment (*Pr*), Excretion (*U*) and Pedal mucus production (*M*). All these parameters were assessed for grouped and individual organisms. Animals fed on formulated diets (energetically rich) and cultured at 18° and 22°C were shown to give better growth rates than the natural diet and preferentially allocated energy to gonad development. Thus, the combination of diet and temperature were factors that contributed to enhance growth rates and gonad development.

The abalone commercial diet (CO) used in this study produced shell deformation in 89 % of the population after four months of feeding juvenile abalone. The information of these studies have important implications when considering the nutritional requirements of cultured abalone when fed on formulated diets, which need to contain not only the energy, but also the necessary micronutients which are required to produce good growth rates and also healthy animals.

Gracias a ti Señor

With all my Love to,

Luis, Ana Karen and Pamela

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CONTENT

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

General Introduction

Abalone are one of the most primitive living marine gastropods in form and structure. *Haliotis* is the only genus in the family and *Haliotis midae* Linne 1758 is the genotype (Cox, 1960). Meglisch (1967) presented a complete classification of abalone, as follows:

Phylum Mollusca (clams, snails, chitons, squids), non-segmented invertebrate with a mantle cavity which typically contains the gills, usually a definite head and a muscular foot.

Class Gastropoda (snails, slugs, nudibranchs), molluscs with one piece spiral shell, or no shell at all, which move by means of a broad muscular foot.

Subclass Prosobranchia (limpets, periwinkles, cowries, whelks, conchs), gastropods which undergo torsion during the veliger larval stage so that the gills, anus and mantle cavity come to lie at the front of the body and the nervous system is twisted into a figure of eight.

Order Archeogastropoda (limpets, abalone), prosobranchs, which have a notch, slit, or holes in the shell over the anus.

Family Haliotidae (abalone), the visceral mass and shell is markedly flattened and the spire is greatly reduced. The opening of the shell is relatively large.

In Haliotis, torsion is accomplished in two stages: in the first, a rapid rotation of 90 degrees occurs during their pelagic stage as a result of muscular action; in the second, the remaining 90 degrees is rotated much more slowly and results from differential growth of the columellar muscle (Cox, 1960).

The principal characteristic of the family Haliotidae is the auriform and approximate circular outline of the shell; it has a convex profile (ranging from highly arched to flattened). A row of rounded shell perforations overlay the respiratory cavity and the large shell aperture. Multi-purpose respiratory pores also have a role as excurrent channels for excretion and the release of gametes. As the shell grows larger, new respiratory pores develop along the growing edge of the shell above the head of the

animal. The number of pores, the colour, texture and shape of the shell are often used to identify different species of abalone (Figure 1.1) (Cox, 1960).

Most of the soft body of abalone is a very large foot, which includes the epipodium and the large columellar muscle. The ventral surface of the foot is used for locomotion and adhesion to the substratum by means of suction. The small head is attached anteriorly to the foot and has a ventral mouth, two stalked eyes and two long retractable sensory cephalic tentacles. The edge of the foot is surrounded by the epipodium, a ruffled flap of tactile sensory tissue. The tentacles, which are sensitive to touch, are also used for chemical sensing of the local environment and histological evidence suggests the tentacles in *Haliotis* can perceive light and dark (Cox, 1962). Abalone are marine herbivores and deposit scrapers with a rhipidoglossan radula (Morton, 1958). The exposed soft parts are heavily pigmented with green, brown and black. This gives a coloration that harmonises with the environment. Probably the pigment serves as protection from strong light (Crofts, 1929).

There are about 100 species of abalone, distributed along rocky shores and sub-tidal water to depths of 400 m (Poore, 1972; Shepherd, 1973), in tropical, sub-tropical and warm temperate seas of all the major continents and among many of the islands in the Pacific, Atlantic and Indian Oceans (Figure 1.2) (Cox, 1960; Lindbergh, 1992). About 22 species are classified as commercially important (Table 1.1). Moreover, the major concentrations both in number of species and individuals are off the coasts of Australia, Japan, western North America, South Africa, West Africa and the Canary Islands. In Europe they are distributed from the English Channel Islands and the west coast of France to the Mediterranean Sea (Figure 1.3) (Cox, 1962; Hayashi, 1980). The major producing countries are Australia, Mexico and Japan. The USA, New Zealand, South Africa, North and South Korea and Canada also land significant quantities (Lindbergh, 1992).

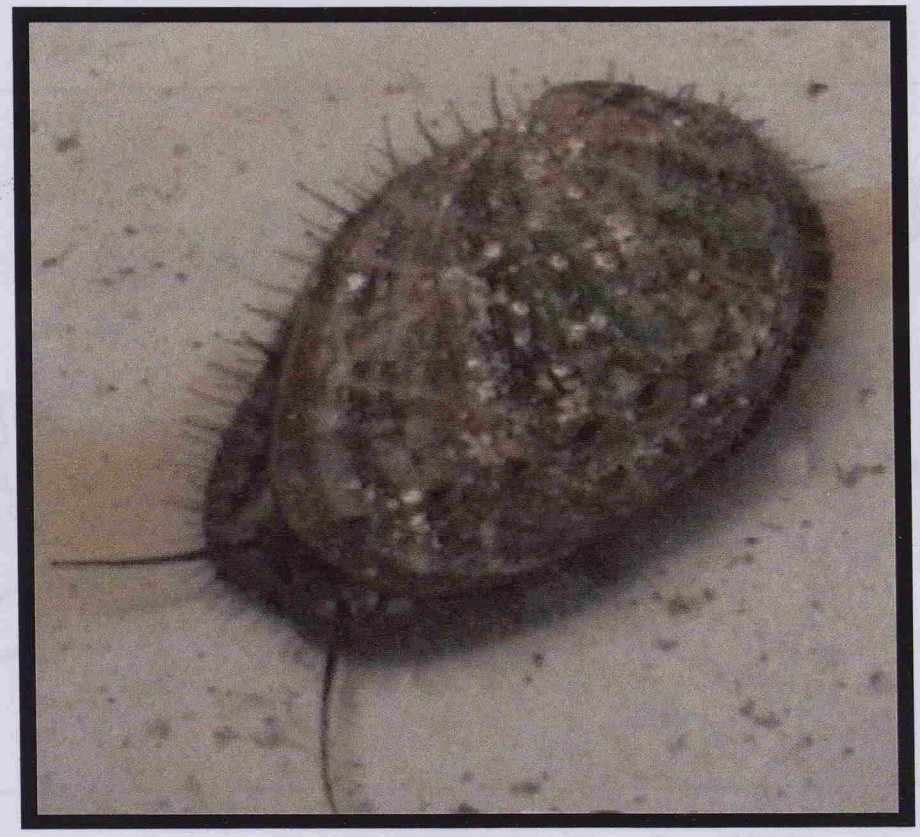

Figure 1.1 Adult abalone *Haliotis tuberculata.*

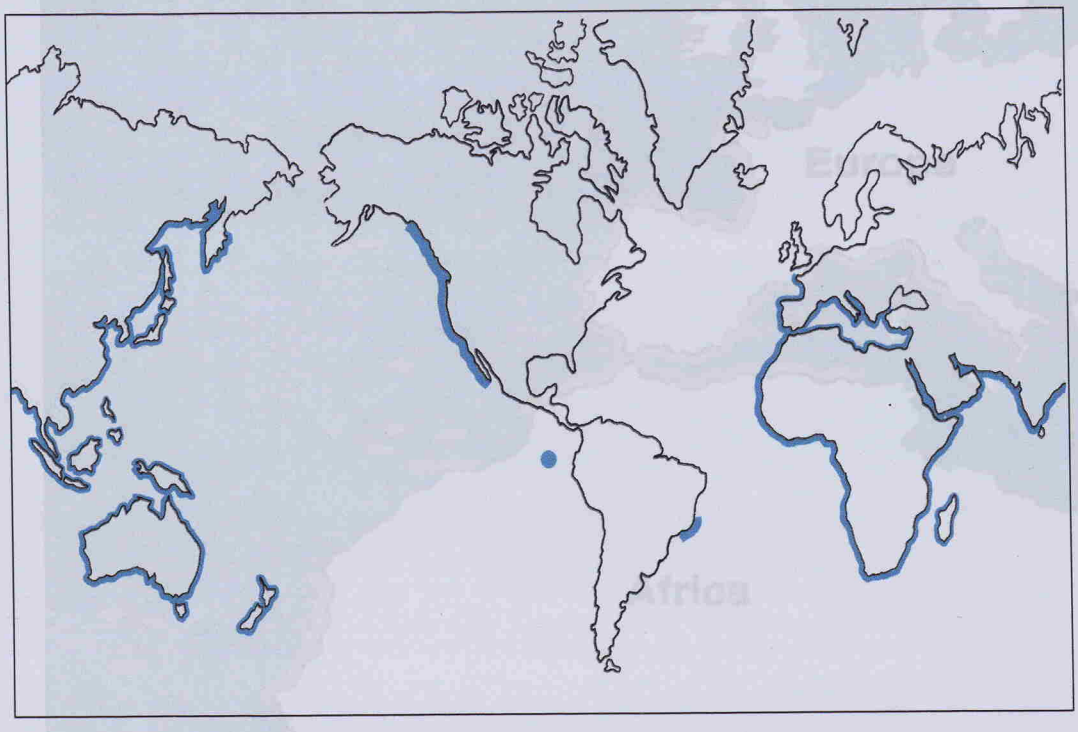

Figure 1.2. Distribution of species of *Haliotis*. From Cox (1962).

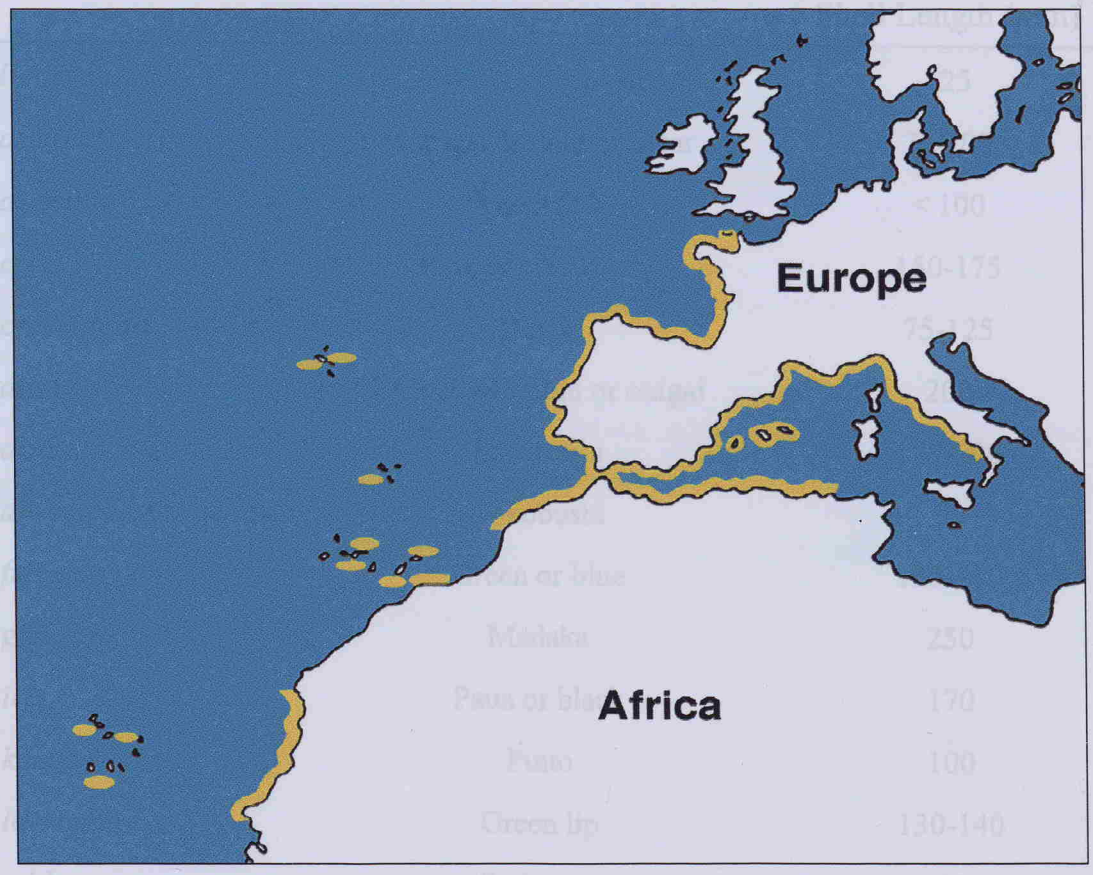

Figure 1.3. Distribution of the abalone *Haliotis tuberculata*. After Gaillard (1958); Nordsiek (1975).

Table 1.1 Commercially important abalone species world-wide.

Scientific name	Common name	Maximum Shell Length (mm)
Haliotis australis	Silver or queen paua	125
H. asinina	Mimigai or donkey's ear	70-100
H. assimilis	Threaded	< 100
H. corrugata	Pink or corrugated	150-175
H. cracherodii	Black	75-125
H. discus	Kuro, awabi, oni or onigai	200
H. discus hannai	Ezo awabi	180-200
H. diversicolor supertexta	Tokobushi	50
H. fulgens	Green or blue	125-200
H. gigantea	Madaka	250
H. iris	Paua or black	170
H. kamtschatkana	Pinto	100
H. laevigata	Green lip	130-140
H. midae	Perlemon	90
H. roei	Roe's	70-80
H. rubra	Black lip	120-140
H. rufescens	Red	>275
H. sieboldii	Megae	170
H. sorenseni	White or sorensen	125-200
H. tuberculata	Ormer	120
H. virginea	Virgin	70
H. walallensis	Flat or northern green	75-125

Summarised from Hahn (1989) and Fallu (1991).

The largest abalone world-wide and the most important North American species extending down to Mexico is the red abalone *H. rufescens*; the white abalone, *H. sorenseni* and the green abalone, *H. fulgens*, are known for their high quality meat. Moreover, Japan has about 10 species of abalone, the most important being *H. discus hannai*, but also important are *H. diversicolor, H. gigantea*, and *H. sieboldii* (Lindberg, 1992).

The ormer (*H. tuberculata*, Linnaeus, 1758), is the most commercially important European abalone found in rocky coasts around the Channel Islands, French and Brittany coasts, as far north as Cherbourg (Figure 1.3) (Hayashi, 1980a, b; Clavier and Richard, 1986). *H. lamellosa* is considered native to the Mediterranean shores of France, Spain, Italy, Yugoslavia, Greece, Syria and Egypt. It is recognised as a subspecies of *H. tuberculata* (Barash and Danin, 1992). It was found that the number (2n = 28) and morphology of chromosomes of *H. tuberculata* and *H. lamellosa* to be similar (Colombera and Tagliaferi, 1983).

Abalone have few parasites. The main predators of abalone are: octopus, large starfish, possibly rays, sea birds (Crofts, 1929; Forster, 1962), crabs, sharks (dogfish) and mesogastropods (Shepherd and Breen, 1992).

Animals under 1 cm in length have protective coloration very different from the adult *Haliotis* species. The diminutive ones are coral-pink, mottled with grey and white, a convincing imitation of the *Lithothamnion* and scattered *Spirorbis* so abundant on the under surfaces of grey granite rocks found at the extreme limit of low tide (Forster, 1962). The shells are grey to red, according to the type of rock in the neighbourhood, and are often encrusted by various epiphytic growths, mainly *Balanus*, *Spirorbis*, *Anomia*, and various polyzoa and sponges; sometimes an algal growth covers so evenly that it seems to be the natural surface of the shell (Crofts, 1929; Forster, 1962).

Abalone larvae are non-feeding (lecithotrophic) and have a relatively brief pelagic phase compared with feeding (planktotrophic) larvae (Mottet, 1978; Olson and Olson, 1989). Lecithotrophic larvae of marine invertebrates cannot ingest particulate food and are assumed to subsist entirely on endogenous reserves (Chia, 1974). Nevertheless, the abalone larvae meet their metabolic requirements by utilising the maternal reserves provided by the egg yolk (Crofts, 1937), plus the absorption of dissolved organic matter (DOM) present in seawater (Jaeckle and Manahan, 1989; Manahan and Jaeckle, 1992).

Benthic diatoms are considered the main diet for early juvenile abalone below the size at which they are capable of consuming macroalgae (Ino, 1952; Ebert and Houk, 1984; Kawamura *et al.*, 1995; Matthews and Cook, 1995). The extracellular substances of diatoms are important food for early juvenile abalone up to the size of 800 μm in shell length (Kawamura and Takami, 1995). In the laboratory, a variety of benthic diatoms have been studied as a food for early juvenile abalone. *Cylindrotheca closterium* has been shown to have the best digestion efficiency and the best growth rates on early juvenile abalone (Kawamura *et al.*, 1995; Kawamura and Takami, 1995).

Adult abalone are almost entirely herbivorous (Leighton, 1961; Cox, 1962), and are found where there is an abundance of drift alga. Each abalone species shows definite preferences for certain types of seaweed (Shepherd and Turner, 1985). Varieties of brown algae are preferred in the Northern Hemisphere; but in Australia and New Zealand where red seaweed are abundant, the reds tend to be selected (Leighton, 1961; Mottet, 1978). Moreover, ormers show a preference for delicate seaweed and in particular red algae such as *Palmaria palmata* (Mercer *et al.*, 1993) and coarser ones such as the brown seaweed *Laminaria* spp., the green seaweed *Ulva lactuca*, *Chondrus crispus* and *Enteromorpha intestinals* (Culley and Peck, 1981; Peck, 1989). Juvenile abalone less than a year old, usually were found eating the encrusting coralline algae and sessile diatoms.

Abalone may consume up to 39 % of their body weight in seaweed per day, but when the nutritional quality of the seaweed is better, 10-20 % is more common (Mottet, 1978). Interestingly, the seaweed, which presents the best nutritional value, is not necessarily the one that is most preferred by the abalone (Leighton and Boolotian, 1963).

The food conversion is the weight of food eaten divided by the increase in abalone weight and it depends on the food quality and the size of the abalone. In young, sometimes as little as 6 grams of good quality of food can give a 1 gram weight gain, but more typically, 10-15 grams or more are required for the same weight gain. The growth of the *Haliotis* species is slow and depends upon the species. Usually, it takes a minimum of four years to reach a shell length of 4-6 cm, (Newman, 1968; Forster, 1967). Gross growth efficiency (K) may be defined as the efficiency with which food is converted into body tissues energy and is the relationships between growth efficiency and ration provided a useful means of comparing growth data for individual animals in relation to body size, ration, temperature and other factors (Hughes, 1971; Widdows, 1978).

In abalone the sexes are separate; the only macroscopic difference in the two sexes is in the colour of the gonad, when it is well developed. In males the colour of the gonad is cream to white; in females the gonad varies from dark-green to yellow-grey (Morse *et al.*, 1980). In warm climates they may spawn continually throughout the year (Boolootian *et al.*, 1962; Leighton and Boolootian, 1963; Weeber *et al.*, 1969), but in colder areas spawning may occur only during the warm months of summer (Crofts, 1937; Forster, 1962; Young and De Martini, 1970; Poore, 1973; Shepherd and Laws, 1974; Clavier, 1992b).

In *H. tuberculata*, spawning occurs when the specimens are 6.5 cm long (Berthou *et al.*, 1985). Spawning of cultured abalone is induced by sudden changes in water

temperature (Webber and Giese, 1969; Clavier, 1992b), exposure to UV-light irradiated seawater and by exposure to Tris-buffered hydrogen peroxide (Morse, *et al.*, 1977), exposure to air or release of gametes by other spawning abalone. A sudden contraction of the foot muscle caused by such factors forces out the eggs and milt. Abalone are highly fecund and a large red abalone may spawn as many as 10 million eggs at a time (Stephenson, 1924). Fertilisation is external and fertilised eggs sink to the seabed, where embryonic development takes place (Webber and Giese, 1969).

Abalone represent to many countries one of the most important hand collected fisheries on the coastline. This is because of its highly commercial value, around 70 USA dollars per kilogram of meat, which make this fishery highly attractive to sports-divers and commercial fishermen. Nevertheless, they are relatively easy to collect, and, as a result, this natural resource has been dramatically in the USA decreased. Under those circumstances, in 1939 the commercial abalone industry in the USA, was asked to offer suggestions for the regulation of the fishery supervised by a group of Fish and Game Biologists in California (Cox, 1962; Harrison, 1969; Tegner, 1992). Moreover, *H. tuberculata* fisheries had a closed season for three years between 1974-1976 (Mgaya and Mercer, 1994) in the hope that populations might recover (Clavier, 1992a).

In the English Channel Islands and France, ormer fishing is restricted to the intertidal zone and the number of days for fishing is limited by appropriate tides. From 1973, divers with scuba gear were prohibited from catching ormers to avoid the possibility of ormer populations disappearing completely (Bossy and Culley, 1976).

There are several methods of fishery regulation, aimed at reducing fishing effort: licensing, bag limits, closed areas and seasons and restrictions on the fishing methods (Bossy and Culley, 1976; Berthou *et al.*, 1985). The minimum legal size of *Haliotis* spp. is 5-7 cm in California (Hahn, 1989) and 8 cm in Europe (Clavier, 1992a; Ebert,

1992). Therefore, to increase the natural stock, the fishery co-operatives, university researchers (Ebert, 1992) and the private sector have been forced to develop abalone hatcheries to produce juveniles (Henderson *et al.*, 1988; Schiel, 1992; Tegner, 1992).

Abalone have a high commercial value; they are marketed in fresh, frozen, canned and dried forms and are eaten cooked or raw. The foot and epipodial muscles are cut into steaks; in some parts of the world the slices are dried in the sun, after partial cooking, but in California and Mexico they are canned on site for international markets, mainly USA, Japan and China (Guzmán del Próo, 1992). The visceral mass and mantle fringe are also of economic importance. Viana *et al.* (1993b and 1996) used silage from abalone viscera as an ingredient for abalone feed, and this proved to be effective in increasing abalone growth. In addition, shells are used for a variety of jewellery.

There are two principal options available for abalone culture. The first, known as ocean ranching, is an extensive form of abalone farming that requires the release of cultured spat into a suitable natural habitat. The abalone is left to grow in the wild until they are large enough to be harvested. Though this form of abalone farming does not have high running costs, it is risky with potentially high losses through predation and poaching (Tegner and Butler, 1985). This method is practised in Japan (Hahn, 1989), and has been conducted experimentally in New Zealand, United States (Tegner and Butler, 1985; Hooker and Morse, 1985) and Mexico (Salas and Searcy, 1992). Moreover, the ormer has been cultured in Guernsey, Channel Island for 15 years with varying degrees of success (Hjul, 1991; Tostevin, personal communication, 1997).

The second option involves the intensive captive spawning and rearing of abalone to marketable size. Hatchery-reared spat are grown out to between 5 to 8 cm. There are two options for rearing abalone intensively. Juveniles can either be kept in shore-based tanks (Figure 1.4), which requires a large investment in land and equipment, with

concomitant high operating costs. Alternately, abalone may be reared in containment systems in sheltered waters. In the latter option abalone are placed in cages or floating rigs (often barrels) (Figure 1.5) suspended from buoys or anchored to the sea bottom. The disadvantages of this system include difficulties of feeding the abalone, continual maintenance, and potential damage from rough seas and stormy weather (Hooker and Morse, 1985; Tostevin, personal communication, 1997).

Figure 1.4. Rearing abalone in shore-based tanks containing 20 l buckets. The abalone are fed on a variety of seaweeds or formulated diets (pellets).

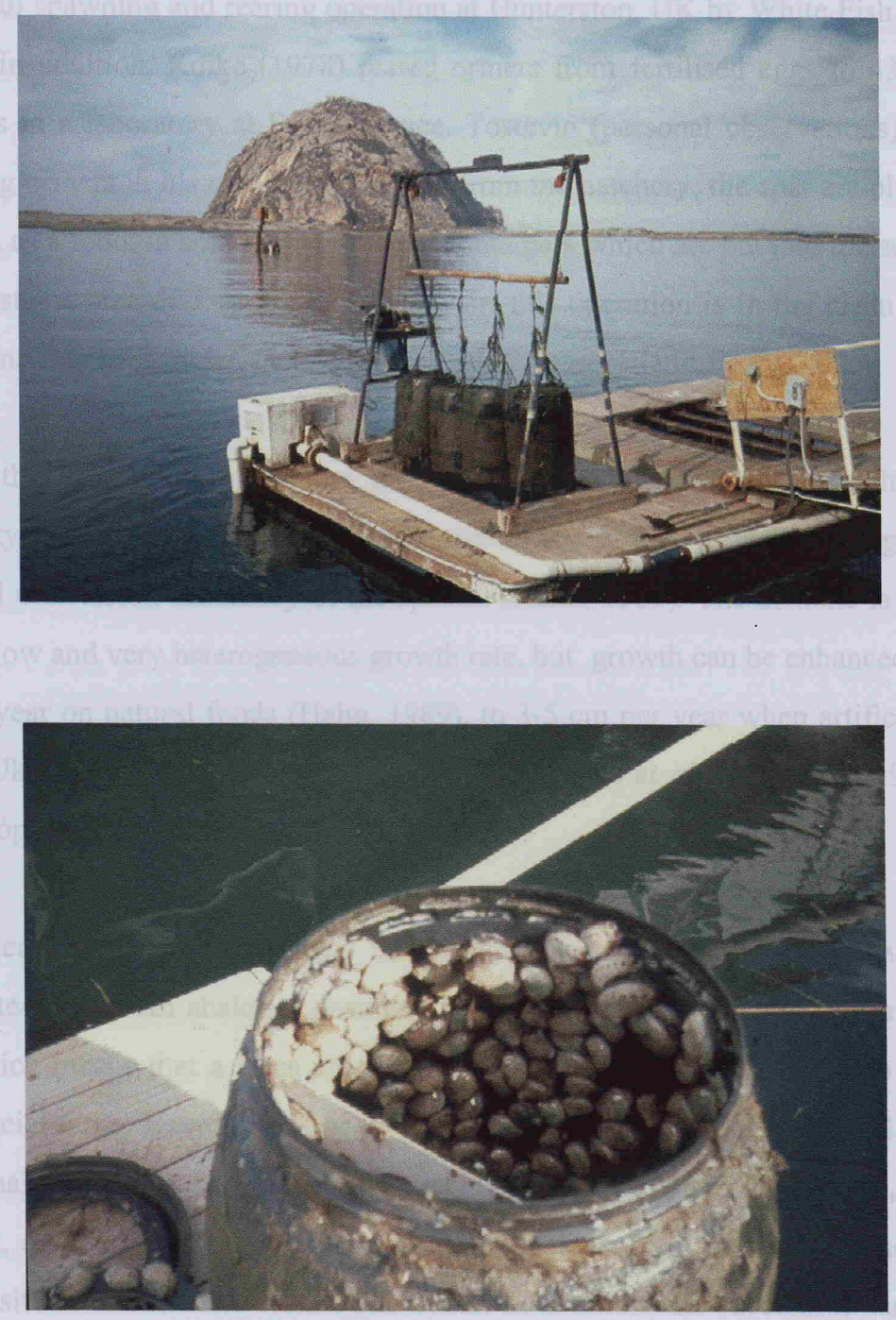

Figure 1.5. Rearing abalone in barrels attached to floating rigs. The abalone can be fed with variety of seaweeds. In these systems is not possible to use formulated diets (pellets).

Several attempts have been conducted on the culture of *H. tuberculata,* with a successful spawning and rearing operation at Hunterston, UK by White Fish Authority (1968). In addition, Koike (1978) reared ormers from fertilised eggs to 435 day-old juveniles in a laboratory at Brest, France. Tostevin (personal observations) has been spawning ormers in his own hatchery, and from the hatchery, the spat are placed, with seaweed as a food, in small barrel-shaped containers which are put into the sea to grow to harvestable size of 7 to 8 cm. The site for this operation is in the clean waters of Rocquaine Bay of Portelet, Guernsey, English Channel Island.

One of the major limiting factors for successful viability of abalone culture is the capability to secure a viable and cost effective source of food as well as the growth rate and feed conversion efficiency of the species (Hahn, 1989). The abalone is an animal with a slow and very heterogeneous growth rate, but growth can be enhanced from 2-3 cm per year on natural foods (Hahn, 1989), to 3-5 cm per year when artificial food is given (Uki *et al.*, 1985a and b; Nie *et al.*,1986; Viana *et al.*, 1993a and 1996; Britz, 1996; López *et al.*, 1998).

The selection of available diets includes harvested and cultured seaweed and formulated feeds. An abalone consumes 10-39 % of its body weight of seaweed each day, which means that a large amount of this food would be necessary to develop a commercial farm. Seaweed has geographical and seasonal variation in availability and nutritional value (i.e. low protein content) (Uki, *et al.*, 1985b; Mai *et al.*, 1994). In addition, difficulties of harvesting, and the high transport cost of wet algae from the harvest site to the abalone culture site occur. In addition, the successful harvesting of seaweed is dependent on the sea conditions. This will obviously present problems with respect to obtaining a regular supply. Furthermore, utilising algae as the principal source of nutrition is costly and labour intensive when the expenses of harvesting, drying and storage are taken into account (Hahn, 1989).

It has been demonstrated that the production costs of seaweed as a diet for *H. midae* are estimated to be more expensive than using a formulated feed. The production costs using seaweed are approximately one and a half times that of using formulated diets (Hahn, 1989). In many countries there is a growing interest in the seaweed industry and competing interest for kelp will make it an unreliable food resource. In addition, it would be expensive to maintain or to culture an adequate supply of kelp (Viana *et al.*, 1993a). Under those circumstances most of the abalone culture facilities in Japan use formulated food after the juveniles are removed from the settled plates (used for rearing post-larval stage with diatoms) (Hahn, 1989).

It has been shown that the abalone *H. discus, H. discus hannai and H. sieboldii* (Nie *et al.*, 1986; Hahn, 1989), *H. fulgens* (Viana *et al.*, 1993a and 1996), *H. midae* (Britz, 1996) and *H. tuberculata* (Mai *et al.*, 1995; López *et al.*, 1998) fed with various formulated diets grow faster than those fed with natural algae. The increase of growth rate is attributed to the higher protein content and protein quality of the formulated feed provided (Uki *et al.*, 1985a and 1985b; Uki and Watanabe, 1992).

Feed costs constitute between 50 and 70% of the operating costs of rearing fish and shellfish from stocking size to market size (Brown *et al.*, 1989). This accentuates the importance of a correct balance of nutrients in the diet to reduce grow-out time. The purpose of formulated diets is to supply the correct nutrient concentration in a readily available form to enable optimum utilisation of nutrients for somatic growth (Phillips, 1972; Lovell, 1989). However, many abalone farmers require more from a feed than nutritional quality and cost-effectiveness.

From the late 1980s there has been a rapid increase in the number of research groups developing formulated diets to supplement or replace seaweed in abalone aquaculture. Formulated diets for use during ongrowing of abalone to marketable size are an important option to enhanced the naturally slow growth rates (~ 40 μm day^{-1}) of

abalone and high cost of holding them in culture systems. For these reasons, a good balanced (averaging around 30% protein, 4% lipid, 47% carbohydrate and 2% crude fibre) diet is seen as essential to successful abalone farming by those countries whose abalone do not only grow on seaweed (Fleming *et al.*, 1996) (Table 1.3). Those who have historically harvested kelp forests as a food source are now keen to supplement this with formulated diets (Fleming and Hone, 1996). Moreover, Japan has formulated diets for abalone for over 30 years, which has culminated in the commercial production of high quality feeds.

Table 1.2. Summary of current and past research into the development of formulated feeds for abalone.

Country	Research Group	Research status	Production status
Australia	-School of Biological and Chemical Sciences, Deakin University	Late 1980s-Early 1990s	R and D, pilot production R and D
	-Marine Sciences Laboratories and Southwest Seafood Pty. Ltd.	Late 1980s-Early 1990s	R and D
	-Key Centre for Aquaculture, University of Tasmania	Current	R and D
	-Fisheries Research and Development Corporation/Co-operative Council	Current	
Canada	-Zoology Department, University of British Colombia	Current	R and D
China	-Yellow Sea Fisheries Research Institute	Late 1980s	R and D/commercial production
	-Dalian Bilong Seafood Co. Ltd.	Current	Commercial production
	-Marine Fisheries Research Institute of Liaoning Province	?	Commercial production
	-Dalian Marine Fisheries Research Institute	?	Commercial production
France	-Argenton Experimental Station	Late 1980s	R and D
Ireland	-Shellfish Research Laboratory, University Collage Galway	Current	R and D
Japan	-Nihon Nosan Kogyo K.K.	Current	R and D/commercial production
	-Nihon Haigo Siryo Co., Ltd.	?	Commercial production
	-Cosmo Venture Inc.	?	Commercial production
	-Hayashikane Sangyo Co., Ltd.	?	Commercial production
	-Nippon Formula Feed Manufacturing Co	?	Commercial production
	-Sakai and Co., Ltd.	?	Commercial production
Korea	-National Fisheries Research and Development Agency	Current	R and D
Mexico	-Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California	Current	R and D
New Zealand	-Promak Technology (NZ) Ltd.	Current	R and D/pilot production
South Africa	-Zoology Department, Rhodes University and Sea Plant Products Ltd.	Current	R and D/pilot production
	-Zoology Department, Cape Town University	Current	R and D
Thailand	-Institute of Marine Sciences, Burapha University	Current	R and D

Summarised from Fleming *et al.* (1996). R research and D developmental.

CHAPTER 2

Reproduction of cultured *Haliotis tuberculata*

2.1. INTRODUCTION

Because of the increasing demand, diminishing natural stocks and regulatory measures used to protect and manage the natural resource, attention has been directed to develop abalone hatcheries. Moreover, to enhance the natural abalone populations, transplantation and stocking of open waters have been carried out mainly in Japan, USA, Australia, Mexico and New Zealand (Tong *et al.*, 1987; McShane, 1992; Salas and Searcy, 1992).

The successful culture of any species relies primarily on the ability to control many aspects of reproduction including spawning, fertilization, hatching, larval care and induction to settle. Control of reproduction remains one of the principal barriers to the economic cultivation of abalone (Ebert and Houk, 1984). For species which are able to mature and spawn under conditions of intensive culture, controlled reproduction can provide seed at precisely those times required by on-growing farms. For most wild animals, spawning is characteristically an annual event with mature eggs and sperm being produced at a time of the year when external conditions, and supplies of available food, are most favourable for the survival of the embryos and larval stages (Cox, 1960; Webber and Giese, 1969; Ebert and Houk, 1984).

In *Haliotis* species the sexes (a single gonad) of mature abalones can be determined by visual inspection if the foot and mantle are forced away from the right side of the shell to expose the horn-shaped conical appendage. The gonad is closely associated with the digestive gland, and it occupies the same position in both sexes. The only macroscopic difference in the two sexes is the colour of the gonad. In males the colour of the gonad is cream to white and in females it is dark-green (Cox, 1962). However, immature or spent ovaries may be cream coloured like the testis, and it may not be possible to

distinguish sexes (Forster, 1962).

Abalone are broadcast spawners and they release their gametes (eggs and sperm) directly into the sea water (Stephenson, 1924; Crofts, 1929), where fertilization takes place. The diameter of the unfertilised egg, including the egg membrane, varies between species from 200 μm to 280μm (Koike, 1978). The eggs are negatively buoyant and generally hatch within 24 hours post-fertilization (Mottet, 1978).

Gametogenesis and spawning period appear to be modulated by environmental differences, including seawater temperature, physical disturbance, food supply, and genetic and hormonal factors (Shepherd *et al.*, 1985) and there is considerable variation even in the same species from one season to the next. In warm climates they may spawn throughout the year (Boolootian *et al.*, 1962; Leighton *et al.*, 1963; Weeber *et al.*, 1969), but in colder areas spawning may be only during the warm months of summer (Crofts, 1937; Forster, 1962; Young and De Martini, 1970; Poore, 1973; Shepherd and Laws, 1974; Clavier, 1992b).

In *H. tuberculata* gonadal maturation has been found to start in animals in the size range 3.8 cm to 5.4 cm shell length at 3 to 4 years old (Hayashi, 1980a; Clavier, 1992b) and they can spawn at 5.55 cm (Stephenson, 1924; Hayashi, 1980a), in their natural environment. Nevertheless, it was found that when ormers are fed on a formulated diet and cultured above 15^{o}C it is possible to start developing the gonad at a smaller size of 1.09 cm length. Moreover, spawning in these animals was reached when they were 13 months of age (Chapter 5).

Wild or hatchery-reared brood stock can be used for controlled production of seed. If wild brood stock are used it is considered essential to condition their gonad for two or three weeks before the spawning season (June-July). Spawning can be achieved with a

combination of temperature and good quality food (Tostevin, personal communication, 1996). Moreover, there are a variety of induction-techniques; abalones that are less receptive can be induced by desiccation, if they are left out of seawater for about 30 to 60 minutes (Genade *et al.*, 1988), and temperature shock by changing the seawater temperature increasing up to 6^{o}C (Imai, 1967; Webber and Giese, 1969).
One of the methods that is considered most reliable involves the use of UV-light, where seawater is irradiated with an ordinary UV-light steriliser, and apparently causing some photo-chemical reaction (breaking up the water molecules and creates small quantities of hydrogen peroxide) to take place in the seawater (Hayashi, 1982). Abalones which are exposed to this irradiated seawater, typically spawn within 2 to 3 hours (Kikuchi and Uki, 1974). The most recent chemical method involves exposing mature abalone to a solution of hydrogen peroxide (to a concentration of ~ 5 mM in alkaline seawater pH ~ 9.1), which causes spawning in both male and female abalones about 3 hours after the first addition (Morse *et al.*, 1977).

In the culture of molluscs, the processes of inducing settlement, metamorphosis and early postlarval survival are probably the most vulnerable phases of the life cycle of abalone (Hahn, 1989). These aspects are considered the critical stage of abalone seed production with mortalities from 90 to 99 % for *H. discus, H rufescens and H. fulgens* (Pyen *et al.*, 1981; Ebert and Houk, 1984; Searcy-Bernal *et al.*, 1988).

In most species, the planktonic trochophore larvae hatch out between 6 to 24 hours under favourable temperature conditions (20°C) (Koike, 1978) . The veliger stage lasts for about a week, by which time the complete shell and operculum have developed (Hahn, 1989). In the absence of a suitable substratum the larvae can prolong the planktonic stage for up to about 3 weeks (Leighton, 1974; Searcy-Bernal *et al.*, 1992), which is probably time enough to be dispersed over considerable distances (Forster *et al.*, 1982; Tegner and Butler, 1985). However, transport of larvae away from suitable

habitat could cause high mortality (Thorson, 1950). Larvae also die because of predation (Pennington *et al.*, 1986) and starvation (Olson and Olson, 1989).

Settlement is defined as contact with substrata leading to metamorphosis from the pelagic to the benthic form (Keough and Downs, 1982). For successful settlement it is necessary to make contact with suitable substrata before the larvae starve to death. Abalone settlement and further metamorphosis are rapid on coralline red algae including species of *Lithothamnium*, *Lithophyllum* and *Hildenbrandia* (Crofts, 1929; Morse and Morse, 1984). This is reported to be a result of a biochemical inducer present on the surface of these algae or because concavities in the coralline algae provide shelter for settling abalone larvae from mobile grazers such as sea urchins, adult abalone, marine snails, or limpets (Crofts, 1929). Moreover, a variety of microalgae, bacteria (Morse *et al.*, 1979; Morse and Morse, 1984) and abalone mucus (Seki and Kan-no, 1981) have been proved to help settlement and metamorphosis.

Artificial metamorphosis may be induced with chemicals, which contain the neurotransmitter gamma-aminobutyric acid (GABA) (Morse and Morse 1984). GABA encourages settlement and metamorphosis by depression of cilia beating in abalone larvae. Abalone larvae do not require the presence of a crustose coralline algal substratum to settle (Morse and Morse, 1984), when GABA is used. GABA was the most efficient method for metamorphosis induction (98%) in *H. rufescens* (Searcy-Bernal *et al.*, 1992). Nevertheless, survival after metamorphosis by this method was low.

Several studies have been conducted on the culture of early juvenile abalone in relation to their main diet. Most of them show that, after settlement and metamorphosis, benthic diatoms and other microscopic algae are a very suitable diet until juveniles reach a shell length of about 20 mm (Norman-Boudreau *et al.*, 1986; Ebert and Houk, 1989; Kawamura *et al.*, 1995; Matthews and Cook, 1995). Naturally-occurring benthic diatoms are allowed to settle on plastic plates for use as an initial food. Gut content

analyses of abalone §post-larvae have shown that prostrate diatoms such as *Cocconeis sublittoralis*, *Amphora proteoides* and *Achnanthes brevipes* (Matthews and Cook, 1995), *Achnanthes brevipes*, *Achnanthes longipes*, *Cocconeis scutellum* and *Cylindrotheca closterium* (Kawamura *et al.*, 1995), *Cylindrotheca closterium* (Kawamura and Takami, 1995), *Navicula* sp and *Cocconeis* sp. (Norman-Boudreau *et al.*, 1986) were the preferred diet for the culture of several species of abalone.

2.2. MATERIALS AND METHODS

2.2.1. Adult conditioning

Two similar experiments were performed in summer 1996 and 1997, at Rocquaine Shellfish Ponds in Guernsey. In mid June 1996 and 1997, about 50 males and 80 females, naturally matured cultured ormers, between 86.20 mm and 121.90 mm length were collected and placed in a buoyant barrel at 8 to 10 m below the seawater surface and fed on a mix of fresh seaweed (*Palmaria palmata* (70%), *Laminaria digitata* (20%) and *Ulva lactuca* (10%). After two to three weeks of conditioning in their natural environment; on the early morning of 29 June and 17 July 1996 and 11 and 25 July 1997, 25 males and 40 females were placed, separated by sexes, in fresh seawater until arrival at the hatchery of Rocquaine Shellfish Ponds (~ 30 min). From this stock 12 males and 8 females were chosen randomly for experimentation; the rest were used by Mr Tostevin to run his annual spawning.

2.2.2. Spawning

Once in the hatchery the abalone were placed into 20 litre plastic containers. Two to three adults of the same sex were placed in each container with fresh heated and aerated seawater (initial temperature 17°C at 09:00 hours) with changes every two

§ The term post-larvae is used for those individuals that have already settled. This term has been used regularly to describe this stage (Kawamura et al., 1995; Matthews and Cook, 1995). Strictly speaking post-larvae are pre-settled once settled the correct term should be juvenile.

hours, until 17:00 hours. At 17:00 hours fully gravid broodstock of each sex were weighed, measured and placed in separate containers filled with exactly 5 litres of heated seawater (20°C) in order to calculate the number of eggs and sperm produced by each animal. A water change was made every hour. During the day culture seawater temperature rose slowly, naturally to 21.5°C.

2.2.3. Fertilization

The quantity of eggs spawned was estimated by analysing three 1 ml samples collected with an automatic pipette from each container. Sperm concentration was determined by progressive dilutions of 5 to 10 aliquots of 0.5 ml using a haemocytometer. From the best stock the fertilization was done within 20 minutes post-spawning in 40 litre plastic containers, adding 3 ml of fresh sperm (20,000 to 80,000 sperms in each millilitre) to each container, with a density of approximately 5,000 eggs, gentle stirring for about one minute using a plunger was conducted to avoid polyspermy.

After fertilization, eggs sank to the bottom and the fertilized ones were passed through a mesh with 100 µm pore size, into round containers. The containers were then decanted and refilled two times to eliminate excess sperm and to keep the fertilized eggs in the cleanest seawater possible. For the next 10 to 13 hours fertilized eggs were left undisturbed. After hatching, the new trochophore larvae swim to the surface, and the seawater was siphoned out removing the debris and replaced by clean seawater. After this, the larvae were not stressed in any way for about 35 hours until operculum formation and the veliger larvae were capable of retracting into the shell.

2.2.4. Larval settlement

Sixty-four hours post-fertilization, the most active larvae were transferred to the settling containers (25 litres) with a set of 10 (40 × 20 mm) glass plates in each one,

standing at about 30 degrees from the vertical. In order to estimate the concentration of the larvae, it was necessary to count the larvae using 1 ml samples collected with an automatic pipette. Three to five aliquots were taken depending on the variation between aliquots. The concentration was about 15,000 to 20,000 larvae in each container. The most active larvae were used first and the settling containers were marked in order to identify these larvae. After introducing the larvae into the settling containers no change of seawater was made for two days to give an opportunity for the larvae to settle in high concentrations. After two days seawater was drained through a 100 μm sieve to check that the larvae were alive from the sieve and placed in new containers (named poorly settled larvae). Change of seawater and food was carried out every other day for ten days, thereafter changes of seawater and food were done every day for the next eight weeks.

For the 1996 experiment, the kind of food offered to the settled larvae followed the traditional culture done by Mr Tostevin (*T. suecica*) and for the 1997 feeding a *Skeletonema costatum* was used at approximately 300 cells ml^{-1}.

2.2.5. Grow-out

At eight weeks old the juveniles were removed gently, with a sponge, from the glass and walls of the containers and put into cartons (25 × 20 ×10 cm) with a one mm nylon mesh to be cultured in the sea on floating rings. The concentration in each carton was approximately 500 juvenile abalone. Prior to transferring the juveniles, the cartons were filled with delicate *Palmaria palmata* as the best nutritional and natural available food for *H. tuberculata*.

2.3. RESULTS

2.3.1. Spawning

The spawning trials were made when the surface of the seawater of Rocquaine Bay

was 16.5°C. Fully gravid broodstock were taken from the floating barrels. The females presented green bulging gonads and the males had creamy gonad. Spawning was induced by raising the temperature of the seawater slowly in the containers with abalones, starting at 09:00 hours at 17°C. By 17:00 hours the seawater temperature had risen to 20°C. After an average of 9 hours, when the seawater temperature was 21.5°C, males started to spawn and females after a further one hour, at the same water temperature.

On 29 June 1996 up to 60% of the males and 62.5% of the females had spawned by 22:00 hours. On 17 July 1996 and 11 July 1997 100% of the males and 87.5% of the females spawned by 21:57 hours. Moreover, on 25 July 1997 100% of males and females spawned by 20:27 hours (all these data from a lot of 12 males and 8 females). The number of eggs ranged from 698,724 (shell length 89.14 cm) to 2,163,141 (shell length 120.34 cm) for an individual (Table 2.1, Figure 2.1). However, in fifty percent of the eggs from the first spawning (29 June 1996) the yolk was not centrally situated and these eggs could not be used for rearing experiments. Spawnings on the next three dates, produced 80 to 100% viable eggs.

Sperm concentration could not be determined from 1996 and 1997 samples. On return to Southampton the samples of sperm had coagulated and could not be counted. However, the same experiment was performed in summer 1999 and it was possible to have the necessary equipment to calculate the number of sperm from each individual spawning during approximately 45 minutes. Number of sperm was variable in relation to animal shell length, $Y = (-2.96) + 0.05(X)$ ($r^2 = 0.51$, $P < 0.005$) and wet body weight, $Y = -0.12 + 0.01(X)$ ($r^2 = 0.35$, $P < 0.005$). A male of 110 mm shell length and 206.44 g body wet weight released 1.26×10^{11} sperms in 45 minutes, whereas an animal with 70% less on body weight released the same quantity of sperm during the same time (Table 2.2, Figure 2.2).

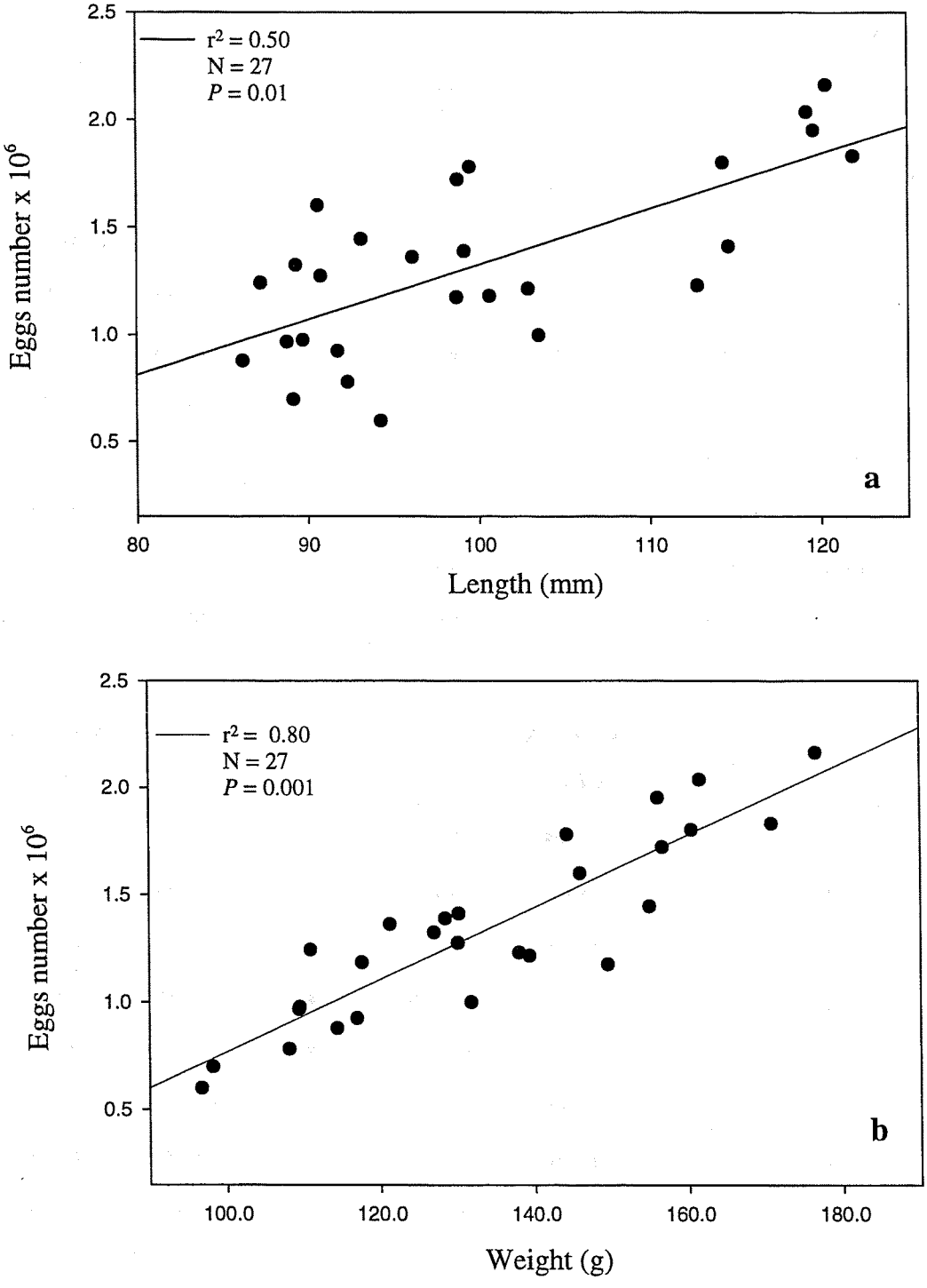

Figure 2.1. Relationship between the number of eggs released and the shell length from the equation $Y = -1.24 + 0.026(X)$ (a); the number of eggs released and the total weight $Y = -0.91 + 0.017(X)$ (b) of *Haliotis tuberculata*. N is the number of observations and r^2 is the correlation coefficient.

Table 2.1. *Haliotis tuberculata*: number of gametes (eggs) released during approximately 45 minutes.

Length (mm)	Weight (g)	Spawn time	Number of eggs	Length (mm)	Weight (g)	Spawn time	Number of eggs
29 June 1996				17 July 1996			
114.30	160.50	20:37	1,799,538	93.17	155.00	20:05	1,445,394
91.62	95.06	---	No spawn	86.20	114.41	20:42	877,664
98.70	149.60	20:45	1,175,550	89.71	109.50	19:55	975,614
87.41	79.11	---	No spawn	102.90	139.40	20:48	1,215,514
91.76	117.00	21:32	924,633	99.50	144.30	20:31	1,779,281
112.60	142.30	---	No spawn	119.20	161.60	19:49	2,037,080
121.90	170.90	21:03	1,830,480	114.31	100.16	---	no spawn
103.50	131.80	21:12	998,633	90.77	130.08	20:17	1,274,489
11 July 1997				25 July 1997			
94.27	96.71	21:02	599,778	89.30	127.00	19:22	1,324,481
102.55	94.40	---	No spawn	114.61	130.20	18:11	1,411,445
119.60	156.15	20:31	1,951,202	92.34	108.12	19:35	780,701
90.60	146.00	20:18	1,599,353	99.17	128.47	20:27	1,389,346
112.80	138.07	20:28	1,229,508	120.34	176.65	20:02	2,163,141
98.78	156.70	21:28	1,719,311	88.79	109.38	18:35	966,629
89.14	98.16	20:57	698,724	96.14	121.34	19:41	1,362,459
87.24	110.95	20:11	1,242,500	100.64	117.70	19:19	1,182,527

Table 2.2. *Haliotis tuberculata*: number of gametes (sperm) released during 45 minutes.

Length (mm) Males	Weight (g)	Number of sperm
110	206.44	1.26×10^{11}
94.5	122.96	1.37×10^{11}
94.0	118.5	2.43×10^{11}
91.5	122.91	8.8×10^{10}
87.0	111.49	1.32×10^{11}
86.0	97.48	1.86×10^{11}
82.0	112.5	1.55×10^{11}
75.1	65.9	2.9×10^{10}
73.4	54.0	1.3×10^{11}
70.7	72.4	4.5×10^{10}
69.6	63.8	9.1×10^{9}
69.2	59.04	1.9×10^{10}

2.3.2. Fertilization

The egg size was 205 ±7 µm diameter in all spawnings. Fertilization was complete within 20 to 25 minutes post spawning, in a seawater temperature of 21 ± 1°C. The eggs took about 15 to 20 minutes to settle to the bottom, and during the next 10 hours two changes of seawater were made. After 1 to 2 hours, the first cleavage took place along the vertical axis of the egg and hatching took place 18 to 20 hours after fertilization. For the next 35 to 40 hours the larvae were not disturbed. After this time veliger torsion was completed and at 64 hours after fertilization the larvae were ready to be transferred to the settling containers. During the seawater changes gentle stirring

was necessary in order to bring debris to the middle of the round container. This was siphoned off through a mesh with 100 µm pore size.

2.3.3. Larval settlement

The abalone larvae were not induced to metamorphose and settle artificially, although the settling holders contained naturally occurring benthic diatoms from running clean filtered seawater from Rocquaine Bay off Portelet, seven days prior the settling day.

Early juveniles reared in 1996 were fed on *Tetraselmis suecica* as a main food at ~ 80 cells ml^{-1} and early juveniles reared in 1997 were fed on *Skeletonema costatum*, at about 300 cells ml^{-1}.

Early juvenile mortality in organisms reared on *T. suecica* (1996) was approximately 35 % higher than those organisms fed on *S. costatum* (1997).

2.4. DISCUSSION

The natural spawning season of *H. tuberculata* in Guernsey populations occurs mainly in the summer over a rather short period, from July to September (Stephenson, 1924; Hayashi, 1980). Our data show that the best time to start with semi-artificial spawning, using a slow increment of seawater temperature, was from mid July. In 1996 and 1997 a lower percent of males and females spawned in June, compared with the spawning in July.

Spawning-induction techniques (desiccation, temperature shock, irradiated seawater using UV-light and hydrogen peroxide) have shown significant contributions to abalone cultivation. However, we found that for an annual production, the most natural way to condition the gametes was using slight increments of seawater temperature throughout the day. This does not induce stress to the animals, thus gametes and larvae quality is high.

The results of the present study suggest that the spawning season starts in July using

cultured abalone from the open sea. This slight difference in the timing of the spawning season compared to Hayashi's (1980) results and those presented here may be a result of the differences between wild animals and cultured ones.

A power relationship between the number of mature eggs in the gonad and body weight has been reported for *Haliotis* species (Newman, 1967; Poore, 1973; Kikuchi and Uki, 1975; Clavier, 1992). A correlation between the number of eggs in the gonad and shell length has been found in *H. tuberculata* (Girard, 1972; Hayashi, 1980).

It might be said that these data had been calculated with a small size range, for females was between 86.2 to 121.9 cm shell length and for males was between 69.2 to 110 cm shell length. Using that size range we found that the number of eggs spawned increased when body weight increased (Figure 2.1a). The present observations for *H. tuberculata* coincide with those of Clavier (1996), who reported that the number of eggs shed during induced spawning ranged from 2×10^5 (20 g body weight) to 1.6×10^6 (145 g body weight) (Table 2.1). The fit of the relationship between the number of eggs spawned and body weight was $Y = -0.91 + 0.017(X)$ ($r^2 = 0.80$; $P = 0.0001$). Nevertheless, the relationship between the number of eggs spawned and the shell length was low $Y = -1.24 + 0.026(X)$ ($r^2 = 0.50$; $P = 0.0001$) (Figure 2.1b). It is different to that of Girard (1972) and Hayashi (1980) for the same species, discussed above.

In males, the number of sperm released appeared to be independent of animal size $Y = -2.96 + 0.05(X)$ ($r^2 = 0.51$, $P < 0.005$) and animal wet body weight, $Y = -0.12 + 0.01(X)$ ($r^2 = 0.35$, $P < 0.005$) (Figure 2.2a and b).

The number of eggs spawned has a weak relationship with the shell length, as our results indicate, although the data is compromised by the restricted nature of the size range. The same relationship was observed in that the number of sperm released was

independent of the shell length and body weight of the animals. The age of the animals used in this study were known and those organisms with a shell length above 90 mm were the oldest ones (5-6 years). In general, females and males at four years old were the best reproducers. This may suggest that mature cultured reproductive organisms could be selected by their age. In wild populations where the age is unknown, it may be easy to confuse either old slow-growing individuals or relatively young fast-growing specimens.

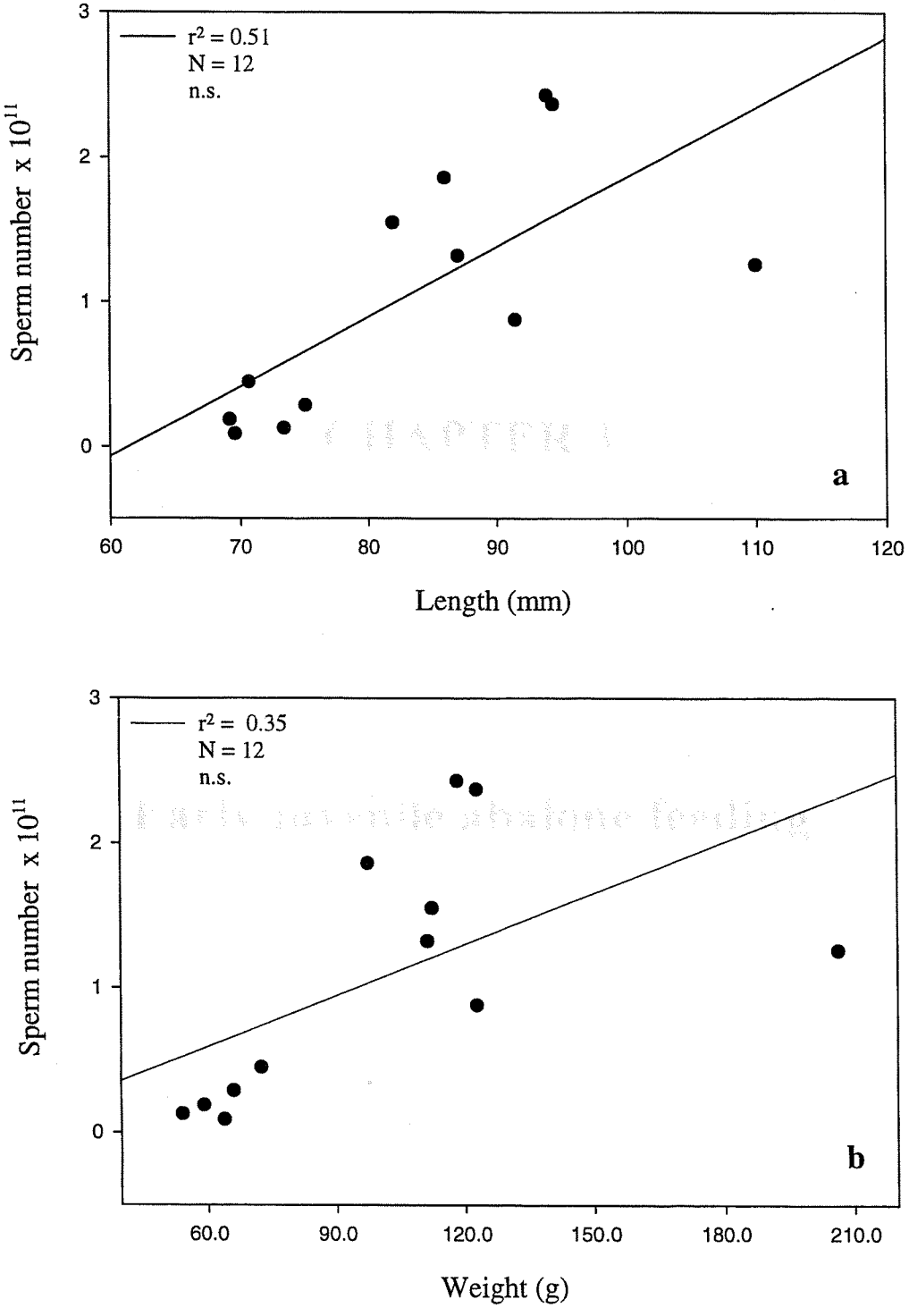

Figure 2.2. Relationship between the number of sperm released and the shell length from the equation $Y = -2.96 + 0.05(X)$ (a); the number of spem released and the total weight $Y = -0.12 + 0.01(X)$ (b) of *Haliotis tuberculata*. N is the number of observations and r^2 is the correlation coefficient. n.s., not significant.

CHAPTER 3

Early juvenile abalone feeding

3.1. INTRODUCTION

In the culture of molluscs, early juvenile mortality has shown high levels, exceeding 90%. There are several factors responsible for early juvenile mortality in nature: seasonal changes in physical conditions, predation, competition for space, disease, and competition for food (Gosselin and Qian, 1977). Throughout the world in abalone seed production some of these factors may be under control, nevertheless reported mortality is from 90 to 99% in *H. discus, H. rufescens*, *H. tuberculata* and *H. fulgens* (Pyen *et al.*, 1981; Ebert and Houk, 1984; Searcy-Bernal *et al.*, 1988; Hahn, 1989).

It has become critical to improve the survival and growth rates of early juvenile abalone by providing them with suitable food (Fleming and Hone, 1996). Inherent to this purpose is the need to determine any selectivity or preference that early juvenile abalone may show for a special type of food. For this reason, several studies have been conducted on the culture of early juvenile abalone regarding their main diet. For most species, after settlement and metamorphosis, benthic diatoms and other microscopic algae are a very suitable diet until juveniles reach a shell length of about 20 mm (Norman-Boudreau *et al.*, 1986; Ebert and Houk, 1989; Kawamura *et al.*, 1995; Matthews and Cook, 1995, Roberts, *et al.*, 1999). Moreover, Kawamura and Takami (1995) showed that the extracellular substances of diatoms are important food for early juvenile abalone up to the size of 800 μm shell length. From this point of view, early juvenile abalone consume a variety of diatoms, nevertheless, the diatoms themselves cannot be digested. This is probably owing to the strong cell walls and size of the diatoms which are not easily deformed or broken by the radula when they are grazing at this stage (Kawamura *et al.*, 1995).

Naturally-occurring benthic diatoms are allowed to settle on plastic plates to use as an initial food. Gut content analyses of abalone post-larvae show that prostrate diatoms

such as *Cocconeis sublittoralis*, *Amphora proteoides* and *Achnanthes brevipes* (Matthews and Cook, 1995), *Achnanthes brevipes*, *Achnanthes longipes*, *Cocconeis scutellum* and *Cylindrotheca closterium* (Kawamura *et al.*, 1995; Roberts, *et al.*, 1999), *Cylindrotheca closterium* (Kawamura and Takami, 1995), *Navicula* sp. and *Cocconeis* sp. (Norman-Boudreau *et al.*, 1986) were the preferred diet for the culture of several species of abalone (Table 3.1). From these diatom species, *Cylindrotheca closterium* has the best digestion efficiency and produces the best growth rates in early juvenile abalone up to 3 mm shell length (Kawamura *et al.*, 1995; Kawamura and Takami, 1995). On the other hand, flagellate microalgae, bacterial-organic species have a higher significance for juveniles (Ryther and Goldman, 1975) owing to their nutritional value.

Juvenile *H. ruber* rasps the surface of crustose coralline algae, removing and ingesting bacteria and the cuticle secreted by the epithelium (Garland *et al.*, 1985). The bacteria, may be benthic or associated with substrata.

In addition to the analysis of feeding, mortality and growth rate of early juvenile abalone have been under investigation since 1952 (Ino, 1952). In Guernsey, English Channel Isles and France abalone farmers are still probably using the wrong kind of food. An investigation concerning the concentration and proper diet for abalone *H. tuberculata* after the settling period was conducted in Guernsey in the summer of 1996. Also in the summer of 1998 the same experiment was repeated at the Southampton Oceanography Centre, in order to have all facilities for early juvenile feeding and for diatom culture.

Table 3.1. Benthic diatom species used to feed early juvenile abalone (>1 mm in shell length) to determine selectivity and feeding behaviours. Ng: not given.

Species	Cell length (µm)	Adhesive strength	Preferred Ingested Diatom	Source
Achnanthes brevipes	28.8	+++	Yes	Kawamura *et al.* (1995)
var. *intermedia*	ng	ng	Yes	Matthews and Cook, 1995
Achnanthes longipes	61.5	+++	Yes	Kawamura *et al.* (1995)
Amphora proteoides	Ng	Ng	Yes	Matthews and Cook, 1995
Amphora angusta var. *ventricosa*	38.8	+	No	Kawamura *et al.* (1995)
Cocconeis sublittoralis	Ng	Ng	Yes	Matthews and Cook, 1995
Cocconeis scutellum	19.2	Ng	No	Kawamura and Takami, 1995
var. *parva*	19.5	+++	Yes	Kawamura *et al.* (1995)
	29.0	+++	Yes	Roberts *et al.* (1999)
Cocconeis sp.	≥10	Ng	Yes	Norman-Boudreau *et al.* (1986)
Cylindrotheca	60.5	Ng	Yes	Kawamura and Takami, 1995
closterium	70.5	+	Yes	Kawamura *et al.* (1995)
	22.0	+	Yes	Roberts *et al.* (1999)
Delphineis karstenii	Ng	Ng	No	Matthews and Cook, 1995
Diploneis placida	Ng	Ng	Yes	Matthews and Cook, 1995
Navicula ramosissima	13.3	Ng	Yes	Kawamura and Takami, 1995
	12.8	+	No	Kawamura *et al.* (1995)
	12.0	+	Yes	Roberts *et al.* (1999)
Navicula sp.	≥10	+	Yes	Norman-Boudreau *et al.* (1986)
	30.0	++	Yes	Roberts *et al.* (1999)
Nitzschia palea	Ng	Ng	Yes	Matthews and Cook, 1995
Nitzschia sp.	46.1	+	No	Kawamura *et al.* (1995)
	ng	ng	No	Matthews and Cook, 1995
Pleurosigma sp.	138.1	+	No	Kawamura *et al.* (1995)
	115.0	+	yes	Roberts *et al.* (1999)
Stauroneis constricta	24.6	Ng	yes	Kawamura and Takami, 1995
Synedra investiens	53.3	++	no	Kawamura *et al.* (1995)
Synedra sp.	≥30	Ng	no	Norman-Boudreau *et al.* (1986)

Early juvenile *H. tuberculata* from Guernsey have been feeding on the food available such as: *T. suecica* and also on the small diatoms *Chaetoceros* spp. and *Thalassiosira pseudonana* as a main source of food after settlement on glass plates. Under this culture system the mortalities are 99% until the juveniles reach eight weeks old (Tostevin, personal communication, 1996). For this reason, it is important to determine the most suitable diet for juvenile *H. tuberculata.*

For all these reasons one of the main aims of this study is to provide ormer farmers with sufficient information to increase the number of seedlings for mass production or to inprove juvenile survival. Central to this aim is the need to establish any selectivity or preference that early juvenile *H. tuberculata* may show towards specific kinds of food supplied from local producers of microalgae and diatoms.

3.2. MATERIALS AND METHODS

3.2.1. Experiment 1

Early juvenile abalone *H. tuberculata* were obtained from the spawning in 1996 at Rocquaine Shellfish hatchery described in Chapter 2. The juveniles utilised in experiments were used after 6 weeks of initial rearing on glass plates covered with naturally occurring benthic diatoms at 20 ± 2°C and fed on extra diet (*Tetraselmis suecica*).

3.2.1.1. Juvenile rearing

In order to avoid stress to the animals when trying to remove them from the glass plates, the glass was cut into small pieces with the exact number of individuals on each piece (Forty-five). Forty-five juveniles between 0.89 to 1.25 mm length were placed in 400 ml beakers filled with clean filtered (Millipore HA filter, 0.45 µm) seawater and

aeration was provided. Three replicates per diet were held in a 12h/12h light-dark cycle and food and seawater was changed every morning for nine days.

3.2.1.2. Diet

The three monoculture species used in this study were originally obtained from Guernsey Sea Farms, and were grown in modified Jorgensen's medium containing 0.05 μgl^{-1} of vitamin B_{12}. *T. suecica* (10-18 μm cell length) was selected as a control, because it has been the main source of food for *H. tuberculata* in Guernsey, at 80 to 100 cells ml^{-1}.

The two marine diatoms available were *Thalassiosira pseudonana* (4-6 μm cell length and 4-5 μm cell wide) and *Skeletonema costatum* (6-9 μm cell length). Four different concentrations of *T. suecica* (40, 80, 160 and 320 cells ml^{-1}) and *T. pseudonana* and *S. costatum* (62.5, 125, 250 and 500 cells ml^{-1}) were used to feed the juveniles.

Before changing food and seawater any dead animals were collected and frozen at -20°C. A light microscope, with a camera lucida and a digitising tablet were used to measure the shell length of the juveniles.

3.2.2. Experiment 2

This experiment was conducted at the Southampton Oceanography Centre in order to have the installations and necessary equipment to follow the survival (during the first six weeks after settlement) and growth rate (during the following six weeks after survival experiment) of early juvenile abalone.

The selection of the specific diatoms used for this experiment was based on the high nutritional value as diatoms are rich in essential amino acids and polyunsaturated fatty acids (Brown, 1991).

3.2.2.1. Juvenile rearing

Larvae of abalone *H. tuberculata* used in this experiment were obtained by "semi-

natural" spawning in our laboratory at the end of July 1998. After sixty-five hours post-fertilization, competent larvae were transferred to settling containers (15 litres) with a set of 5 (15 x 30 mm) glass plates in each one, standing at about 30 degrees from the vertical. In order to know the concentration of the larvae, it was necessary to count the larvae using 1 ml samples collected with an automatic pipette. Three to five aliquots were sampled depending on the variation between aliquots.

The concentration was about 5,000 larvae in each container. These containers were stored in a controlled light-temperature room 12:12 light- dark cycle at 20 ± 1°C and aeration was provided.

Clean filtered (Millipore HA filter, 0.45 µm) seawater was provided and after two days the early larvae were fed on a mix of *S. costatum, Cylindrotheca closterium* and *Navicula ramosissima*. Food and seawater were changed every morning for six weeks duration.

3.2.2.2. Diet

Three marine diatom species were grown in monoculture and used to feed early juvenile *H. tuberculata* after 5 days settlement. *S. costatum* was originally obtained from Guernsey Sea Farms, and were grown in modified Jorgensen's medium containing 0.05 μgl^{-1} of vitamin B_{12}. *C. closterium* (~ 28 µm cell length) and *N. ramosissima* (~ 12 µm cell length) were obtained from Provasoli-Guillard National Centre of Culture of Marine Phytoplankton (CCMP) where diatoms were supplemented with f_2 medium containing a mix of trace metals and vitamin solution (Guillard and Ryther, 1962).

First six weeks

Three replicates of fifty juveniles with a mean shell length of 483 ±54 µm were fed on three different concentrations of *S. costatum* (500, 1000 and 1500 cells ml^{-1}), *C.*

closterium (500, 1000 and 1500 cells ml^{-1}) and *N. ramosissima* (500, 1000 and 1500 cells ml^{-1}) during the six week period. Before changing food and seawater, any dead animals were collected and frozen at -20°C. The number of dead individuals was registered in order to assess cumulative mortality for each treatment.

Second six weeks

The remaining animals from the feeding treatments at concentration 1500 cell ml^{-1} from each diet were used to assess daily growth rates over 42 days. Twenty animals per replicate were held in a controlled light-temperature room under the same conditions as the first six-week experiment.

A light microscope, with a camera lucida and a digitising tablet was used to measure the shell length of each juvenile. Every fourteen days, measurement of growth in shell length was carried out. The piece of glass with early juveniles attached was removed from the beaker and immersed in a petri dish with a thin layer of seawater in order to diminish desiccation and stress on the animals. This glass was then placed under the microscope and the shell lengths recorded.

3.3. RESULTS

3.3.1. Experiment 1

All juvenile abalone fed on the three species of phytoplankton showed active feeding behaviour throughout the experimental period. Animals were examined under an inverted microscope and the food was observed in their stomachs. After the second day the animals reared on no food showed empty stomachs and started to die. However, marked differences were observed between the mortality of abalone fed on the three-phytoplankton species (K-W test, $H = 26.9$, df = 11, $P = 0.0048$) (Figure 3.1).

Animals reared on no food reached 92 % mortality at the end of the experimental trial.

Juveniles fed on *T. suecica* show the highest mortality at all concentrations of food, ranging from 52 to 59%. Nevertheless, when the early juvenile abalone were fed on *T. pseudonana* and *S. costatum* the mortality was reduced with increasing concentrations. *T. pseudonana* at concentrations of 250 to 500 cells ml^{-1} produced an average of 15 to 16 % mortality, respectively. Juveniles reared on *S. costatum* showed the lowest mortality (5 %) at 500 cells ml^{-1} concentration at the end of the experimental period (Figure 3.1).

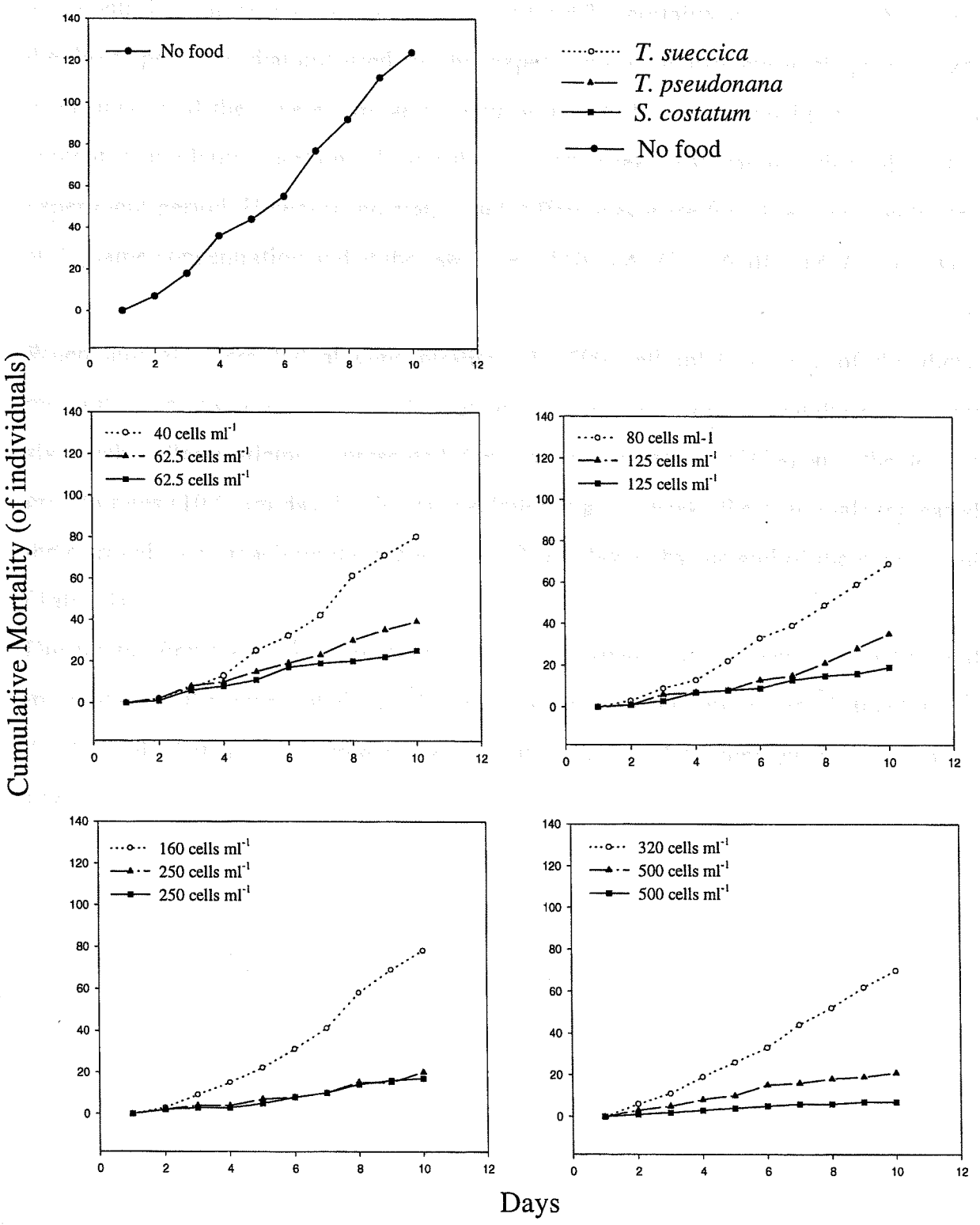

Figure 3.1. Cumulative mortality of juvenile *Haliotis tuberculata* fed on the larger phytoflagellate (*Tetraselmis sueccica*) and on the two species of marine diatom (*Thalassiosira pseudonana* and *Skeletonema costatum*). Four different concentrations were used.

3.3.2. Experiment 2

The control group reared on no food reached 100% mortality by day nine. Although the three species of diatoms used for this experiment were different in shape and size, when measured they were similar in both protein (27 - 31%) and lipid (12 - 15%) content. Cumulative mortality during the six first weeks was variable throughout the experiment period. However, no significant differences were found between replicates at the same concentration and at the same diet (ANOVA, $F = 1.6$, df = 18, $P = 0.194$).

When animals were fed at concentration of 1500 cell ml^{-1} on any of the diets, mortality was low (Figure 3.2). Although animals fed on *C. closterium* during the first six weeks after settlement presented the highest mortalities (56%) and the lowest growth rates (10.6 μm day^{-1}), during the following six weeks these animals increased their growth rates reaching the highest (27.88 μm day^{-1}) by the end of the experiment (Table 3).

During the first six weeks of rearing on *N. ramosissima*, animals were very active and mortality was the lowest at about 34% when diatom concentration was 1500 cell ml^{-1}. At the end of this period animal size was the highest (1.42 mm mean shell length, Figure 3.3).

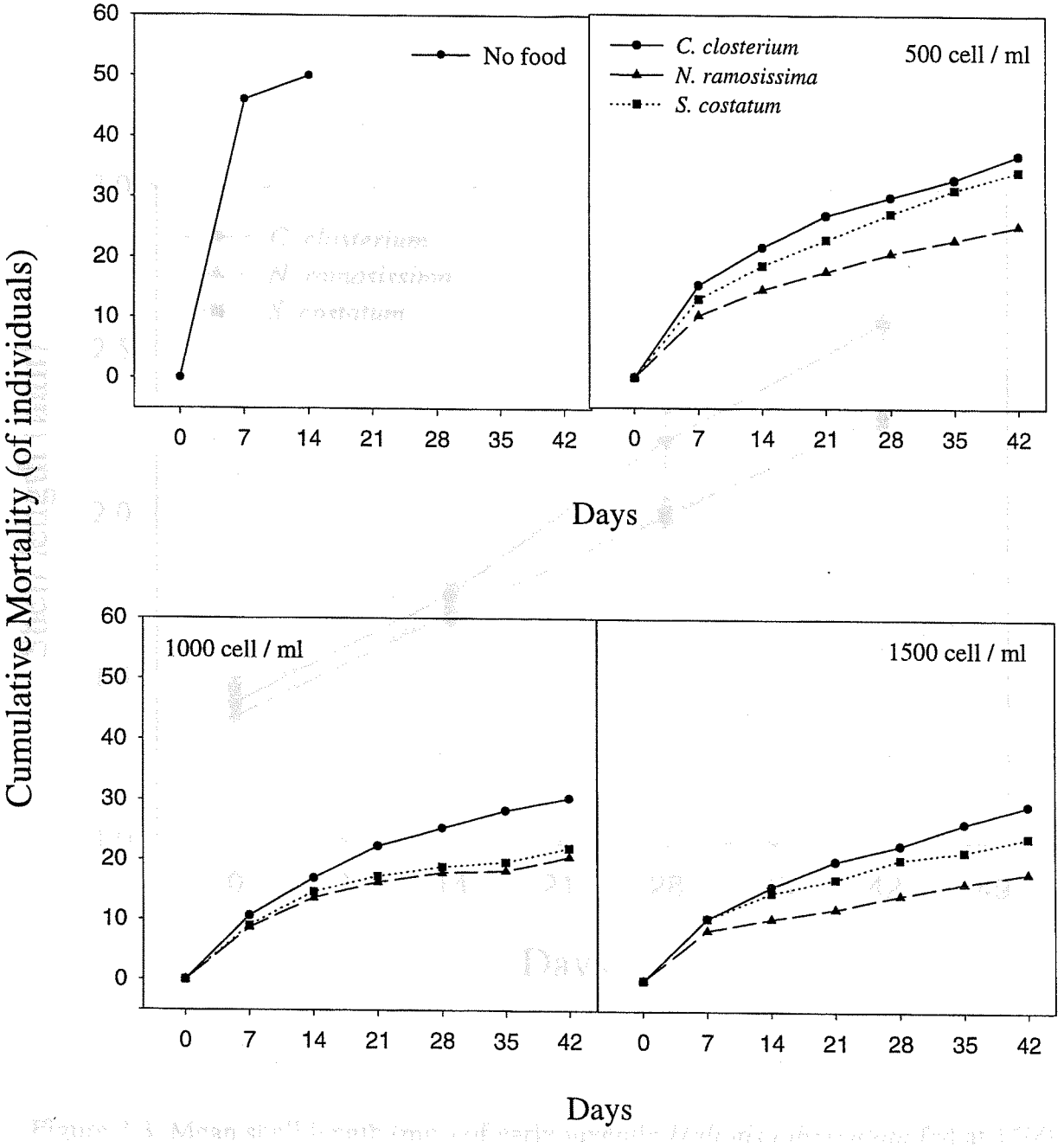

Figure 3.2. Cumulative mortality of juvenile *Haliotis tuberculata* fed on three marine diatom: *Cylindrotheca closterium*, *Navicula ramosissima* and *Skeletonema costatum*. Three different concentrations for each diet (500, 1000, 1500 cell/ml) were used. No food as a control.

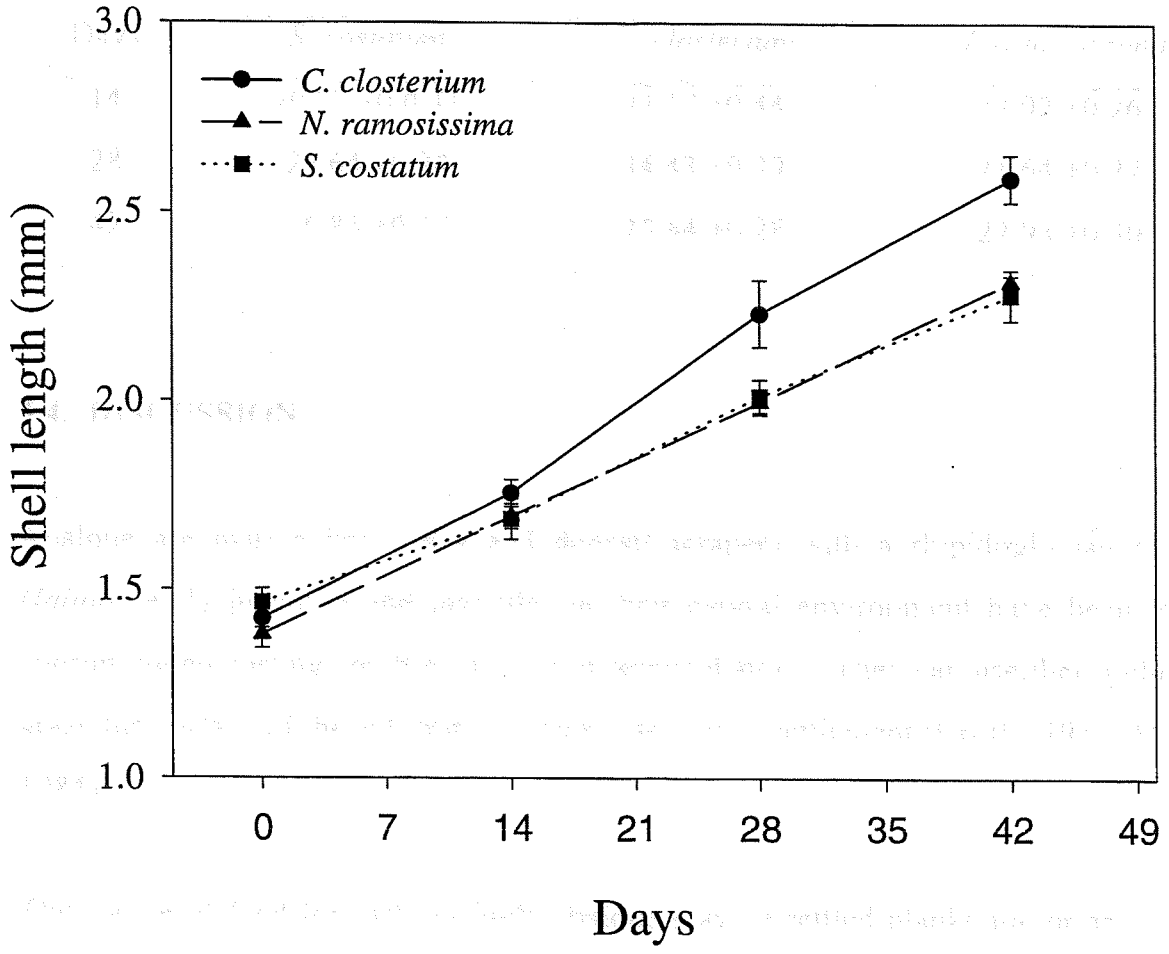

Figure 3.3. Mean shell length (mm) of early juvenile *Haliotis tuberculata* fed at 1500 cells ml^{-1}during six weeks with *Cylindrotheca closterium*, *Navicula ramosissima* or *Skeletonema costatum*. Bars indicate standard errors.

Table 3.2. Daily growth rate of early juvenile *H. tuberculata* fed for 42 days on *Skeletonema costatum, Cylindrotheca closterium* and *Navicula ramosissima* and. Animals mean shell length of 483 ± 54 μm at the beginning of the experiment. Mean values and standard errors ±.

	Growth Rate μm day^{-1}		
Days	*S. costatum*	*C. closterium*	*N. ramosissima*
14	20.07 ±0.0.31	23.57 ±0.44	22.02 ±0.26
28	25.64 ±0.28	34.43 ±0.39	21.64 ±0.22
42	16.85 ±0.37	25.64 ±0.28	22.93 ±0.30

3.4. DISCUSSION

Abalone are marine herbivores and deposit scrapers with a rhipidoglossan radula. *Haliotis* early juveniles and juveniles in their natural environment have been found consuming encrusting coralline algae and sessile diatoms. They can use their radula to graze the surface of the substratum immediately after settlement (Crofts, 1937; Morse, 1984).

The source of food for early juvenile abalone may be settled planktonic or associated with substrata. Crofts (1937) reported that newly settled *H. tuberculata* were found feeding on diatoms and foraminiferans present on the surface of stones in English Channel waters. Also early juvenile *H. tuberculata* were able to rasp off fragments of delicate red seaweed. Nevertheless, Koike (1978) reported that *T. suecica* was found to be a good source of food for juvenile *H. tuberculata*. The larger green flagellates such as *Tetraselmis suecica* have been shown to be an excellent diet, supplying both energy and essential nutrients for bivalve molluscs (Chu *et al.*, 1982; Whyte, 1987). Nevertheless, this species is a phytoflagellate and probably is not an adequate food for

juvenile abalone, as it swims actively in the water column. It only settle once it dies. Results from the first experiment suggest that *T. suecica* as a sole food is not sufficient for successful development and growth of early juvenile *H. tuberculata,* but in natural pelagic communities it may play a major role as an additional energy source. Nevertheless, *S. costatum* shows the lowest mortalities and it can be a potential source of food for early juvenile *H. tuberculata* in the Guernsey population. However, further research is required on this matter before concluding which kind of food is more suitable for this species.

Despite the nutritional value the three diatoms used for the second experiment being similar, although mortality and growth rates were variable between treatments. Early juveniles reared on the diatom *C. closterium* exhibited the highest mortality and the lowest growth during the first six weeks of culture. This was probably owing to the food not being consumed and digested. *C. closterium* was the largest diatom in terms of cell length and also the strongest in adhesion compared with the other two diatoms. It has been observed that diatoms with highly adhesive prostrate forms are difficult to consume and digest by abalone below the size of 800 μm (Kawamura *et al.*, 1998a). Alternatively, very young abalone may lack the digestive capabilities to fully use diatoms cell contents (Kawamura *et al.*, 1998b; Roberts *et al.*, 1999). The size of diatom cells has been seen as a critical factor determining the dietary value of diatoms, especially for very small abalone (Hahn, 1989; Fleming *et al.*, 1996).

Notwithstanding that early juvenile abalone possess an extraordinarily long radula in relation to total body size, they are able to remove only a layer of diatoms 1-3 μm thick (Garland *et al.*, 1985). Early juvenile feeding on largely indigestible diatoms probably get much of their nutrition from the extracellular secretions of diatoms, which consist mainly of polysaccharides (Hoagland *et al.*, 1993).

In addition, during the following six weeks the same animals (reared on *C. closterium*)

started to increase in growth rate and by the end of this period mortality was at its lowest (10%). This behaviour may be a result of selective feeding. During the first six weeks the abalone may have been too small to consume the biggest diatoms whereas during the following weeks when they reached shell length above 1mm the best food was *C. closterium*. On the other hand, *N. ramosissima* and *S. costatum* were easily ingested and assimilated by early juvenile abalone during the first six weeks. Nevertheless, animals reared on those two diatoms showed decreased growth rates by the end of the experiment.

Norman-Boudreau *et al.* (1986) showed that newly metamorphosed abalone (one week old) *H. kamtschtkana* x *H. kamtschtkana* or *H. kamtschtkana* x *H. rufescens* ingested primarily diatoms less than 10 μm width from a mixed species diatom film. In the same context, Matthews and Cook (1995) reported that diatoms in the gut were detected by day seven, which indicates that feeding began between days two and six, ingesting first the smallest diatoms. However, ingestion of diatom cells does not imply that abalone are utilised as food, in that case many diatom cells pass through the gut of very young abalone *H. discus hannai* alive and unbroken. Suggesting that the abalone could not utilise diatom cell contents for nutrition unless the diatom was broken during grazing (Kawamura *et al.*, 1995).

Further research is needed in order to determine the most beneficial diet for early juvenile abalone *H. tuberculata* depending on the size of the animal and conditions of culture. One way is to focus on the digestibility of diatoms as those have been shown to be a good source of food for these organisms. The ability to increase the digestibility of diatom cultures (specific size and strain) would increase the consistency of production and will benefit abalone hatcheries world-wide.

CHAPTER 4

Juvenile *Haliotis tuberculata* feeding on formulated diets

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4.1. INTRODUCTION

The ormer (*Haliotis tuberculata*), the most commercially important European abalone, is a temperate species. In the natural environment it grows slowly, reaching a mean shell length of 45 mm in a minimum of three years (Forster, 1967; Clavier and Richard, 1986). Abalone stocks have been declining around the world with a concomitant increase in the price of this important delicacy. Previous works have examined the culture of the ormer in Europe (Koike *et al.*, 1979; Hayashi, 1980a, 1982; Culley, 1981; Culley and Peck, 1981; La Touche *et al.*, 1993; Mgaya and Mercer, 1994; Mgaya, 1995). To date, the only research concerning nutritional requirements of *H. tuberculata* has been conducted by Peck (1989) using natural food, and by Mercer *et al.* (1993) and Mai *et al.* (1994, 1995 and 1996) feeding with natural and formulated food. However, recent research demonstrated that the growth rate of *H. tuberculata* fed with kelp and cultured at temperatures above 20°C was higher than reported in the literature (Shpigel *et al.*, 1996a, b).

The economic viability of abalone aquaculture depends on good growth rates. In culture, feed conversion ratio (FCR) is greatly influenced by factors such as quality of feed, feed intake and water temperature (Hahn, 1989). In particular, temperature is the most important environmental factor that influences metabolic rate and energy expenditure (Fry, 1971).

Dixon (1992) and Britz *et al.* (1997) have determined growth rates of *H. midae* using formulated diets at different temperatures of culture. They concluded that abalone growth increased significantly with temperature. Formulated diets have been evaluated; they can improve the growth rates of juveniles and young adults (e.g., Uki *et al.*, 1985a; Nie *et al.*, 1986; Hahn, 1989; Uki and Watanabe, 1992; Viana *et al.*, 1993a, 1996; Mai *et al.*, 1994, 1995; López and Viana, 1995; Knauer *et al.*, 1996).

This study was designed to assess growth rates (length and weight gain), (FCR), and soft tissue weight/shell weight (Stw/Sw) ratio of abalone with two formulated diets with different sources of protein (fish meal and casein). Three different temperatures were used in conjunction with the diet treatments.

4.2. MATERIALS AND METHODS

4.2.1. Abalone rearing

Four month old juveniles of *H. tuberculata* used in this experiment were obtained via semi-natural spawning at Rocquaine Shellfish Pounds, in Guernsey, Channel Islands, at the end of July, 1996. Two months after fertilization juveniles were shipped (by air) to the Southampton Oceanography Centre (within one hour) and placed in the rearing system for acclimatisation. Two batches of 99 animals were randomly taken from a group of 500. Mean length and wet weight were 3.22 ±0.81 mm and 15.98 ± 6.88 mg, respectively. During two months acclimatisation, temperature was gradually raised to that used in the experiment: 15º, 18º and 22ºC.

4.2.2. Preparation of diets

Formulations of the two experimental diets are presented in Table 4.1. Diets were prepared with different sources of protein, one based on casein (CA) and the other on fishmeal (FM). In order to obtain the same proximate content (protein, lipid and carbohydrate) between diets it was necessarily to add different percentage of ingredients between diets (fishmeal, casein meal, and corn meal and cod oil). Vitamin and mineral mixtures were formulated as recommended by Special Diets Services (SDS), Company, England. All ingredients were individually ground, using a Warring Blender and passed through a mesh with 240 μm pores size and mixed to obtain a homogeneous paste. The paste was flattened using a kitchen roller to a thickness of 1.5 - 2.0 mm. Pieces of 5×5 mm were cut and stored at -20ºC until required.

Table 4.1. Composition of two formulated diets for juvenile abalone *Haliotis tuberculata*.

Ingredients	Fish meal based diet	Casein meal based diet
Fish meal ❶	35	----
Casein meal ❶	----	25
Seaweed meal ❷	20	20
Corn meal ❸	10	18
Soybean meal ❶	8	8
Cellulose ❶	5	5
Sodium alginate ❶	8	8
Gelatine ❶	4	4
Vitamins mix ❹	3	3
Minerals mix ❹	5	5
Cod oil ❶	2	4

All ingredients expressed as percent dry weight. Sigma Chemical Co., UK ❶

Palmaria palmata from the English Channel Islands ❷

Maseca, produced in Mexico ❸

Mix based on the requirements for fish in mg (SDS) ❹. Vitamins: A, 4.9; B_1, 1.3; B_2, 1.6; B_6, 1.8; B_{12}, 0.0015; C, 40; D, 0.30; E, 5.0; H, 0.025; K, 2.25; Choline chloride, 75; PABA, 5; Folic acid, 0.075; Inositol, 5. Minerals: Calcium, 1600; Cobalt, 0.005; Copper, 0.09; Iodine, 0.01; Ferric citrate, 0.06; Potassium, 3.5; Selenium, 0.005; Zinc, 0.9.

4.2.3. Proximate analysis

All analyses were carried out using five replicates (0.5 - 1.5 g each) on diets and on samples of ormer (soft body) at the beginning and at the end of the experimental period. Moisture content was determined by weighing samples before and after drying at 70°C for 24 hours. Ash content was measured using remaining dried samples by incinerating at 500°C in a muffle furnace for 8 hours. Crude protein content was analysed using the Kjeldhal method (AOAC, 1990) for nitrogen content of the samples. The value obtained was then multiplied by 6.25 to estimate crude protein. Fat content was determined by a column procedure using methanol-chloroform/water as

the eluting solvent (Bligh and Dyer, 1959). The percentage of carbohydrate was determined by difference where % carbohydrate = 100 - (% protein + % lipid + % ash + % moisture).

4.2.4. Experimental procedure

Groups of eleven juveniles between 2.42 - 4.02 mm shell length and 9.1 - 22.86 mg body weight, were transferred to 1000 ml plastic containers. Three replicates per diet were held in a constant temperature bath (± 0.5°C) at each of the following temperatures 15°, 18° and 22°C. UV-sterilised, filtered and heated seawater was changed every morning for 105 days. Aeration was provided and every container was covered to prevent animals from escaping. The light-dark sequence was 12h/12h and tanks were covered with a 4 mm mesh net to reduce ambient light. Salinity was 32 ±3.

The experimental group was fed every afternoon and any uneaten food was collected the following morning to estimate the food consumed (*Fc*) and feed conversion ratio (FCR). Growth was measured monthly as gain in weight and length. Weights were obtained using an electronic balance (to 0.1 mg). A light microscope with a camera lucida and a digitising tablet was used to measure the shell length in smallest abalone. Vernier calliper was used to measure the shell length in animals bigger than 1 cm.

Food stability is defined as the amount of dry matter lost in seawater. This evaluation was conducted at intervals through a 35 day period prior to the experiment, using containers that did not contain abalone, under the same conditions as those of the growth experiments. Food was collected after having remained in the aquaria for 12 h. The dry weight (samples dried at 70°C for 24 h) for each trial (15°, 18° and 22°C), was measured.

The feed consumed *(Fc)* was calculated in terms of dry weight with the following equation:

$$Fc = \frac{GS}{100} - R \qquad \text{Equation 4.1}$$

where G is the weight of food offered per animal per day (in milligrams); S, the percentage of food recovered as the stability of the pellet, obtaining a factor for each diet and for each temperature (from the controls without abalone); and R, the remaining food (in grams) after the abalone had fed.

The feed conversion ratio was calculated in the following manner:

$$FCR = \frac{Fc}{W} \qquad \text{Equation 4.2}$$

where Fc is grams of food (dry basis) consumed per animal per day and W, grams of body weight gained (wet basis) per animal per day, as in Britz (1996). At the end of this phase, three animals from each replicate (54 animals in total: nine animals from each temperature, from each diet) were processed to find the soft tissue weight: shell weight ratio (Stw/Sw), on a wet weight basis.

4.2.5. Statistical analyses

If the case, data for experimental replicates were pooled as no significant difference was found between them using a one-way analysis of variance at a significance level of $P < 0.05$. In proximate analysis, a two-sample t-test was used to compare the means between diets and the same analysis was performed for proximate analysis of soft tissue of abalone. A two-way analysis of variance was used in order to compare the shell length between diets and temperatures followed by a multiple comparison of the means (SNK, Student-Newman-Keuls method), the same analysis was performed for the data of the body weight. A one-way analysis of variance was used to correlate the

food stability between diets and temperatures followed by comparison of the means by SNK method. The daily growth rates were analysed by Kruskal-Wallis one-way analysis of variance on ranks method. All statistics were calculated using the Sigmastat package (1996).

4.3. RESULTS

Diets were significantly different in crude protein (t = -10.7, df = 8, P = 0.0001), crude lipids (t = 2.05, df = 8, P = 0.0074), moisture (t = -4.01, df = 8, P = 0.0039) and ash (t = 9.27, df = 8, P = 0.0001) content between diets (Table 4.2). Analysis of the soft tissue of *H. tuberculata* juvenile showed differences between samples for crude protein (t = -3.58, df = 8, P = 0.0072), crude lipids (t = -6.29, df = 8, P = 0.0002), ash content (t = -3.81, df = 8, P = 0.0051) and moisture (t = 14, df = 8, P = 0.0001) content (Table 4.2).

Table 4.2. Proximate analysis of the two diets used and soft tissues of juvenile abalone *Haliotis tuberculata*. Data are expressed as percentages. Standard errors ±. n =3.

Sample	Crude Protein	Crude Lipid	Carbohydrate	Ash	Moisture
Fish meal diet	31 ±.0.8	5.6 ±.0.7	21.54 ±.0.7	12.1 ±.0.5	29.2 ±.0.9
Casein diet	36 ±.0.7	4.9 ±.0.4	19.06 ±.0.82	8.1 ±.0.9	31.5 ±.0.8
Soft tissue [a]	9.2 ±.0.6	2.7 ±.0.5	1.96 ±.0.1	4.8 ±.0.5	82.3 ±.0.7
Soft tissue [b]	10.7 ±.0.7	4.6 ±.0.5	2.82 ±.0.1	6.0 ±.0.5	76.7 ±.0.5

[a]Soft body tissue from juveniles before the start of the experimental trial, [b]Soft body tissue from juveniles at the end of the experimental trial.

The food stability test indicated a significantly greater (ANOVA, $F_{5,30}$ = 184.1; P < 0.0001) mean dry matter loss derived at 18°C and 22°C compared with 15°C for the CA and FM diets, after 12 h submersion in seawater (Table 4.3). Survival of juvenile

H. tuberculata during the feeding trial was not affected by dietary treatment and averaged 90% for animals cultured at 15°C (K-W test, $H = 2.65$; df = 2; $P = 0.26$) and 100% for animals cultured at 18° and 22°C 15°C (K-W test, $H = 5.86$; df = 5; $P = 0.32$). The relationship of Stw/Sw ratio is shown in Table 4.3, significantly greater differences (ANOVA, $F_{5,12} = 10.4$; $P = 0.0005$) were found between temperatures (15°, 18° and 22°C). Feed conversion ratio was related to temperature ($P < 0.05$), decreasing from 3.52 and 3.56 at 15°C to 0.76 and 0.76 at 22°C for the CA diet and the FM diet, respectively (Table 4.3).

Table 4.3. Survival of the juvenile during the feeding trial as a percentage.

Diet	°C	S (%)	Survival (%)	Stw/Sw ratio	Fc (mg) day^{-1}	W (mg) day^{-1}	FCR
Casein meal	15 °	95.4 ±0.2	90	1.04 ±0.5	0.16 ±0.01	0.08 ±0.03	3.52
	18 °	92.0 ±0.3	100	1.77 ±0.4	0.95 ±0.10	0.87 ±0.08	0.84
	22 °	87.2 ±0.55	100	2.49 ±0.2	1.57 ±0.16	1.80 ±0.27	0.76
Fish meal	15 °	97.4 ±0.75	90	1.1 ±0.5	0.19 ±0.01	0.12 ±0.02	3.56
	18 °	93.5 ±0.63	100	1.9 ±0.2	1.32 ±0.12	1.66 ±0.09	0.91
	22 °	89.5 ±0.85	100	2.6 ±0.4	1.62 ±0.14	1.97 ±0.20	0.76

Average values of soft tissue weight/shell weight ratio (Stw/Sw). Stability (S) of the diets (dry matter remaining in seawater without abalones over 12 h) is given as a percentage. Feed consumed (Fc) and average weight gain (W) are estimated. Feed conversion ratio (FCR) indicated as feed consumed/weight gain. Standard errors ±..

4.3.1. Shell growth

Significant effects of diets (ANOVA, $F_{9,946} = 466.1$; $P < 0.0001$) and temperatures (ANOVA, $F_{2,946} = 407.2$; $P < 0.0001$) were found on the shell length. During the first

month the mean growth of abalone was low (Figure 4.1a); mean values were 3.25, 3.62 and 3.58 mm per month for the CA diet and 3.21, 3.78, and 3.81 mm per month for the FM diet cultured at 15º, 18º and 22ºC, respectively. From January, the mean length increased markedly, the maximum value at the end of the four month experiment was 14.16 mm for abalone on the FM diet at 22ºC, about two and a half times the value for the same diet at 15ºC (Figure 4.1a).

4.3.2. Body weight

A two-way ANOVA indicated highly significant effects of diets ($F_{9,946} = 312.3$; $P < 0.0001$) and temperatures ($F_{2,946} = 485.9$; $P < 0.0001$). During the first month mean growth on both diets were similar ($P < 0.05$; Figure 4.1a and 4.1b). Following January, animals cultured at 18º and 22ºC grew faster than animals cultured at 15ºC ($P < 0.05$), showing significant differences between temperatures by April ($P < 0.05$; Figure 4.1b). By the end of the experimental trial, mean weights of the FM diet were 173.95 mg and 222.85 mg for 18ºC and 22ºC, respectively.

For the CA diet means were 131.15 mg and 203.84 mg at the same temperatures. Juveniles cultured at 15ºC on the CA diet exhibited a significant (ANOVA, $P < 0.05$) lower weight gain (0.08 mg per day), than juveniles cultured at 18ºC (1.1 mg per day) and 22ºC (1.8 mg per day). The same tendency was observed for juveniles fed the FM diet. Mean length and weight increased through time (Figure 4.1a and 4.1b) on both diets at each of the three temperatures (15º, 18º and 22ºC). At the beginning of the experiment the average length and weight of juvenile *H. tuberculata* did not differ significantly (ANOVA , $P > 0.05$; Figures 4.1a and 4.1b). After January these differences became significant (ANOVA, $P < 0.05$) between temperatures, to peak at 22ºC.

Significant differences occurred between diets and temperatures on the daily growth rates of the shell (Kruskal-Wallis Method, $H = 212.9$, df = 23, $p \leq 0.0001$) and body weight ($H = 442.9$, df = 23, $P \leq 0.0001$) for animals cultured at 18º (and 22ºC (Table 4.4). Low growth rates were found at the lowest temperature. The growth rates for animals cultured at 15ºC were low with null length increase and loss of weight at the beginning (0.04 µm day^{-1} and -176.6 µg day^{-1}) to the highest values in April (39.82 µm day^{-1} and 196.4 µg day^{-1}) which are still low compared with the other treatments (Table 4.4).

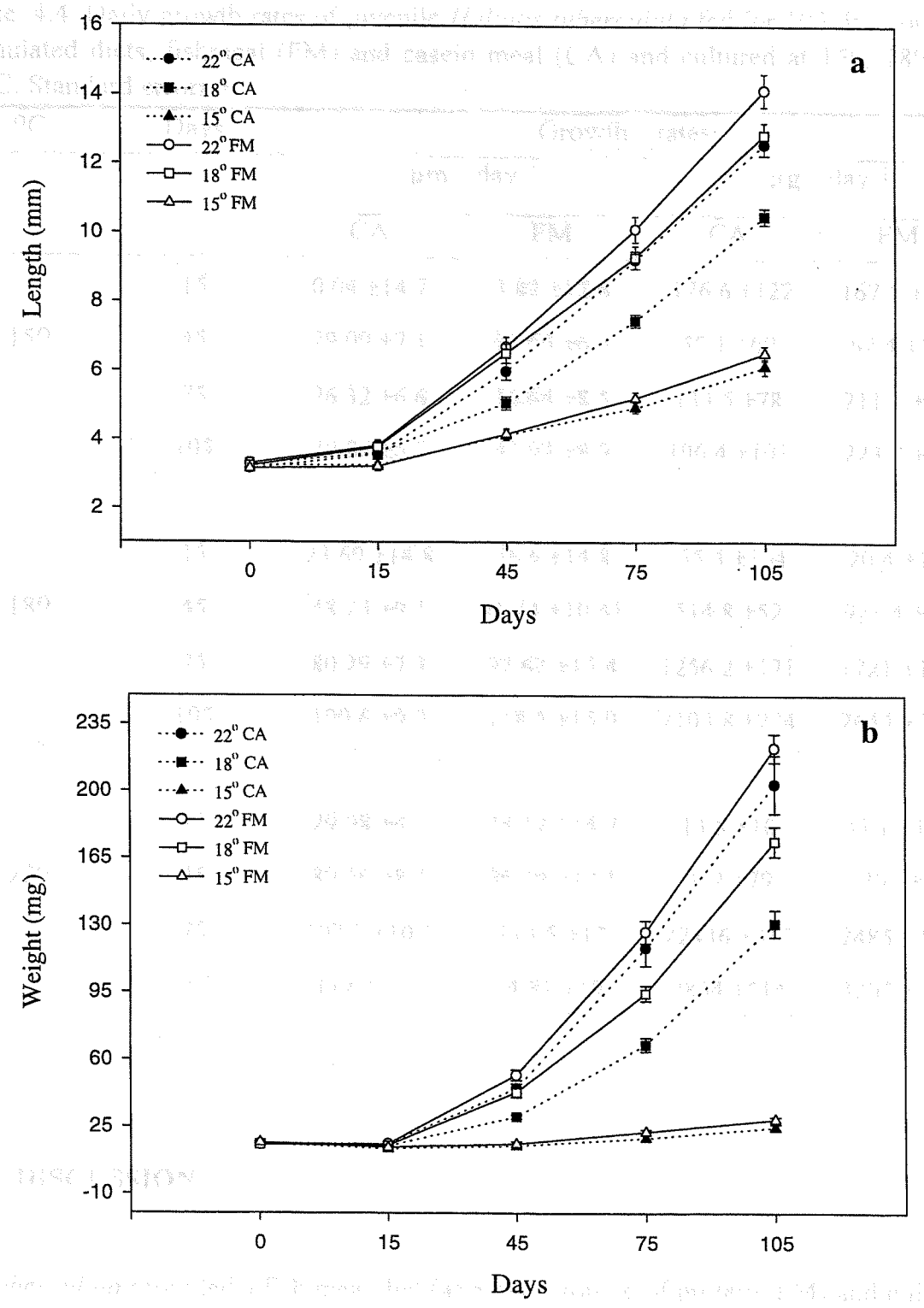


Figure 4.1. Mean growth of *Haliotis tuberculata* provided during 105 days (December to April) with two formulated diets (CA and FM) and cultured at 15°, 18° and 22°C. Bars indicate standard errors. a) shell length means and b) body weight means.

Table 4.4. Daily growth rates of juvenile *Haliotis tuberculata* fed for 105 days on two formulated diets, fishmeal (FM) and casein meal (CA) and cultured at 15°, 18° and 22°C. Standard errors ±.

°C	Days	Growth rates			
		µm day-1		µg day-1	
		CA	FM	CA	FM
15°	15	0.04 ±14.7	3.82 ±12.6	-176.6 ±122	-167.5 ±130
	45	29.09 ±7.1	31.60 ±6.6	55.1 ±60	62.4 ±59
	75	26.32 ±6.6	36.64 ±8.5	133.5 ±78	211.1 ±69
	105	39.82 ±5.5	43.92 ±8.9	196.4 ±101	223.7 ±67
18°	15	23.69 ±14.8	36.6 ±14.8	-55.4 ±104	-20.4 ±105
	45	48.23 ±9.1	91.44 ±10.41	514.8 ±52	921.4 ±76
	75	80.29 ±7.3	92.62 ±15.4	1256.2 ±121	1721 ±175
	105	100.6 ±9.9	118.5 ±15.9	2103.8 ±274	2644 ±321
22°	15	29.98 ±4.7	34.12 ±14.7	13.8 ±10	33.1 ±101
	45	80.56 ±8.5	96.39 ±12.1	962 ±79	1201 ±95
	75	107.5 ±10.5	113.5 ±15	22436 ±315	2485 ±199
	105	112 ±13.3	134.81 ±19.7	2854 ±514	3205 ±322

4.4. DISCUSSION

H. tuberculata provided a fish meal diet (as a main source of protein; FM) and cultured at 18° and 22°C, produced superior growth rates for shell length and body weight compared to juveniles fed a casein diet (CA) and cultured at 18° and 22°C. Although

the CA diet contained a slightly higher protein content than did the FM diet, a significantly greater growth rate was observed in juveniles fed the FM diet. Our results are different from those reported by Uki *et al.* (1985a), who found that a casein diet resulted in a better growth than a fish meal diet in *Haliotis discus hannai*. Diet palatability is an important consideration when formulating diets for aquaculture species. Abalone show a preference for certain ingredients, resulting in better food acceptance and consumption (Harada, 1992; Sakata and Ina, 1992; Shepherd and Steinberg, 1992; Viana *et al.*, 1994, 1996). However, the nutritional value of a diet is of major importance especially in terms of the content of those elements necessary to give a proper balance between energy and growth. In this study, fishmeal was more efficient than casein, resulting in better overall growth rates.

Water temperature is one of the most important environmental factors influencing metabolic rate and energy expenditure. Strict comparison of our results, on the effects of temperature and diets on growth, with data on other species is difficult because experimental conditions vary widely, including environmental factors and the initial sizes used. Nevertheless, growth rates of *H. tuberculata* have been shown to be much higher when cultured at temperatures between 18° and 22°C (Peck, 1989; Shpigel *et al.*, 1996a, b).

The daily growth rates for animals cultured at 15°C were low, with no measurable length increase and loss of weight at the beginning. Highest values for this group occurred in April, which are still low compared with the other treatments. According to several authors (Peck, 1989; Mercer *et al.*, 1993; Mai *et al.*, 1995), *H. tuberculata* fed natural, mixed, and formulated diets, cultured at temperatures of 12° - 15°C, produced acceptable rates of growth for shell length and body weight. We found low growth rates at low temperature (15°C). In the same context, Peck (1989) found best growth rates when *H. tuberculata* was reared at 22.5°C. Animals during winter have lower

food intake rates and also have reduced metabolism and energy requirements. Most of the energy absorbed sustains metabolic needs, and any deficit drawn from its own nutrient reserves (Widdows, 1973). Individuals would be expected to lose weight (Peck, 1989).

From the beginning of the experimental trial, the animals from the control group fed on dry *P. palmata* began to lose weight with no shell length increase. Mortalities were high, reaching the highest levels (up to 85 %) by the third month. We believe the dry seaweed did not supply adequate nutrient. Dried and rehydrated marine algae often are not as acceptable and supportive of growth, as are fresh (living) plants (Leighton, personal communication).

We were not able to use fresh seaweed in this experiment because *Palmaria palmata* is a seasonal species, disappearing during the coldest months of the year. Indeed, *P. palmata* is one of the major constituents of the natural diet of juvenile *H. tuberculata*, which also includes a variety of delicate seaweed, such as *Ulva lactuca* and *Enteromorpha intestinalis*, and coarser ones, such as *Laminaria* spp., *Chondrus crispus*, encrusting coralline algae, and sessile diatoms (Culley and Peck, 1981; Mercer *et al.*, 1993). Combination of these algal foods should provide all nutrients necessary for maximum growth. Moreover, reported observations suggest that abalone fed on seaweed supporting colonies of bryozoan or hydroids grew faster than did that fed the same species without such growths (Hahn, 1989). These metazoans could play an important role in abalone nutrition, as a supplementary source of protein and lipid. In our control group, the use of dried seaweed cleaned of all fouling organisms may have reduced its nutritive value and contributed to its poor performance as a food. The high mortalities may be related to bacterial growth and degradation of the rehydrated alga.

A mixed algal diet may have been more appropriate for our study. Among other researchers, Day and Fleming (1992) and Viana *et al.* (1993a and 1996) found that

single species of seaweed, provided as exclusive food items, did not sustain growth over extended periods, failing to provide a nutritionally balanced diet and thus reflected in low growth rates in *Haliotis rubra* and *Haliotis fulgens*. Furthermore, some recent studies on formulated diets (Britz, 1996; Britz *et al.*, 1997) have not included a seaweed-fed control group. This could be a matter of choice after finding low growth and high mortalities when offering dried seaweed under the same experimental conditions as maintained in feeding trials with formulated diets.

CHAPTER 5

Our results clearly show a significant increase in body and shell growth rate at higher temperatures. FCRs also improved markedly at higher temperatures. The diet providing protein from FM supported better growth in *H. tuberculata* than did the CA-based diet. We would recommend that those involved in culture of this abalone control seawater temperature to remain in the range of 18-22°C and provide a FM-based formulated diet.

CHAPTER 5

METABOLISED ENERGY EXPENDITURE AND GROWTH RATES OF JUVENILE *Haliotis tuberculata* (L.) WHEN FED ON DIFFERENT DIETS AND CULTURED AT DIFFERENT TEMPERATURES

5.1. INTRODUCTION

Growth rate studies are necessary to estimate the sustainable production of commercially important species. Thus, measurements of food required are necessary to know the gain in body weight and length. Nevertheless, occasionally the gain or loss in body weight of organisms varies with a particular kind of food. It is possible, therefore, that seaweed grazers are selective in their diet (Leighton, 1966).

5.1.1. Food preferences

Research on seaweed preferences in abalone has been investigated in a variety of countries. The Australian greenlip abalone *H. leavigata*, shows strong preference for red seaweed over brown (Poore, 1972; Shepherd, 1973; Wells and Keesing, 1989). Conversely, the Japanese abalone *H. discus hannai* prefers the brown (Imai, 1978; Uki *et al.*, 1986). *H. cracherodii*, *H. corrugata*, *H. fulgens* and *H. rufescens* from California also prefer browns (Cox, 1962; Leighton, 1966, 1968; Leighton and Boolotian, 1963; Tutschulte and Connell, 1988), and the South African abalone *H. midae* prefers a variety of brown seaweed (Barkai and Griffiths, 1986). Moreover, the European abalone *H. tuberculata* shows strong preferences for a certain variety of seaweed (Culley and Peck, 1981), depending on the energy content. In addition, *H. tuberculata* tends to optimise its food sources (Peck, 1983).

With respect to feeding on formulated diets, *Haliotis* species favours certain ingredients. In most of the studies, the main protein sources are fish meal and casein meal. The Japanese abalone *H. discus hannai* has a preference for, and shows the highest growth rates, when fed on a diet with casein (Uki *et al.*, 1986; Uki and Watanabe, 1992). Nevertheless, *H. fulgens* (Viana *et al.*, 1994) and *H. midae* (Britz, 1996) have a preference for fishmeal as the main protein source.

It is clear that abalone show a selection for certain ingredients, resulting in better food

acceptance and consumption (Harada, 1992; Sakata and Ina, 1992; Shepherd and Steinberg, 1992; Viana *et al.*, 1993, 1994, 1996; Corazani and Illanes, 1998; López *et al.*, 1998).

5.1.2. Metabolic demand

The continuous flow of energy through organisms is required for life. As with all animal farming systems, the growth and production of cultured abalone is dependent upon the supply and intake of dietary nutrient inputs (Barkai and Griffiths, 1986 and 1988). All animals require an adequate supply of food if they are to satisfy their metabolic demand for energy and nutrients. The diet must supply appropriate amounts of substrates (carbohydrates, protein and lipid) and cofactors (vitamins and minerals) if it is to be handled efficiently and the nutrients made available to the body for maintenance metabolism, growth and reproduction (Jobling, 1993).

It has been recognised that an insufficient dietary intake of macronutrients, such as protein, results in profound disturbance in growth. It is also well recognised that many micronutrient (vitamins and minerals) deficiencies result in an inhibition of growth. In many animals intake of food is remarkably well adjusted to energy expenditure. If energy needs are increased because of physical activity, food intake is adjusted accordingly (Odum, 1971; Brett and Groves, 1979).

Positive energy balance occurs when metabolised energy intakes exceed expenditure and there is net tissue deposition; this condition occurs in the growing animal. Conversely, negative energy balance occurs when the metabolic demand for energy is such that expenditure is superior to intake. In situations where the demand for energy frequently exceeds supply, growth will cease, body substrate reserves will be sacrificed, eventually with the loss of functional tissue, and the metabolic processes that maintain normal function will be compromised (Jobling, 1993).

All the energy acquired through the ingestion of food is ultimately lost as waste, in faeces or by excretion, or used in metabolic processes or deposited as a new body tissue (Bayne and Newell, 1983).

5.1.3. Energy balance

Numerous studies on grazing gastropods deal with flow and allocation of energy in biological systems within a population (Odum, 1963; Hughes, 1971; Paine, 1971; Branch and Newell, 1978; Huebner and Edwards, 1981; Wright and Hartnoll, 1981; Horn, 1986; Blandenier and Perrin 1989; Davies *et al.*, 1990; Lucas, 1996; Plaut *et al.*, 1996) and on individual organisms (Hughes, 1971; Emberton, 1982; Barkai and Griffiths, 1986, 1988; Peck *et al.*, 1987). Nevertheless, in several cases some components of the energy budget have not been measured and only obtained by subtraction or interpolating from the other terms (Paine, 1971; Wright and Hartnoll, 1981; Plaut *et al.*, 1996) or by making assumptions from existing data (Phillipson, 1966). For example, nitrogenous waste (U) is rarely measured and is often assumed to be negligible (Hughes, 1970, 1971).

It is important to determine the energy partitioning for an individual organism, energy flow through a population of a single species, or energy transfer between trophic levels. Such energy partitioning may be useful to make inferences about physiology or ecology in an organism or population (Davies and Hatcher, 1998).

There are limited data of the energy partitioning in *Haliotis* species (Barkai and Griffiths, 1986, 1988). Data are available for the effect of different diets and for individuals reared at different temperatures (Emberton, 1982; Peck *et al.*, 1987).
The study of the energy flow as an individual energy budget through a population of a single species involves the partitioning of ingested energy into the major physiological components using the energy budget equation:

$$I = E + Pg + Pr + R + U + M$$ Equation 5.1

where I is energy value of the food ingested, E is energy egested as faeces, P_g is energy allocated to production of somatic growth (in this study using whole body weight), P_r is energy incorporated into the reproductive investment (gamete production, in this study using the whole gonad), R is energy assessed for metabolic purposes (respiration), U is energy value of waste materials, as ammonia excretion, and M is energy value of pedal mucus production (Peck, 1987; Jobling, 1993; Davies and Hatcher, 1998).

5.1.3.1. Absorption

Part of the food consumed by the abalone will pass through the gastrointestinal tract without being digested and absorbed and is lost in the faeces. Thus, food and faecal analysis can provide an accurate analysis of how much of the nutritional content of the food has been absorbed by the animal (Crisp, 1984).

Absorption efficiency or digestive efficiency is usually defined as the energy ratio of nutrients in the food remaining after faecal losses have been accounted for (Crisp, 1984; Jobling, 1993). The following equation can be used:

$$Ab = I - E = Pg + Pr + R + U$$ Equation 5.2

where Ab is absorbed ration, and the others terms are as described above.

Also food absorption can be determined by the ash ratio method of Conover (1966), which does not require the quantitative collection of faeces and can be calculated as:

$$Ac = \frac{(Fr - Er)}{(I - Er)\,x\,(Fr)}\,x\,100$$

where *Fr* is ash free dry weight of the food ration, *Er* is the ash free dry weight of the egested ration. The Conover method considers only organic material absorbed from the food and assumes that none of the inorganic or ash fraction is utilised. However, some organisms are capable of absorbing inorganic material from food (Conover, 1966). Also, Peck (1983) suggested that *H. tuberculata* might absorb inorganic material from the food ingested. Thus, this method was not applied in this study.

6.1.3.2. Assimilation

Assimilation is that part of the ingestion that is retained for physiological purposes, such as somatic growth (*Pg*), reproduction (*Pr*) and respiration (*R*), but excluding egested and excreted materials.

$$As = Pg + Pr + R \qquad \text{Equation 5.3}$$

5.1.3.3. Somatic growth

Growth represents a combination of an increase in body mass and alterations in body composition, which together are described as changes in the form of the animal. In addition, as an animal matures its functional capacity increases. The pattern of growth and development of an individual is primarily determined genetically. However, a variety of environmental factors may influence whether an individual actually achieves its potential growth. Growth results when energy acquisition is in excess of energy expenditure. If energy intake is more than expenditure, positive growth occurs, and endogenous reserves of energy will not be utilised to support the body maintenance metabolism.

Thus metabolic demand for energy is increased during growth and will be dependent upon the growth rate. In addition to the energy costs associated with laying down new tissue, the resulting changes in both form and function will increase the metabolic demand for the maintenance of these tissues.

The requirement for energy during growth may be separated into two component parts; firstly, there is a requirement for substrates from which the new tissue will be formed, and secondly, energy is needed to meet the metabolic cost for growth, including the synthesis of the new tissue.

Growth in abalone is most frequently measured by recording progressive changes in body weight over a period of time, and gives an indication of the growth rates.

5.1.3.4. Reproductive investment

In many mollusc species, reproduction is not initiated until somatic growth is near completion; in others, growth continues after the age of first maturity, but an increasing proportion of spare energy is allocated to gametogenesis. Also, overall growth efficiency may remain relatively constant under these conditions, inasmuch as somatic growth efficiency declines (Bayne and Newell, 1983).

Gonad maturation in males of *H. tuberculata* has been reported to be earlier than in females, usually occurring after the second year at 25 to 40 mm shell length (Girard, 1972; Hayashi, 1980a).

In the present study juveniles cultured at the highest temperatures (18° and 22°C) and fed on a rich diet (Fish meal and Casein meal) start to invest in gonad development after three to four months expending most of the energy in growth (body weight and shell length). It was possible to follow the gonad development and the final spawning.

Reproductive investment is a reflection of the total investment in the gonad (gametogenic or somatic). It was decided to use the complete gonad to get the total energy investment for females and males of different sizes.

In *Haliotis*, reproductive growth has been assessed by the collection of eggs from ripe females and obtaining the calorific content of the eggs. Assuming that gonad development is complete within 90 days (Hayashi, 1980a; Peck, 1983; Barkai and Griffiths, 1988). No data are available for reproductive investment in males. Nonetheless, the production of eggs, sperm and the material associated with them (gonadal tissue, egg capsules and seminal fluid), may be accounted as part of the assessment of total gonadal output (Crisp, 1984).

5.1.3.5. Respiration

Energy metabolism may be measured by either direct or indirect calorimetry. In a resting individual in a thermoneutral environment the amount of heat released by the oxidative activities of all the cells of the body (measured by indirect calorimetry) is the same as the amount of heat lost to the environment (measured by direct calorimetry). Measurement of the rate of metabolic heat production by indirect calorimetry depends on two assumptions:

(1) that the end result of all the biochemical reactions, which occur in the body, amounts effectively to the combustion or synthesis of the three substances: carbohydrate, protein and lipid.

(2) that for each of these substances, when it is oxidised in the body, there are fixed ratios between the quantities of oxygen consumed, carbon dioxide produced and heat produced (McLean and Tobin, 1987).

Indirect calorimetry exploits the relationship between energy metabolism and the rate of oxygen consumption. It may be reliably used for both short term measurements of resting energy expenditure (over periods of less than an hour) and measurements of total energy expenditure over longer periods (up to 24 hours or more).

In molluscs, metabolic energy losses by respiration are well documented in the literature (Hughes, 1971; Uki and Kikuchi, 1975; Huebner and Edwards, 1981; Jan *et al.*, 1981; Wright and Hartnoll, 1981; Houlihan and Innes, 1982; Mace and Ansell, 1982; Bayne and Newell, 1983; Jan and Chang, 1983; Carefoot, 1987). Measurements of the losses of metabolic energy have been determined by direct calorimetry (Paine, 1971; Hammen, 1979 and 1980), and also by respirometry via oxygen consumption (Barkai and Griffiths, 1988; Huebner and Edwards, 1981). The oxygen content of seawater has been measured by the Winkler titration technique described by Strickland and Parsons (1968). To convert oxygen uptake to energy loss it is necessary to use an oxy-calorific coefficient, that depends upon the type and concentration of metabolic substrate respired: protein, lipid and carbohydrate (Jobling, 1993).

5.1.3.6. Excretion

The protein of a diet with natural ingredients is usually assimilated to a greater degree than other components of the food (Fleming et al., 1996). This assumes that the protein is available for assimilation. In this context, diminution of amino acids may lead to the release of amino groups that cannot be recycled through other metabolic processes and must therefore be excreted as end-products, together with ammonia and urea (Brett and Groves, 1979).

Many factors are known to influence rates of nitrogenous excretion by marine animals, and attempts are often made to distinguish between excretion of endogenous and exogenous origin. Endogenous nitrogen excretion is measured as the rate of excretion of fish, which have been deprived of food for a number of days. On the other hand, exogenous excretion is considered to result from the direct deamination of amino acids ingested and absorbed from the food. The exogenous component of nitrogen excretion will therefore be influenced by factors such as feeding rate, protein quality of the food, the composition of the diet with respect to the levels of essential and non-essential amino acids, and also the animal size, and temperature of culture (Jobling, 1993).

Nitrogen excretion, as ammonia, can be analysed by the spectrophotometric method of Solorzano (1969). To obtain the energy loss by ammonia excretion a coefficient of 68.9 kcal mol^{-1} (Brafield and Solomon, 1972), can be used. Nevertheless, the proportion of nitrogen excreted in other metabolic end products may reach more that 30%. Horn (1986) found that in *Chiton pelliserpentis* nitrogen excretion was 66% ammonia and 34% urea in high-shore chitons. Also Clarck *et al.* (1990) found that *Nacella concinna* excreted an average of 88.8% ammonia, 7.9% urea, and 3.3% fluorescamine-positive substances (FPS).

5.1.3.7. Pedal mucus production

Pedal mucus, as one of the secreted products for locomotion in gastropod molluscs, has been shown to play an important role in the energy budget (Horn, 1986; Peck *et al.*, 1987; Davies *et al.*, 1990; Kideys and Hartnoll, 1991; Riegl and Branch, 1995; Navarro and Torrijos, 1995; Smith and Davies, 1995). In *Haliotis* species there is only one study of the estimation of mucus production (Peck *et al.*, 1987). Their results show that *H. tuberculata* expend up to 27.7 % of the assimilated energy in a 1 g dry weight animal. Pedal mucus has been demonstrated to be energetically rich but recent studies have not considered that aspect in energy budget determination (Wilbur and Hilbish, 1989; Grant, 1996).

5.2. MATERIALS AND METHODS

5.2.1. Abalone rearing

Seven-month-old juveniles of *H. tuberculata,* used in this experiment, were obtained by semi-natural spawning in our laboratory at the Southampton Oceanography Centre, at the end of July 1997. Three batches of 135 animals were selected based on shell length and whole body weight. Mean length and wet weight were 5.07 ± 0.12 mm and 22.22 ± 5.34 mg, respectively. During acclimatisation, animals were housed in a

constant temperature baths under similar conditions and temperature was gradually raised to that used in the experiment: 15°, 18° and 22°C. Groups of fifteen juveniles between 4.16 - 5.97 mm length and 14.33 - 30.12 mg wet weight, were transferred to 1000 ml glass beakers. Three replicates per diet were held in a constant temperature bath (± 0.5°C) at each of the following temperatures 15°, 18° and 22°C.

Food, filtered and heated seawater were changed four times a week for 150 days (March to August). From August to October the experimental group were fed every afternoon, and any uneaten food was collected the following morning to estimate the food consumed.

5.2.2. Diet

Three different diets were used: the first diet (SW) was a mix of the red seaweed *Palmaria palmata* and the green seaweed *Ulva lactuca*, in the proportion of two thirds and one third, respectively. This diet was used as a control.

The formulation of the second diet (FM) is presented in Table 5.1. It was prepared with fishmeal as a main protein source. Vitamin and mineral mixtures were formulated as recommended by Special Diets Services (SDS) Company, England. All ingredients were individually ground with a Warring blender, passed through a mesh with a 200 µm pore size, and then mixed to obtain a homogeneous paste. The paste was flattened with a kitchen roller to a thickness of 1.0-1.5 mm. Pieces of 5 x 5 mm were cut. Pellets were dried in a convection oven for 18 hours at 50°C and stored at -20°C until required.

The third diet (CO, abalone commercial diet) was obtained from Gulf Feeds, Australia, and was formulated with casein as a main protein source, with good stability up to 12 hours after submerging in seawater. The exact formulation of this diet is held by Gulf

Feeds and not available. Pellet size was 8 x 3 mm and 2 mm thickness, approximately. This diet was also stored at -20°C until required.

Table 5.1. Composition of the fishmeal diet (FM). All ingredients given as a percentage of dry matter.

Ingredients	Percentage
Fish meal ❶	35.0
Seaweed meal ❷	14.0
Corn meal ❸	10.0
Starch ❶	20.0
Sodium alginate ❶	8.0
Gelatine ❶	4.0
Vitamin mix ❹	1.0
Mineral mix ❹	1.0
Cod oil ❶	6.0

Sigma Chemical Co., UK ❶
Palmaria palmata from the English Channel Islands ❷
Maseca, produced in Mexico ❸.
Mix based on the requirements for fish in mg (SDS) ❹. Vitamins: A, 4.9; B_1, 1.3; B_2, 1.6; B_6, 1.8; B_{12}, 0.0015; C, 40; D, 0.30; E, 5.0; H, 0.025; K, 2.25; Choline chloride, 75; PABA, 5; Folic acid, 0.075; Inositol, 5. Minerals: Calcium, 1600; Cobalt, 0.005; Copper, 0.09; Iodine, 0.01; Ferric citrate, 0.06; Potassium, 3.5; Selenium, 0.005; Zinc, 0.9.

5.2.3. Proximate analysis

Moisture, protein, lipid and ash content of each diet, faeces, whole abalone body from each treatment, female and male whole ripe gonad were determined with three replicates (0.35 - 1.1 g, depending on the amount available of each sample) as follows:

- Moisture content was determined by weighing samples before and after drying at 70°C for 24 hours. Gonads and mucus samples were dried in a freeze dryer for 24 hours at - 54°C.
- Ash content was measured using the remaining dried samples by incinerating at 600°C in a muffle furnace for 8 hours.
- Crude protein content was analysed using the Kjeldhal method for nitrogen content of the samples. The nitrogen content of protein varies from 15 - 18%. If an average level of 16% N is assumed, crude protein is estimated by N x 6.25 (100/16). Non-protein N content such as urea and nucleic acids were considered in this study (AOAC, 1990).
-Lipid content was determined by a column procedure using methanol-chloroform-water as the eluting solvent (Bligh and Dyer, 1959).
- The percentage of carbohydrate was determined by difference where:
% carbohydrate = 100 - (% protein + % lipid + % ash).
- The energy content of three replicates was obtained by burning a preweighed dry sample in a Gallenkamp ballistic bomb calorimeter.

5.2.4. Growth

Shell growth and body weight

Growth rates were obtained from monthly measurements as gain in whole body weight and shell length from March to October 1998. Weights were obtained using an electronic balance (to 0.1 mg). A light microscope with a camera lucida and a digitising tablet were used to measure shell length in small abalone (<1 cm). Prior to weighing, each organism was dried with absorbent tissue until no more water came out from the foot and shell pores.

5.2.5. Determination of the components of the energy budget

5.2.5.1. Estimation of energy

The Gross Energy (*GE*) content of dried diets, faeces, whole abalone body from each treatment, whole female and male ripe gonad of the juveniles and mucus was estimated by ballistic bomb calorimetry (Miller and Payne, 1959).

Calibration of the bomb calorimeter was conducted by firstly combusting at least three cotton wicks of the standard length (7 cm) and a mean blank deflection was calculated. Three standard benzoic acid tablets with known energy density of 26.434 kJ g^{-1}, were combusted in turn and the mean calibration constant (the amount of energy per unit deflection - k) was determined by the following equation:

$$k\,(kJ / deflection) = \frac{26.434\ (kJ / g) \times weight\ of\ benzoic\ acid\ (g)}{deflection\ benzoic\ acid - mean\ deflection\ of\ blank} \qquad \text{Equation 5.4}$$

Triplicate dried samples were weighed and then combusted to obtain the *GE* content as:

$$GE\,(kJ / g) = \frac{k\ x\ (deflection\ of\ sample - deflection\ of\ blank)}{weight\ of\ sample\ (g)} \qquad \text{Equation 5.5}$$

Because *GE* is not the same amount of energy that is physiologically available, it must be corrected for bioavailability across the gastrointestinal tract to give the Digestible Energy (*DE*). The content per gram of dried sample assuming 95% availability as:

$$DE = (kJ / g) = GE\,(kJ / g)\ \text{x}\ 0.95 \qquad \text{Equation 5.6}$$

metabolisable Energy (*ME*) content per gram of the dried sample allowing for an assumed availability of 95% and the incomplete metabolism of protein as:

$$ME\ (kJ/g) = DE\ (kJ/g) - (N\% \times 0.075) \qquad \text{Equation 5.7}$$

where N% is the percent nitrogen in the sample calculated from Kjeldhal analysis as:

$$N\% = \frac{weight\ of\ N(g)}{weight\ of\ sample\ (g)\ x\ 100} \qquad \text{Equation 5.8}$$

However, the final estimation of *ME* content also includes the energy contained in unavailable carbohydrate, which constitutes a high proportion of the dry weight of the samples as:

$$ME\ (kJ) = ME\ (kJ/g\ dry\ weight)\ x\ total\ dry\ weight\ of\ sample\ (g) \qquad \text{Equation 5.9}$$

5.2.2. Somatic growth (*Pg*)

Somatic growth (*Pg*) was obtained from the August, September and October measurements of the daily gain in dry body weight.

At the end of the experiment representative samples of abalone from each treatment were weighed, using whole body values for those animals that did not develop gonad from 15°C on the three diets (SW, FM and CO).

Animals that did develop gonads from the treatments of 18° and 22°C on the three diets were dissected and the gonad separated from the rest of the body, in order to acquire the energy expended on the rest of the body components (shell, pedal and adductor muscles, head, tentacles, complete visceral mass, hemolymph, mucus and drained water). In order to avoid double assessment on reproductive investment, female and males gonads were not taken into account for somatic growth because to

assess the energy expended in reproduction (*Pr*) whole gonads were used.

Energy gain in growth was assessed by combusting a sample from each treatment in a bomb calorimeter. The other variables of the energy budget were also determined for the August to October samples.

5.2.5.3. Ingestion (*I*)

The experimental group was fed every afternoon and any uneaten food was collected the following morning to estimate the food consumed. Daily rations were related to wet body mass at about 5% on seaweed and 1% on formulated diets. The dry weight samples (dried at 60°C for 24 h) of each trial (15°, 18° and 22°C), were measured.

The feed ingested (*I*) was calculated in terms of dry weight with the following equation:

$$I = (GS / 100) - R \qquad \text{Equation 5.10}$$

where *G* is the weight of food offered per animal per day (in grams); *R*, the remaining food (in grams) after the abalone had fed; and *S*, represents a factor for the dissolution of the pellet and is calculated from the weight differences (*G* - *R*) in the tanks not containing abalone, thus a factor from each treatment was assessed.

To obtain the energy ingested through food it was necessary to obtain the calorific value for each diet to be applied for the specific treatment.

5.2.5.4. Faecal losses (*E*)

Juvenile abalone were held in the same conditions as that of the experiment for feed consumed (*I*). A known amount of food from each diet was given at 20:00 h and faeces were collected the following morning at 8:00 h. Faecal material was picked up with a plastic 10 ml pipette, placed in to a petri dish and examined under dissecting microscope. Any remaining food was removed and transferred to a 250 ml centrifuge

tube. The samples were then centrifuged at 2000 rpm for 5 min and excess water was removed and rinsed with sterilised water to eliminate seawater drops. In order to collect enough faecal material it was necessary to run a 20 day trial. Each day samples were labelled and frozen until the end of the faecal collection period. This material was dried to constant weight at 60°C. Dry faeces were used to analyse protein, lipid, ash and energy content.

Thus absorption (*Ab*) was calculated for each treatment from the total ingested food (*I*) minus the total egested faeces (*E*).

5.2.5.5. Reproductive investment (*Pr*)

During the monthly measurements of length and weight, organisms were examined, sexed and assigned to one of the 5 reproductive stages depending on the level of gonad development: stage 1 = indeterminate, 2 = immature males and females, 3 = small gonad bulk, 4 = large gonad bulk or ripe and 5 = empty collapsed gonad lumen. The following equation was applied:

$$5\lambda_5 = \frac{\lambda_1 + 2\lambda_2 + 3\lambda_3 + 4\lambda_4}{N} \qquad \text{Equation 5.11}$$

where λ = number of stages and N = total number of stages.

Because reproductive investment determined from the number of eggs does not give a true reflection of the total investment in gonad production, organisms representative from each stage and from each treatment (diets and temperatures) were dissected and the complete gonad separated, frozen (-70°C) and dried in a freeze dryer (IEC Lyoprep 3000) at 0.5 M bar per 24 hours at -54°C. Thus, female and male gonads were used to determine the calorific content for each sex and for each stage. For this analysis digestive abalone gland was not separated from the total gonad weight. The freeze-dried gonads were then ground and representative samples were used for proximate

analysis and energy content. Calculations were based on the number of days taken to reach a determined stage, and energy consumed reported as calories per day per animal dry weight.

5.2.5.6. Respiration (*R*)

To convert oxygen uptake to energy losses it is necessary to use an oxy-calorific coefficient for each diet, and it depends upon the type and concentration of metabolic substrate respired: protein, lipid and carbohydrate (Jobling, 1993). The denominate used was animal dry weight, in order to determine the maintenance energy required for juvenile abalone *H. tuberculata*.

The size of animals used in the respiration experiment varied. As a result a preliminary experiment was set to determine the volume of seawater used and the experiment duration needed at a set temperature. Animals of a specific size that consume between 20 and 40% of the oxygen in no more than one hour were used for the experiment. If they used less it was difficult to measure the oxygen consumed and if greater than 40% the animals were stressed and oxygen consumption was increased considerably.

In respiration, all batches of the juveniles from the experiment for growth, from each treatment (15°, 18°, 22°C and SW, FM, CO diets) were used and labelled "Main group". Additionally 3 organisms of the same size (approximately) were selected from each treatment and labelled "Subgroup", in order to find the respiration rate in both light (starting at 08:00 h) and dark (starting at 22:00 h) as day and night over grouped and individual animals. Organisms were transferred to 11 respirometer vessels under the same conditions as those of the growth experiment. Prior to use, seawater (32 ± 3 ppt.) was filtered and aerated for 2 to 3 hours or until oxygen saturation and stored at the required temperature for the day trial.

As a preliminary test the dissolved oxygen content and temperature were monitored in the respirometer vessels with polarographic probes connected to a computer (Endeco, pulsed D.O. Sensor Controller), data were recorded every 5 minutes. At the same time oxygen consumption (µl O_2 day^{-1}) was measured using the Winkler method (Strickland and Parsons, 1968), and by the end the Winkler method was more readable, thus data from this method was used to obtain oxygen consumption. Three samples of seawater from each vessel were carefully siphoned into 110 ml BOD bottles avoiding the production of bubbles in the samples. An extra set of samples was used in each trial as a control without abalone, to measure the oxygen consumption resulting from microbial respiration.

The relationship between body size and oxygen consumption established that metabolism is proportional to a constant power of the body weight as described by the equation:

$$Ln\,R = a + b \times Ln\,W \qquad \text{Equation 5.12}$$

where R is oxygen consumption in µl O_2 day^{-1} $animal^{-1}$, W is dry body weight in mg, a and b are constants. The value of a denotes the level of the metabolic rates of an organism of unit body weight, and varies according to a wide variety of factors, including temperature of culture and diets. The value b is less variable, when the data for all organisms are pooled, it is found that b (related to weight) approaches a value of 0.86 for use with fish species (Brett and Groves, 1979; Bayne and Newell, 1983). However, any factor that affects large and small individuals differently, e.g. seasonal reproductive processes, will alter the value of the slope of the regression.

5.2.5.7. Excretion (*U*)

Experimental conditions for ammonia production were the same as those for oxygen

consumption. Seawater used for this experiment was previously boiled for 1 hour and aerated for 2 to 3 hours (thus the final ammonia concentration was a mean of 0.075 µg-atoms N l^{-1}) and stored at the required temperature for the day trial. One litre vessels were filled with this seawater and incubated in temperature-controlled rooms for one to three hours depending on the organism weight. Measurements of ammonia production were performed again both on light and dark as a day and night excretion.

As the experiments were for a maximum of three hours no feeding was necessary, thus avoiding the possibility of altering the ammonia concentration with food presence. Analyses of seawater in the incubator tanks was carried out at the beginning and at the end of each experimental period.

The total ammonia excretion was calculated as the difference between experimental and blank readings and expressed as µg-atoms N l^{-1} (Solorzano, 1969) from a regression obtained from a series of ammonia standards, thus the followed equation was obtained: µg-atoms NH_4 l^{-1} = -0.99 + (76.7 x absorbance). A 50 ml sample of seawater was mixed with 2 ml of phenol solution (10 mg phenol in 100 ml of 100 % v/v ethyl alcohol), plus 2 ml of nitroprusside (0.5 g of sodium nitroprusside in 100 ml of de-ionised water) and 5 ml of oxidising solution (Table 5.2), mixing after each addition by swirling the flasks. All glassware was acid washed before use.

This end solution was then incubated in a dark room at 27°C and after approximately 2 hours the incubated samples were read on a spectrophotometer at 640 nm.

Fresh solutions were prepared for each batch of measurements, and ammonia concentration was calculated from regression obtained from a series of ammonia standard solutions. And finally, to obtain the energy loss by ammonia excretion a coefficient of 68.9 kcal mol^{-1} was used (Brafield and Solomon, 1972).

Table 5.2. Oxidising solution used, for ammonia determination (from Solorzano, 1969).

Alkaline Solution	Oxidising Solution
100 g of sodium citrate	5 ml of sodium hypochlorite (about 1.5 N)
5 g sodium hydroxide	20 ml of alkaline solution
500 ml de-ionised water	

The relationship between body size and ammonia excretion established that metabolism is proportional to a constant power of the body weight as described by the equation:

$$Ln\ U = a + b \times Ln\ W \qquad \text{Equation 5.12}$$

where U is ammonia excretion in µg-atoms N l^{-1} day^{-1} $animal^{-1}$, W is dry body weight in mg, a and b are constants.

5.2.5.8. Pedal mucus production (*M*)

To avoid abnormal mucus production, the determination of mucus was performed three days before the corresponding measurements of length and weight in September, thus reducing an extra stress levels for this month.

The entire experiment was repeated three times every day. Each container (1l beakers for main group and 400 ml beakers for subgroup) was cleaned of food and faeces, and refilled with filtered seawater and settling under the same conditions as that for growth. Pedal mucus production was measured for each experimental trial (15°, 18° and 22°C and on SW, FM and CO diets).

In order to achieve a large enough quantity of mucus the total number of abalone from the experiment for growth were left for six hours. After six hours the animals were removed and each container was rinsed with distilled water to clean off faeces and seawater. The pedal mucus adhered to the walls along with that from the foot of each abalone was carefully scraped off onto a pre-weighed beaker. Each beaker was dried in a freeze drier at 0.5 M bar per 24 hours at -54°C and then re-weighted to determine the dry weight of samples. The dried samples of pedal mucus were used for proximate analysis and energy content.

5.2.6. Statistical analyses

When no significant differences were found between experimental replicates, data were pooled and one-way or two-way analysis of variance at a significance level of $P < 0.05$ were applied. In proximate analysis a two-sample *t*-test was used to compare the means between samples. A two-way analysis of variance was used in order to compare the shell length and body weight between diets and temperatures followed by a multiple comparison of the means (SNK, Student-Newman-Keuls method). The daily growth rates were analysed by Kruskal-Wallis one-way analysis of variance on ranks method. All statistics were calculated using the Sigmastat package (1996).

5.3. RESULTS

5.3.1 Proximate analysis and energy content

The Kruskal-Wallis test shown that the three diets (SW, FM and CO) were significantly different in the protein ($H = 7.2$, df = 2, $P = 0.00357$; 15.52, 29.14 and 33.05%, respectively), lipid (K-W test, $H = 7.2$, df = 2, $P = 0.00357$; 3.1, 9.46 and 11.51%, respectively) and ME2 (ANOVA, $F_{2,6} = 91.8$; $P < 0.0001$; 11.2, 15.4 and 15.9%, respectively) content. No significant differences were found in carbohydrate

content between SW and CO diet (K-W test, $H = 5.42$, df = 2, $P = 0.0714$; 51.27 and 51.99%, respectively) (Table 5.3).

Faeces from individuals feeding on the three diets (SW, FM and CO) were significantly different in protein (K-W test, $H = 5.96$, df = 2, $P = 0.0250$; 5.09, 5.66 and 12.8%, respectively), lipid (ANOVA, $F_{2,6} = 108.2$, $P < 0.0001$; 0.1, 1.02 and 2.3%, respectively) and ME2 (K-W test, $H = 7.2$, df = 2, $P = 0.00357$; 6.7, 8.4 and 11.0%, respectively) content. However, no significant difference in carbohydrate content was found between SW-faeces and FM-faeces (K-W test, $H = 4.36$, df = 2, $P = 0.132$; 47.5 and 49.14%, respectively).

The whole animal samples fed on the three diets showed significant differences in protein (K-W test, $H = 5.96$, df = 2, $P = 0.025$; 41.18, 55.5 and 58.3%, respectively), lipid (K-W test, $H = 5.96$, df = 2, $P = 0.025$; 2.8, 9.84 and 8.59%, respectively), carbohydrate (K-W test, $H = 6.49$, df = 2, $P = 0.0107$; 28.9, 12.16 and 11.0%, respectively) and ME2 (K-W test, $H = 5.6$, df = 2, $P = 0.005$; 13.9, 15.9 and 16.3%, respectively) content. Samples of ripe female and male gonads of the matured abalone from this study are significantly different in protein ($t = -12$, df = 4, $P = 0.0003$; 54.22 and 82.8%, respectively), lipid ($t = 26.6$, df = 4, $P \leq 0.0001$; 32.4 and 6.75%, respectively), carbohydrate ($t = 8.13$, df = 4, $P = 0.0012$; 7.73 and 5.06%, respectively) and ME2 ($t = 6.02$, df = 4, $P = 0.0038$; 22.0 and 17.6%, respectively) content (Table 5.3). Diets, faeces and whole animal tissue from the CO treatment showed the highest metabolised energy values with 4,063 ± 141, 2,629 ± 181 and 3,895 ± 191 cal g^{-1} dry weight animal, respectively.

Table 5.3. Proximate analyses as percent of diets, faeces, whole body, mucus, female and male ripe gonad from each treatment of abalone *Haliotis tuberculata*. Gross Energy (GE1 and GE2) and Metabolisable Energy (ME2) per gram dry weight of each sample. Mean values and standard error ±.

Sample	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)	Moisture (%)	Nitrogen (%)	GE1 (kJ/g)	GE2 (kJ/g)	ME2 (kJ/g)
Seaweed diet	15.52 ±0.5	3.10 ±0.4	51.27 ±1.7	30.15 ±0.6	86.01 ±1.5	2.63 ±0.00	11.53 ±0.3	12.0 ±0.44	11.2 ±0.32
Seaweed faeces	5.09 ±0.6	0.10 ±0.03	47.50 ±2.2	47.40 ±1.7	82.8 ±3.8	0.81 ±0.03	8.27 ±0.7	7.1 ±0.8	6.7 ±0.51
Whole animal	41.18 ±0.9	2.80 ±0.7	28.9 ±1.7	27.30 ±1.0	60.5 ±2.2	6.56 ±0.44	12.98 ±0.6	15.1 ±0.29	13.9 ±0.23
Fishmeal diet	29.14 ±1.1	9.46 ±0.8	45.18 ±2.3	16.22 ±0.5	27.4 ±1.9	5.64 ±0.63	15.47 ±0.8	16.7 ±0.72	15.4 ±0.61
Fishmeal faeces	5.66 ±0.6	1.02 ±0.3	49.14 ±4.9	42.46 ±1.4	85.0 ±4.1	0.92 ±0.1	8.62 ±0.5	8.9 ±0.60	8.4 ±0.20
Whole animal	55.50 ±3.8	9.84 ±1.1	12.16 ±1.1	22.5 ±1.0	59.50 ±2.3	8.95 ±0.36	15.73 ±0.9	17.5 ±0.69	15.9 ±0.48
Commercial diet	33.05 ±1.6	11.51 ±1.0	51.99 ±3.6	3.45 ±0.3	1.2 ±0.1	5.35 ±0.30	17.93 ±0.6	18.4 ±0.85	17.0 ±0.59
Commercial faeces	12.80 ±0.6	2.30 ±0.1	54.52 ±3.5	30.38 ±2.2	88.7 ±3.7	2.1 ±0.39	11.21 ±0.4	11.75 ±0.9	11.0 ±0.76
Whole animal	58.30 ±1.6	8.59 ±1.3	11.0 ±0.45	21.78 ±1.2	59.06 ±2.2	9.28 ±0.35	15.64 ±0.7	17.9 ±0.92	16.3 ±0.80
Mucus	2.10 ±0.1	0.70 ±0.06	22.07 ±0.9	75.06 ±1.2	90.50 ±3.3	0.35 ±0.04	10.02 ±0.8	10.47 ±1.0	9.77 ±0.40
Female ripe gonad	54.22 ±3.1	32.4 ±1.5	7.73 ±0.36	5.67 ±0.5	67.48 ±2.9	8.65 ±0.49	22.87 ±0.8	23.8 ±0.95	22.0 ±0.79
Male ripe gonad	82.8 0±2.7	6.75 ±0.8	5.06 ±0.42	5.29 ±0.4	70.06 ±3.9	13.5 ±0.78	18.73 ±0.3	19.6 ±1.1	17.6 ±0.90

GE1 is the energy calculated from the proximal analysis (protein, lipid and carbohydrate) and GE2 was calculated burning samples in a bomb calorimeter. ME2 was calculated from GE2. To convert J to calories, multiply by 4.184.

5.3.2 Growth

Mean wet growth in shell length and whole body weight increased rapidly from day 30 until 90 day and to day 120 when abalone was fed on formulated diets and reared at 18° and 22°C (Figures 5.1 and 5.2). By the end of the experimental period, abalone fed on formulated diets and reared at 22°C had gained an average weight of 1,928.5 mg and an average of 18.85 mm length, whereas animals fed on SW had grown to an average of 798.0 mg and 13.7 mm at the same temperature of culture (Appendixes 1 - 16).

Shell length

At 15°C there was no significant effect of diet on the growth rates in terms of shell length on the organisms (ANOVA, $F_{20, 42} = 1.61$; $P = 0.0974$). Growth was steady and also showed the lowest mean growth rates of the entire experiment. At 18° and 22°C a significant differences between formulated and SW diets were found (ANOVA, $F_{20, 42} = 30.1$; $P < 0.0001$). The highest rates were for those organisms fed on FM diet and cultured at 22°C by 90 day. Additionally, juveniles fed on formulated diets (FM and CO) and cultured at 22°C show a rapid growth during the first 90 days of the study, with an average shell length growth rate of 134.8 ± 5.89 μm day^{-1} and 127.90 ± 3.27, respectively (Table 5.4 and Figure 5.3).

After 90 days the growth decreased notably reaching less than half of the highest rates (58.22 ± 7.43 μm day^{-1}). The decrease in growth rates at this time by the juveniles cultured at 22°C and fed on formulated diets coincided with the investment in production of gametes.

Overall, growth of juveniles *H. tuberculata* reared at 15°C and fed on the three diets is similar. Growth increased at higher temperatures and the best growth in shell length was reached with FM and CO diets reared at 22°C.

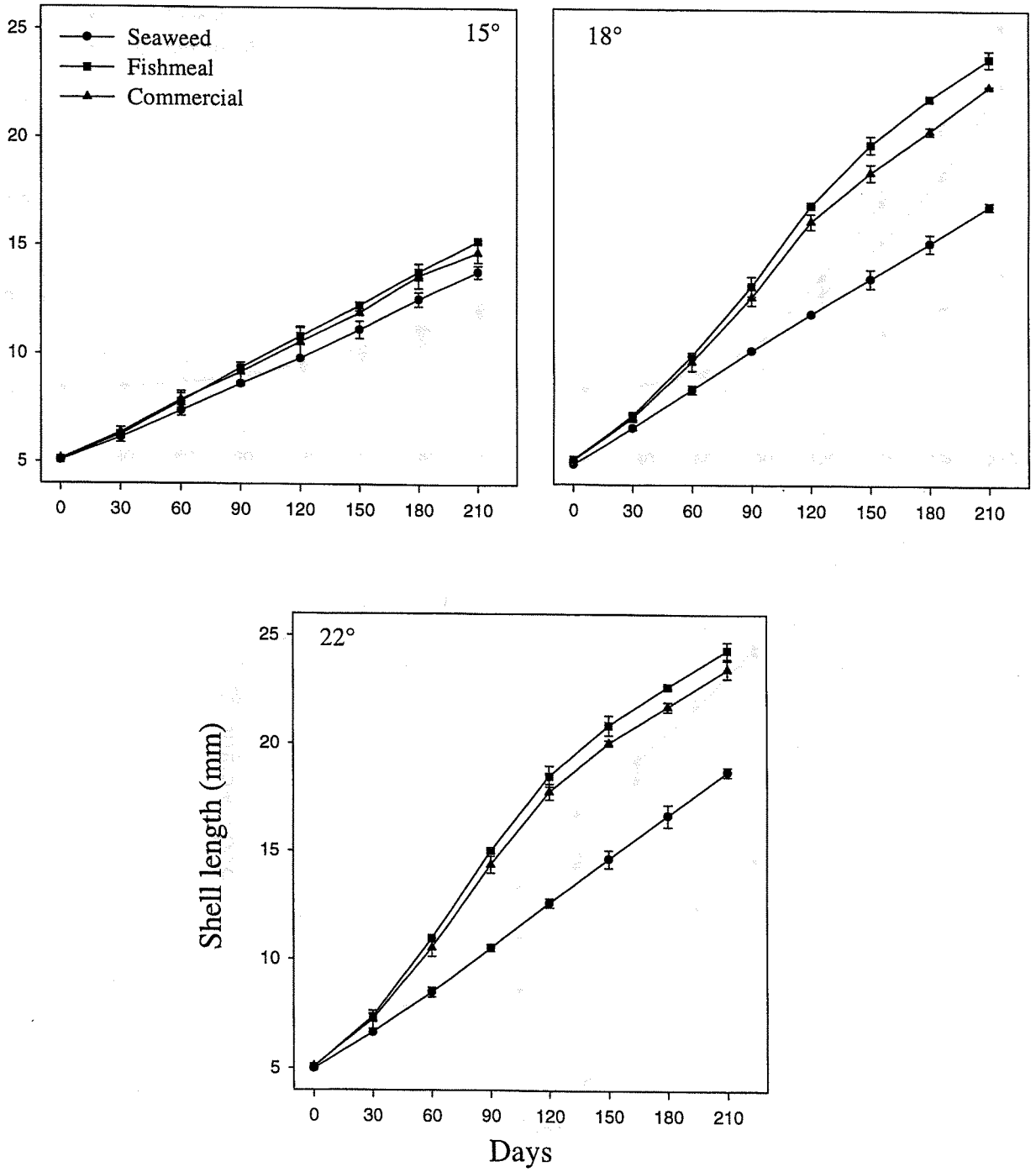


Figure 5.1. Mean shell length (mm) of *Haliotis tuberculata* fed during 210 days (March to October) with seaweed (SW) and two formulated diets (FM and CO) and cultured at 15°, 18° and 22 °C. Bars indicate standard errors.

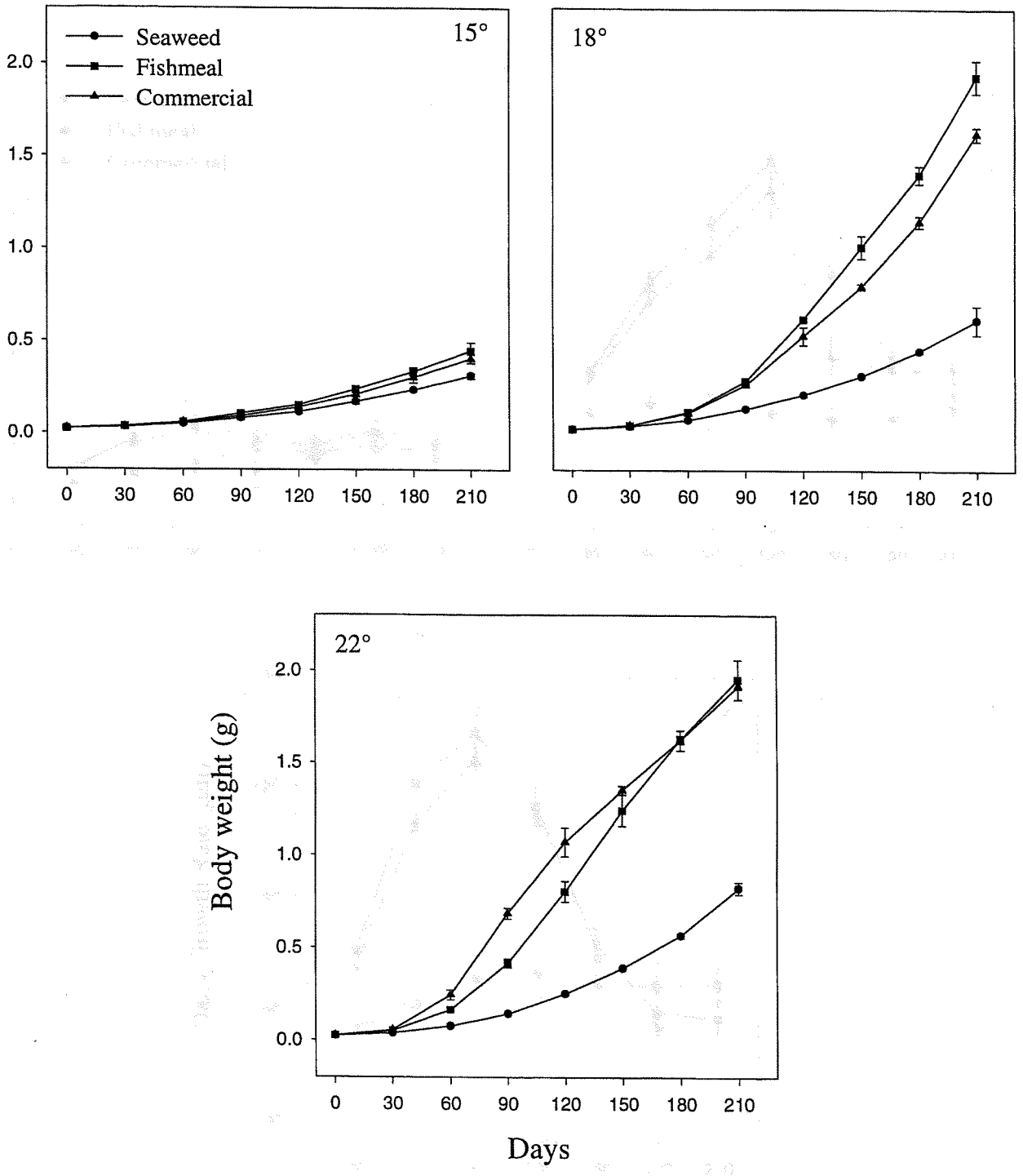


Figure 5.2. Mean body weight (g) of *Haliotis tuberculata* fed during 210 days (March to October) with seaweed (SW) and two formulated diets (FM and CO) and cultured at 15°, 18° and 22 °C. Bars indicate standard errors.

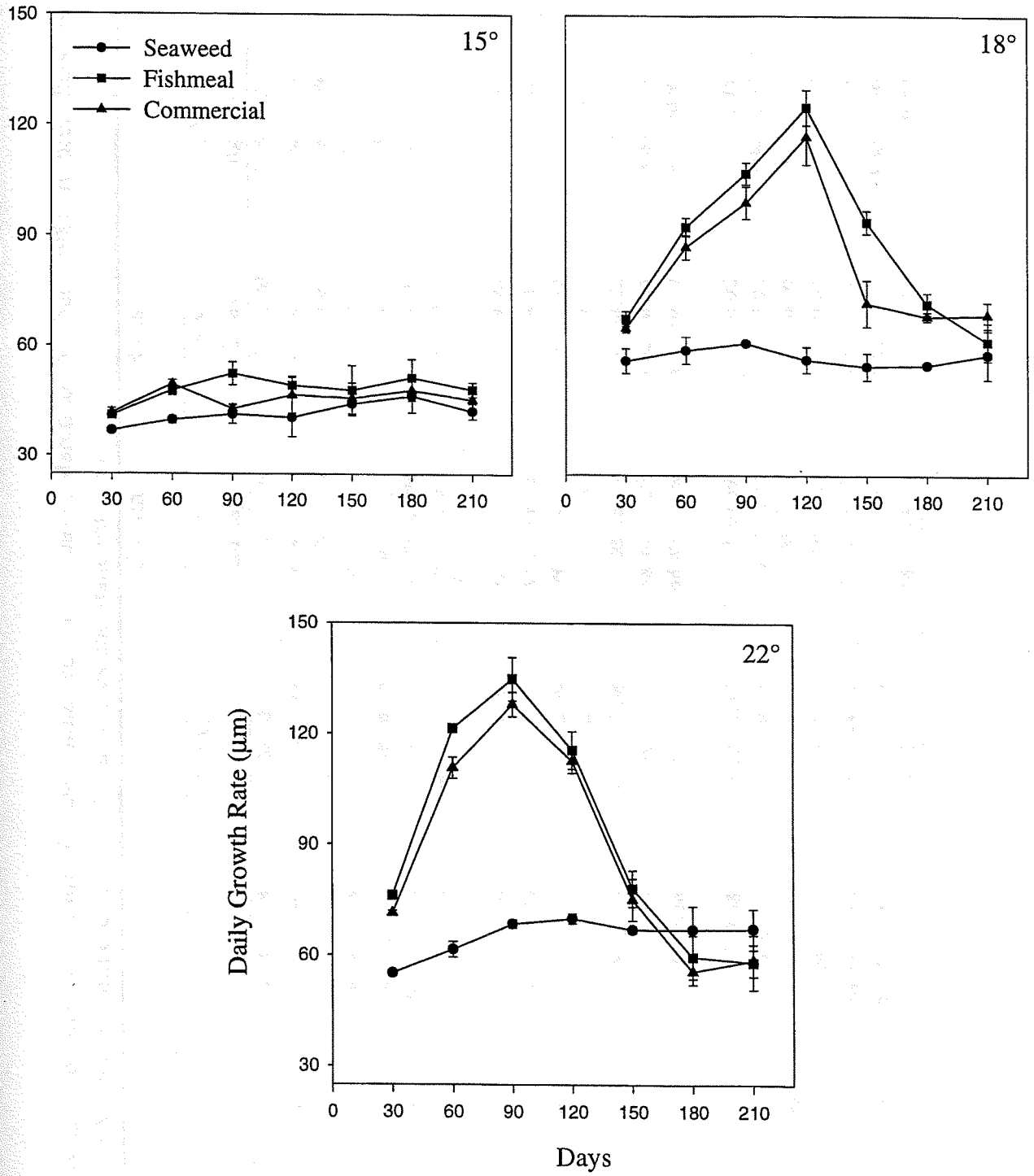


Figure 5.3. Mean daily growth rates (µm) of *Haliotis tuberculata* fed during 210 days (March to October) with seaweed (SW) and two formulated diets (FM and CO) and cultured at 15°, 18° and 22 °C. Bars indicate standard errors.

Table 5.4. Daily growth rates of juvenile *H. tuberculata* fed for 210 days (March to October 1998) on three diets, mix of seaweed (SW), fishmeal (FM) and a commercial (CO). Mean values and standard error ±.

		Growth Rates					
Temperature of culture	Days	SW $\mu m\ day^{-1}$	FM $\mu m\ day^{-1}$	CO $\mu m\ day^{-1}$	SW $mg\ day^{-1}$	FM $mg\ day^{-1}$	CO $mg\ day^{-1}$
15 °C	30	36.78 ±0.48	41.00 ±0.51	41.56 ±1.26	0.31 ±0.00	0.36 ±0.01	0.38 ±0.02
	60	39.67 ±0.96	47.78 ±1.44	49.44 ±1.11	0.61 ±0.02	0.82 ±0.01	0.80 ±0.07
	90	41.22 ±2.50	52.33 ±3.18	42.78 ±1.18	1.01 ±0.01	1.38 ±0.09	1.12 ±0.06
	120	40.44 ±5.28	49.11 ±2.44	46.44 ±4.83	1.20 ±0.10	1.81 ±0.07	1.64 ±0.03
	150	44.11 ±3.12	47.89 ±6.76	45.67 ±4.18	2.11 ±0.21	2.28 ±0.12	2.37 ±0.04
	180	46.33 ±4.54	51.33 ±5.05	47.89 ±2.80	2.03 ±0.00	3.62 ±0.29	3.33 ±0.09
	210	42.22 ±4.54	48.11 ±1.95	45.33 ±2.65	2.64 ±0.12	5.83 ±0.11	5.92 ±0.36
18 °C	30	56.22 ±3.39	67.67 ±2.03	65.11 ±1.13	0.52 ±0.02	0.79 ±0.03	0.72 ±0.02
	60	59.11 ±3.70	92.67 ±2.52	87.22 ±3.32	1.22 ±0.03	2.43 ±0.03	2.28 ±0.04
	90	61.11 ±0.73	107.2 ±2.99	99.33 ±4.35	2.04 ±0.03	5.48 ±0.10	5.08 ±0.19
	120	56.56 ±3.55	125.1 ±4.73	117.2 ±7.61	2.55 ±0.13	11.27 ±0.19	8.88 ±0.49
	150	54.78 ±3.70	94.10 ±3.08	72.00 ±6.33	3.42 ±0.22	13.05 ±0.19	8.94 ±0.85
	180	55.11 ±0.56	71.89 ±3.01	68.44 ±1.25	4.62 ±0.24	14.63 ±0.54	11.70 ±0.66
	210	58.00 ±6.68	61.67 ±5.17	68.89 ±3.56	6.77 ±0.10	14.27 ±0.91	12.49 ±0.58
22 °C	30	55.11 ±0.68	76.22 ±0.62	71.33 ±0.69	0.51 ±0.02	1.21 ±0.05	1.07 ±0.02
	60	61.56 ±2.13	121.4 ±0.29	110.70 ±2.85	1.25 ±0.03	6.94 ±0.07	6.23 ±0.01
	90	68.44 ±1.06	134.8 ±5.89	127.90 ±3.27	2.21 ±0.06	14.9 ±0.31	14.56 ±0.10
	120	69.89 ±1.35	115.6 ±5.13	112.56 ±3.27	3.69 ±0.05	12.9 ±0.40	13.00 ±0.19
	150	66.89 ±0.80	78.11 ±4.92	75.11 ±5.72	4.57 ±0.02	9.47 ±0.24	9.19 ±0.51
	180	67.00 ±6.43	59.67 ±5.90	55.67 ±3.51	5.82 ±0.39	8.83 ±0.46	10.19 ±0.14
	210	67.22 ±5.49	58.22 ±7.43	58.78 ±4.36	7.94 ±0.63	9.17 ±0.46	8.58 ±0.28

Body weight

Growth rates of body weight (mg day^{-1}) of abalone cultured at 15°C were statistically similar (K-W test, $H = 61.2$, df = 20, $P \leq 0.1$) between diets, from the beginning of the experiment up to150 days. However, from day 150 growth rates (mg day^{-1}) became different between formulated and SW diets (K-W test, $H = 70.1$, df = 20, $P \leq 0.001$). Thus, during the last two months organisms fed on formulated diets grew faster than those fed on SW reaching up to 5.92 ± 0.36 mg day^{-1}.

Abalone fed on formulated diets and cultivated at 18°C were statistically significantly different (K-W test, $H = 61.3$, df = 20, $P \leq 0.0001$) in body weight during the first 90 days. After day 120 those organisms fed on FM diet started to develop faster growth, reaching up to 14.63 ± 0.54 mg day^{-1}. On the other hand, abalone cultured at 22°C and fed on formulated diets were similar (K-W test, $H = 60.3$, df = 20, $P \leq 0.05$) in body weight. Moreover, the highest growth rates were reached after 90 days, and from this time the growth fell suddenly from 14.9 ± 0.31 mg day^{-1} to 9.17 ± 0.46 mg day^{-1} by the end of the experiment when abalone were fed on FM and CO diets and cultured at 22°C (Table 5.4 and Figure 5.4).

Comparing the reductions in daily growth rates (from figures 5.3 and 5.4) of abalone fed on formulated diets and cultured at 18°C, it can be seen that possibly gonad maturation did decrease more growth rates on shell length than that of body weight.

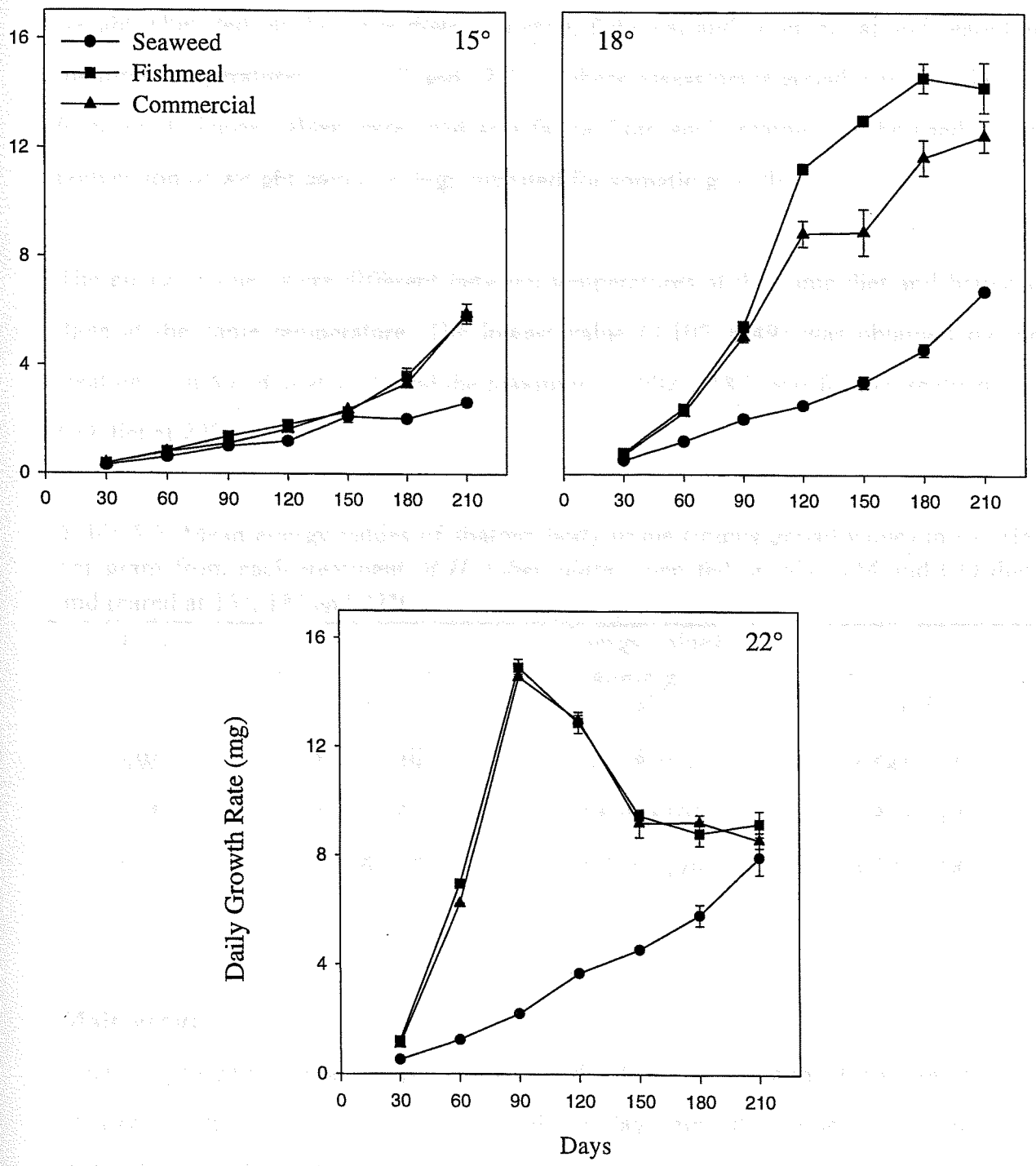


Figure 5.4. Mean daily growth rates (mg) of *Haliotis tuberculata* fed during 210 days (March to October) with seaweed (SW) and two formulated diets (FM and CO) and cultured at 15°, 18° and 22 °C. Bars indicate standard errors.

5.3.3. Somatic growth

Table 5.5 shows the mean energy values in calories per gram of abalone dry body weight when fed on the three diets: seaweed, fishmeal and commercial and reared at the three temperatures: 15°, 18° and 22°C. In these assessments gonad was not taken in to account. Those values were used as a factor from each treatment to be used in the conversion of weight gain to energy invested for somatic growth.

The energy values were different between temperatures at the same diet and between diets at the same temperature. The lowest value (3,105 ± 49) was obtained by the treatment on SW diet at 15°C and the maximum (3,902 ± 187) was for the treatment on CO diet at 22°C.

Table 5.5. Mean energy values of abalone body tissue (minus gonad value) in calories per gram from each treatment *of H. tuberculata* when fed on SW, FM and CO diets and reared at 15°, 18° and 22°C.

Diets	Energy values calorie g^{-1}		
	15°	18°	22°
SW	3,105 ±49	3,518 ±71	3,581 ±112
FM	3,477 ±62	3,690 ±105	3,877 ±172
CO	3,536 ±48	3,705 ±126	3,902 ±187

Main group

Final weight gained in grams per animal per day from each treatment was converted to energy investment in calories per animal per day using the value factors shown in Table 5.5, in order to determine the energy utilised on somatic growth.

By the end of the experimental period animals cultured at 18°C and fed on CO diet

were spending 39.7% more energy on growth than those cultured at 22°C and fed on the same diet (Table 5.6).

Table 5.6. Weight gained from the Main group in grams per animal per day and then converted to calories per animal per day. Values determined for the September and October samples.

°C	Diet	Shell Length (mm)	Body Weight (g)	Weight gained grams x 10^{-3} animal^{-1} day^{-1}	Energy investment calories animal^{-1} day^{-1}
15°	SW	12.2 ±0.3	0.11 ±0.01	0.88 ±0.06	2.7 ±0.2
	FM	13.9 ±0.3	0.13 ±0.01	1.61 ±0.09	5.5 ±0.3
	CO	13.4 ±0.2	0.13 ±0.01	1.46 ±0.12	5.1 ±0.5
18°	SW	15.2 ±0.4	0.23 ±0.01	1.75 ±0.19	6.1 ±0.6
	FM	21.9 ±0.5	0.59 ±0.05	6.15 ±0.26	22.5 ±0.9
	CO	20.5 ±0.5	0.47 ±0.03	5.04 ±0.20	18.5 ±0.7
22°	SW	16.7 ±0.4	0.23 ±0.01	2.23 ±0.14	7.9 ±0.4
	FM	22.6 ±0.5	0.67 ±0.05	3.51 ±0.16	13.6 ±0.6
	CO	21.9 ±0.4	0.66 ±0.03	3.74 ±0.17	14.5 ±0.6

Subgroup

To obtain the energy invested in somatic growth by abalone of the same size the value factors show on Table 5.5 were used.

Animals fed on formulated diets and cultured at 18° and 22°C invested more energy in somatic growth compared with those fed on SW and cultured at 15°C. Furthermore, by the end of the experiment abalone cultured at 18°C and fed on formulated diets were investing more energy in growth than those cultured at 22°C and fed on formulated diets. This is probably owing to animals from the 22°C group diverting more energy into reproduction and spawning than those from the 18°C group (Table 5.7). In other words, abalone from FM 18°C with mean dry body weight of 0.24 g were spending

39.42 ± 2.1 cal per day compared with abalone FM-22°C with mean dry body weight of 0.34 g that were spending 25.21 ±1.0 cal per day.

Table 5.7. Weight gained from the Subgroup in grams per animal per day and then converted to calories per animal per day. Values determined for the September and October samples.

°C	Diet	Shell Length (mm)	Body Weight (g)	Weight gained Grams x 10^{-3} Animal^{-1} day^{-1}	Energy investment calories animal^{-1} day^{-1}
15°	SW	16.2 ±0.4	0.20 ±0.02	2.51 ±0.21	7.75 ±0.7
	FM	16.2 ±0.4	0.21 ±0.01	3.15 ±0.20	10.63 ±0.7
	CO	16.7 ±0.4	0.23 ±0.01	2.68 ±0.22	9.65 ±0.8
18°	SW	16.3 ±0.6	0.22 ±0.02	3.30 ±0.11	11.29 ±0.4
	FM	16.4 ±0.2	0.24 ±0.02	10.67 ±0.54	39.42 ±2.1
	CO	16.7 ±0.5	0.21 ±0.02	9.33 ±0.50	34.44 ±1.8
22°	SW	16.4 ±0.9	0.23 ±0.04	4.91 ±0.14	17.05 ±0.5
	FM	16.5 ±0.8	0.34 ±0.02	6.52 ±0.25	25.21 ±1.0
	CO	16.4 ±0.7	0.33 ±0.02	8.09 ±0.32	31.43 ±1.2

5.3.4 Absorption

The metabolisable energy content (kJ g^{-1}) of diets and faeces are given in Table 5.3 (page 88). In this experiment feeding frequency was once every day, and the amount of food consumed tended to increase with temperature and animal size. Means of ingested, egested and absorbed energy are presented on Table 5.8 and 5.9.

Main group

Organisms fed on the SW diet and cultured at 15°C presented the lowest metabolised energy ingested with only 14.2 ±0.5 cal day^{-1} animal^{-1}. Those fed on formulated diets ingested up to 40 % more at the same temperature. Moreover, abalone fed on formulated diets did present significant differences (K-W test, H = 24.7, df = 8, P =

0.0018) across temperatures, showing the highest ingested energy by organisms fed on CO diet (up to 123 ±7.1 cal day^{-1} $animal^{-1}$).

Energy egested in those animals cultured at 15°C did show significant differences between SW and formulated diets (K-W test, $H = 24.7$, df = 8, $P = 0.0023$). However, organisms fed on formulated diets and reared at 18°C were not significantly different in terms of energy egested with values of 8.8 ± 0.6 for FM and 8.7 ± 0.5 for CO diet (K-W test, $H = 24$, df = 8, $P > 0.05$) (Table 5.8).

Table 5.8. Metabolized energy from the Main group (calories per day per animal per gram dry weight) ingested (*I*), egested (*E*) and absorbed (*Ab*) from the three diets: mix of seaweed (SW), fishmeal (FM) and a commercial (CO). Values determined for the September and October samples. Mean values and standard error ±.

°C	Diets	n	Shell Length (mm)	Body Weight (g)	Ingestion	Egestion	Absorption
15°	SW	42	12.2 ±0.3	0.11 ±0.01	14.2 ±0.5	2.4 ±0.3	11.8 ±0.2
	FM	39	13.9 ±0.3	0.13 ±0.01	23.2±1.2	2.8 ±0.3	20.4 ±0.6
	CO	40	13.4 ±0.2	0.13 ±0.01	23.8 ±2.9	3.0±0.5	20.8 ±1.9
18°	SW	42	15.2 ±0.4	0.23 ±0.01	24.0 ±3.2	2.7 ±0.1	21.3 ±2.8
	FM	42	21.9 ±0.5	0.59 ±0.05	76.7 ±3.1	8.8 ±0.6	68.0 ±2.2
	CO	40	20.5 ±0.5	0.47 ±0.03	97.0 ±4.2	8.7 ±0.5	88.9 ±3.1
22°	SW	41	16.7 ±0.4	0.23 ±0.01	32.2 ±2.7	3.7 ±0.2	28.5 ±1.8
	FM	43	22.6 ±0.5	0.67 ±0.05	98.7 ±4.4	6.5 ±0.4	92.7 ±3.7
	CO	41	21.9 ±0.4	0.66 ±0.03	123 ±7.1	7.6 ±0.6	115 ±4.2

Metabolized energy of the three diets and faeces determined by burning samples in a bomb calorimeter: 2,677 ±76 and 1,601 ±122 for SW; 3,680 ±146 and 2,629 ±47.8 for FM; 4,063 ±141 and 2,629 ±182 calories for CO.

Abalone fed on CO diet cultured at 22°C present the highest energy intake and the highest energy egested (123 ± 7.1 and 7.6 ± 0.6 cal day^{-1} animal^{-1}, respectively) reaching the highest proportion in absorption of the entire experiment. The proportion of the daily energy absorbed from formulated diets was significantly different between temperatures (K-W test, H = 24.8, df = 8, P = 0.0017). Organisms fed on SW diet showed the lowest proportions of the energy absorbed at all temperatures (11.8, 21.3 and 28.5 cal day^{-1} animal^{-1} dry weight, respectively; Table 5.8).

Subgroup

Ingestion, egestion and absorption were assessed using 3 organisms of the same size (16.36 ±0.17 mm and 0.24 ±0.03 dry weight) from each treatment. Energy across ingestion of SW diet is significantly different between temperatures at 15° 25.0 ± 0.5, at 18° 33.9 ± 3.2 and at 22°C 45.8 ± 2.7 cal day $^{-1}$ animal^{-1} dry weight (K-W test, H = 23.8, df = 8, P = 0.0025).

The highest energy intake was observed at 18° and 22°C with values up to 70.8 ± 3.1 and 75.8 ±7.1 cal day^{-1} animal^{-1} dry weight, respectively, when feed on formulated diets (Table 5.9).

Faeces were difficult to collect and data for egestion show big deviation values on all treatments (Table 5.9). This was possibly a result of experimental error in the collection of the faeces as some days the amount was different even in the same replicate. One-fifth of the energy ingested was lost as faeces from those cultured at 18° and 22°C.

Results of the energy egested as faeces show differences (K-W test, H = 43.1, df = 8, P = 0.0017) between diets and temperatures. The lowest mean percentage of egested energy was when juveniles were fed on SW and FM diets, with a range of 11.2 to 14.3% and also it was not affected by temperature.

Table 5.9. Metabolized energy from the Subgroup (calories per day per animal per gram dry weight) ingested (*I*), egested (*E*) and absorbed (*Ab*) from the three diets: mix of seaweed (SW), fishmeal (FM) and a commercial (CO). Values determined for the September and October samples. Mean values and standard error ±.

°C	Diet	n	Shell Length (mm)	Body Weight (g)	Ingestion	Egestion	Absorption
15°	SW	3	16.2 ±0.4	0.20 ±0.02	25.0 ±0.5	3.0 ±0.4	22.1 ±0.3
	FM	3	16.2 ±0.4	0.21 ±0.01	29.4 ±1.2	4.2 ±1.0	25.2 ±0.7
	CO	3	16.7 ±0.4	0.23 ±0.01	32.1±2.9	6.8 ±1.1	25.3 ±2.1
18°	SW	3	16.3 ±0.6	0.22 ±0.02	33.9 ±3.2	4.0 ±0.5	29.9 ±1.7
	FM	3	16.4 ±0.2	0.24 ±0.02	70.8 ±3.1	7.9 ±0.8	62.9 ±2.3
	CO	3	16.7 ±0.5	0.21 ±0.02	69.4 ±4.2	11.2 ±0.6	58.2 ±3.0
22°	SW	3	16.4 ±0.9	0.23 ±0.04	45.8 ±2.7	5.9 ±0.2	40.0 ±2.0
	FM	3	16.5 ±0.8	0.34 ±0.02	68.5 ±4.4	9.0 ±1.2	59.5 ±2.9
	CO	3	16.4 ±0.7	0.33 ±0.02	75.8 ±7.1	14.9 ±1.9	60.9 ±4.6

Metabolized energy of the three diets and faeces determined by burning samples in a bomb calorimeter: 2,677 ±76 and 1,601 ±122 for SW; 3,680 ±146 and 2,629 ±47.8 for FM; 4,063 ±141 and 2,629 ±182 calories for CO.

The commercial diet made with casein as a main protein source presents the highest values of energy egested, reaching up to 21.2% in abalone cultured at 15°C (Table 5.9).

At 15°C very little energy was consumed and absorbed, where the energy absorbed by juveniles reared at 15°C was not significantly different for formulated diets (K-W test, $H = 23.9$, df = 8, $P = 0.24$). However, those organisms fed on SW diet showed some differences in absorption with temperature, and again a significant increase (K-W test, $H = 30.9$, df = 8, $P = 0.002$) in absorption with increasing temperature was achieved with values of 22.1 ± 0.3, 29.9 ± 1.7and 40.0 ± 2.0 cal day $^{-1}$ animal^{-1} dry weight,

respectively. Moreover, the energy absorbed was not influenced by formulated diets at 18° and 22°C (Table 5.9).

5.3.5. Reproductive investment

A significant result of the fast-growing tendency, was that the gonad began to develop in all animals cultured at 18º (after 120 day) and 22ºC (after 90 day) (Figures 5.5 and 5.6). Spawning occurred in these individuals (animals fed on formulated diets and reared at 22ºC) at the beginning of September. Successful fertilization was achieved following the spawning and larvae were found swimming four days after the fertilization. However, none of these larvae reached the metamorphic stage; massive mortality was found in all containers by the end of the fourth day.

The spawning had a significant depressing effect on growth rates. Daily growth rates of shell length and body weight dropped dramatically (Figures 5.3 and 5.4). In the same context, animals cultured at 15ºC became gravid late on. However, this observation was after the experimental period. For this reason, gonad investment by abalone cultured at 15ºC were not taken in account to assess reproductive investment.

In female and male abalone gonad is closely associated with the digestive gland and this gland occupies ~12% of the total gonad weight. These results (12 ±1.6%) were obtained from the dissection of 25 animals of different sizes, in order to get more accuracy in the gonad weight.

There are significant differences of the ME2 content between female (5,258 ±189 cal g^{-1}) and male (4,207 ±215 cal g^{-1}) ripe gonads ($t = 6.02$, $df = 4$, $P = 0.0038$) (Table 5.3).

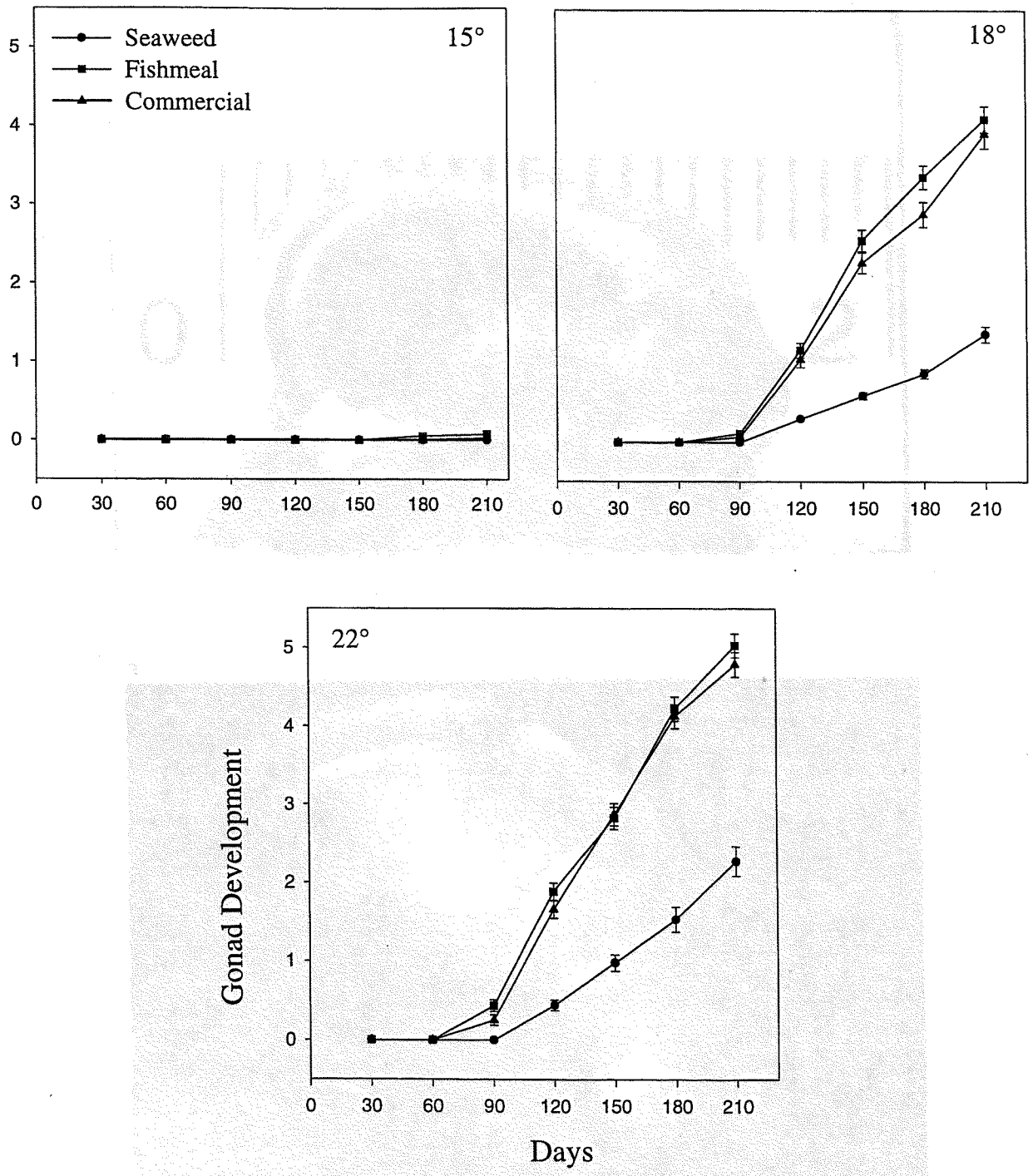


Figure 5.5. Gonad development of *Haliotis tuberculata* fed during 210 days (March to October) with seaweed (SW) and two formulated diets (FM and CO) and cultured at 15°, 18° and 22°C. Gonad development: stage 1 = indeterminate, 2 = immature males and females, 3 = small gonad bulk, 4 = large gonad bulk or ripe and 5 = empty collapsed gonad gonad lumen. Bars indicates standard errors.

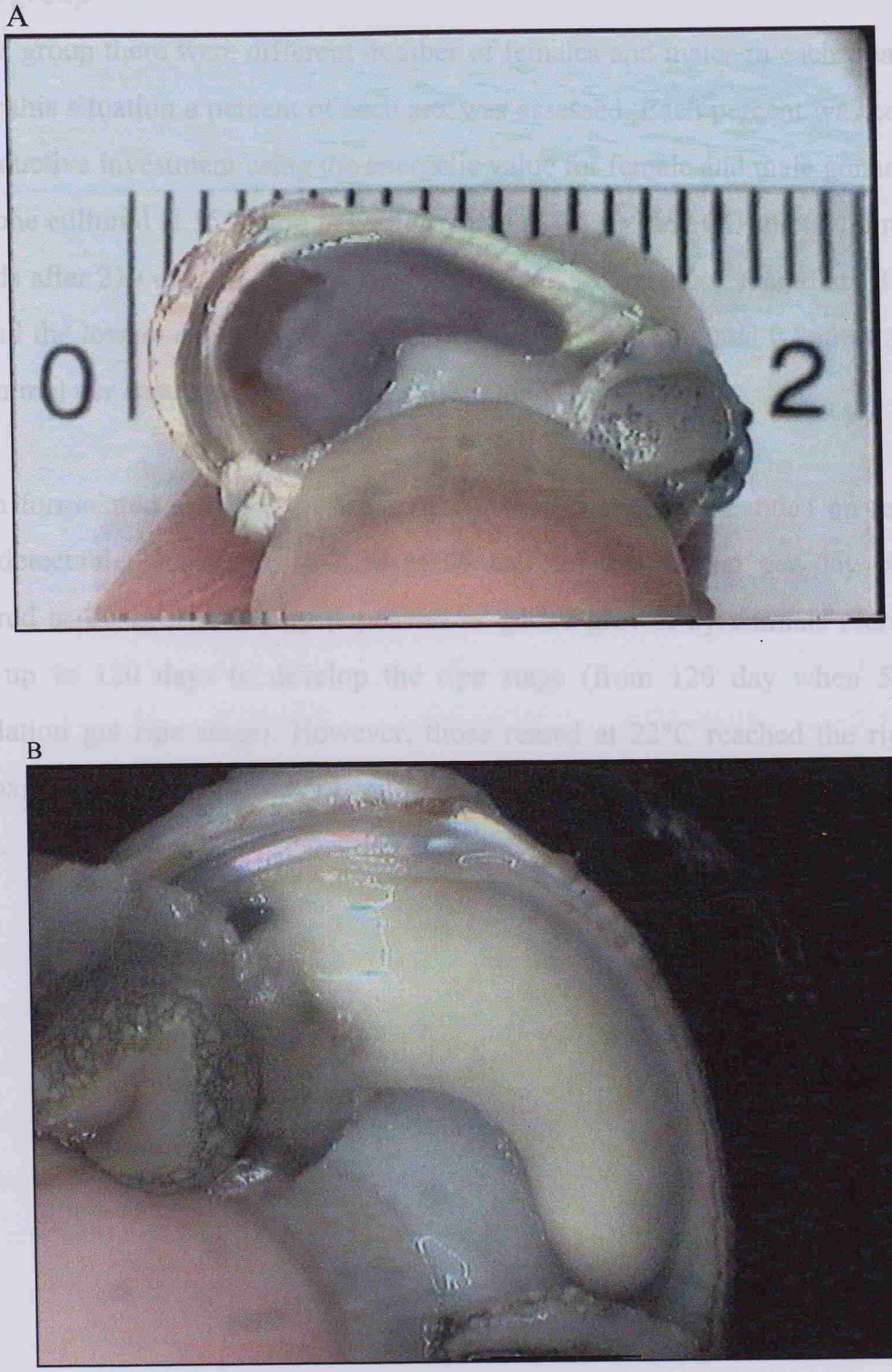


Figure 5.6. Early development of the abalone *Haliotis tuberculata*. A.- Female gonad started at 1.32 cm length. B.- Male gonad started at 1.09 cm length. Both cultured at 22°C and fed on fishmeal diet.

Main group

In this group there were different number of females and males in each treatment, and under this situation a percent of each sex was assessed. Each percent was converted to reproductive investment using the energetic value for female and male gonad.

Abalone cultured at 15°C and fed on the three (SW, FM and CO) diets did not develop gonads after 210 days of culture. Juveniles fed on seaweed and reared at 18° and 22°C expend the lowest energy on reproduction with 0.675 ± 0.04 and 0.866 ±0.03 calories per animal per day, by the end of the experimental period.

When formulated diets were used, an increment in energy expended on reproduction was detectable, increasing to 2.28 ±0.09 calories per animal per day on FM diet cultured at 22°C. The energy deposited as gonad growth by animals reared at 18°C took up to 120 days to develop the ripe stage (from 120 day when 55% of the population got ripe stage). However, those reared at 22°C reached the ripe stage in approximately 90 days (from 90 day when 79% of the population got ripe stage) (Table 5.10).

Table 5.10. Reproductive investment in gonad production and energy utilized by juvenile *H. tuberculata* from the Main group. Data was obtained using dry weigth samples.

°C	Diets	n	Shell Length (mm)	Body Weight dry weight (g)	Investment ($g \times 10^{-3}$)	Energy invested (calories)
15°	SW	42	12.2 ±0.3	0.11 ±0.01	null	null
	FM	39	13.9 ±0.4	0.13 ±0.02	null	null
	CO	40	13.4 ±0.4	0.13 ±0.02	null	null
18°	SW	42	15.2 ±0.6	0.23 ±0.03	0.16 ±0.007	0.9 ±0.02
	FM	42	21.9 ±0.9	0.59 ±0.05	0.65 ±0.01	3.1 ±0.05
	CO	40	20.5 ±0.8	0.47 ±0.03	0.52 ±0.009	2.5 ±0.03
22°	SW	41	16.7 ±0.8	0.23 ±0.02	0.26 ±0.009	1.2 ±0.01
	FM	43	22.6 ±0.9	0.67 ±0.05	0.74 ±0.01	3.6 ±0.04
	CO	41	21.9 ±1.0	0.66 ±0.04	0.72 ±0.01	3.5 ±0.06

To convert from gonad investment to energy utilised a factor for females (5,258 ±189 cal g^{-1}) and male (4,207 ±215 cal g^{-1}) were used.

Subgroup

To determine energy budget components for organisms of the same size, females were selected because not enough males were available. To convert the energy expended on reproduction a female gonad mean value of 5,258 ±189 cal g^{-1} was used.

When females were selected from the group of 15°C the gonad stage was indeterminate, thus it was null reproductive investment for this group.

The energy investment on gonad development is affected by temperature. Abalone cultured at 22°C and fed on formulated diets expend the highest energy values (Table 5.11).

Table 5.10. Reproductive investment in gonad production and energy utilized by juvenile *H. tuberculata* from the Main group. Data was obtained using dry weigth samples.

°C	Diets	n	Shell Length (mm)	Body Weight dry weight (g)	Investment ($g \times 10^{-3}$)	Energy invested (calories)
15°	SW	42	12.2 ±0.3	0.11 ±0.01	null	null
	FM	39	13.9 ±0.4	0.13 ±0.02	null	null
	CO	40	13.4 ±0.4	0.13 ±0.02	null	null
18°	SW	42	15.2 ±0.6	0.23 ±0.03	0.16 ±0.007	0.9 ±0.02
	FM	42	21.9 ±0.9	0.59 ±0.05	0.65 ±0.01	3.1 ±0.05
	CO	40	20.5 ±0.8	0.47 ±0.03	0.52 ±0.009	2.5 ±0.03
22°	SW	41	16.7 ±0.8	0.23 ±0.02	0.26 ±0.009	1.2 ±0.01
	FM	43	22.6 ±0.9	0.67 ±0.05	0.74 ±0.01	3.6 ±0.04
	CO	41	21.9 ±1.0	0.66 ±0.04	0.72 ±0.01	3.5 ±0.06

To convert from gonad investment to energy utilised a factor for females (5,258 ±189 cal g^{-1}) and male (4,207 ±215 cal g^{-1}) were used.

Subgroup

To determine energy budget components for organisms of the same size, females were selected because not enough males were available. To convert the energy expended on reproduction a female gonad mean value of 5,258 ±189 cal g^{-1} was used.

When females were selected from the group of 15°C the gonad stage was indeterminate, thus it was null reproductive investment for this group.

The energy investment on gonad development is affected by temperature. Abalone cultured at 22°C and fed on formulated diets expend the highest energy values (Table 5.11).

Table 5.11. Reproductive investment and energy utilized by juvenile *H. tuberculata* from the Subgroup, using only females in all treatments. Data was obtained using dry weigth samples.

°C	Diets	N	Shell Length (mm)	Body Weight (g)	Investment ($g \times 10^{-3}$)	Energy invested (calories)
15°	SW	3	16.2 ±0.08	0.20 ±0.01	null	null
	FM	3	16.2 ±0.1	0.21 ±0.01	null	null
	CO	3	16.7 ±0.1	0.23 ±0.01	null	null
18°	SW	3	16.3 ±0.3	0.22 ±0.01	0.13 ±0.007	0.68 ±0.04
	FM	3	16.4 ±0.2	0.24 ±0.02	0.22 ±0.01	1.15 ±0.06
	CO	3	16.7 ±0.3	0.21 ±0.02	0.19 ±0.02	1.01 ±0.08
22°	SW	3	16.4 ±0.1	0.23 ±0.01	0.17 ±0.006	0.87 ±0.03
	FM	3	16.5 ±0.2	0.34 ±0.02	0.44 ±0.02	2.28 ±0.09
	CO	3	16.4 ±0.2	0.33 ±0.02	0.42 ±0.03	2.22 ±0.11

To convert from gonad investment to energy utilised a factor for females (5,258 ±189 cal g^{-1}) were used.

Comparison with data in Tables 5.10 and 5.11 show that when energy investment is assessed on a single animal, it is lower than in a grouped one.

5.3.6. Respiration

Metabolic energy losses by respiration were calculated by using an oxy-calorific coefficient for each of the diets used during the feeding trial of the juveniles as follows: 0.00492 cal μlO_2 consumed for SW, 0.00484 cal μlO_2 consumed for FM and 0.00483 cal μlO_2 consumed for CO. Those values were obtained from the concentration of protein; lipid and carbohydrate on each of the diets used (SW, FM and CO).

To calculate the oxy-calorific coefficient it was necessary to use the following values: for protein (Crisp, 1984) respiration: 0.00457 cal μlO_2 with NH_3 end product, for lipid respiration: 0.00469 cal μlO_2 and for carbohydrates respiration: 0.00504 cal μlO_2 consumed (Brafield and Solomon, 1972; Elliott and Davidson, 1975). All data were then expressed in terms of animal dry weight of a representative sample from each treatment in order to know the maintenance energy required for juvenile abalone *H. tuberculata*.

Abalone in the field usually forage more intensively during the night (Uki and Kikuchi, 1975) and organisms from this study were fed strictly according to the natural period; food was available from 20:00 h until 08:00 h.

Table 5.12 (animals from Main group) and Table 5.13 (animals from Subgroup) show the results of oxygen consumption in $\mu l\ O_2$ animal^{-1} day^{-1} and in calories animal^{-1} day^{-1} again during day and night. Respiration rates by oxygen consumption measured during the night tend to be higher (from 6 to 18%) than the rates during the day, in all treatments and in both groups.

Main group

Data from main group suggest that abalone were in average more active during night-time than the daytime (16-34% at 15°C, 12-16% at 18°C and -1.9-2.7% at 22°C).

The results of measurement of oxygen consumption for juvenile *H. tuberculata* show differences in the energy expended through respiration between formulated and SW diets at 15°C, measured during day (ANOVA, $F_{4,\ 18} = 130.5$, $p < 0.0001$) and during the night (ANOVA, $F_{4,\ 18} = 79.0$, $p < 0.0001$).

The proportion of energy used in respiration increased dramatically with temperature, although some of the temperature effects are affected by differences in animal size.

When abalone were maintained at 18° and 22°C and fed on formulated diets a significant increment in oxygen consumption was detected at night, with mean maximum of 19.45 cal animal^{-1} day^{-1} during the day and 22.51 cal animal^{-1} day^{-1} during the night, for those abalone cultured at 22°C and fed on FM diet (Table 5.12).

Subgroup

The level of oxygen consumption increased with temperature by x 2.12 (SW), x 2.83 (FM) and x 2.60 (CO) for the 7°C rise from 15-22°C. These equate to Q_{10} values of 2.7, 4.4 and 3.9 respectively, and indicate a very high temperature sensitivity in the metabolic rate of *H. tuberculata* over this range. Differences (ANOVA, $F_{4,\ 18} = 67$, $P < 0.1$) were not detected between formulated diets at the same temperature (Table 5.13).

Although the size of the animals used was the same for each treatment (SW, FM and CO diets) the energy consumed was 200, 245 and 233% higher in animals cultured at 22°C compared with those cultured at 15°C. Comparisons of respiration rates between animals fed on the three diets showed nutritional influence on the energy expended through oxygen consumption.

The effect of body size on rates of oxygen consumption was determined from data of the day and night respiration at the same temperature and also between groups of each temperature treatment as shown in Tables 5.14. The regression results from the main group show high values of the constant *b* ranging from 0.78 to 1.09 (Figure 5.7).

Table 5.12. Data obtained using all batches of those juveniles *H. tuberculata* reared in the experiment for growth from each treatment after six months of culture (September). Mean values and standard error in ±.

°C	Diets	N	Shell Length (mm)	Dry Weight (g)	Respiration Day	Respiration in Night	Energy lost as respiration in Day	Energy lost as respiration in Night
					μlO_2 animal^{-1} day^{-1}	μlO_2 animal^{-1} day^{-1}	calories animal^{-1} day^{-1}	calories animal^{-1} day^{-1}
15°	SW	42	12.2 ±0.3	0.11 ±0.01	386.5 ±62	584.6 ±41	1.92 ±0.3	2.91 ±0.2
	FM	39	13.9 ±0.3	0.13 ±0.01	1,035 ±43	1,236 ±59	5.01 ±0.2	5.94 ±0.3
	CO	40	13.4 ±0.2	0.13 ±0.01	1,034 ±79	1,351 ±62	5.12 ±0.4	6.57 ±0.3
18°	SW	42	15.2 ±0.4	0.23 ±0.01	887.6 ±77	1,059 ±85	4.39 ±0.3	5.28 ±0.4
	FM	42	21.9 ±0.5	0.59 ±0.05	4,015 ±132	4,647 ±167	19.45 ±0.7	22.51 ±0.5
	CO	40	20.5 ±0.5	0.47 ±0.03	3,341 ±201	3,794 ±149	16.19 ±0.9	18.44 ±0.7
22°	SW	41	16.7 ±0.3	0.23 ±0.01	1,172 ±71	1,204 ±82	5.78 ±0.4	5.95 ±0.6
	FM	43	22.6 ±0.3	0.67 ±0.05	6,688 ±281	6,565 ±212	32.40 ±1.3	31.82 ±1.0
	CO	41	21.9 ±0.3	0.66 ±0.03	5,869 ±229	6,870 ±205	28.48 ±1.1	33.29 ±0.9

To convert from μlO_2 animal^{-1} day^{-1} to calories animal^{-1} day^{-1} the followed oxy-calorific coefficients were used: 1 μlO_2 is equivalent to 0.00492 calories for SW, 0.00484 for FM and 0.00483 for CO diet

Table 5.13. Data obtained using those juveniles *H. tuberculata* with same size reared in the experiment for growth from each treatment after six months of culture (September). Mean values and standard error in ±.

°C	Diets	n	Shell Length (mm)	Dry Weight (g)	Respiration in Day	Respiration in Night	Energy lost as respiration in Day	Energy lost as respiration in Night
					μlO_2 animal^{-1} day^{-1}	μlO_2 animal^{-1} day^{-1}	calories animal^{-1} day^{-1}	calories animal^{-1} day^{-1}
15°	SW	3	16.2 ±0.08	0.20 ±0.01	773.2 ±57	993.5 ±85	3.87 ±0.3	4.96 ±0.2
	FM	3	16.2 ±0.1	0.21 ±0.01	1,295 ±85	1,582 ±77	6.32 ±0.2	7.89 ±0.1
	CO	3	16.7 ±0.1	0.23 ±0.01	1,450 ±43	1,791±65	7.08 ±0.2	8.93 ±0.3
18°	SW	3	16.3 ±0.3	0.22 ±0.01	1,100 ±80	1,357 ±61	5.44 ±0.2	6.85 ±0.6
	FM	3	16.4 ±0.2	0.24 ±0.02	3,271 ±127	3,659 ±140	16.22 ±0.6	17.82 ±0.7
	CO	3	16.7 ±0.3	0.21 ±0.02	3,481 ±148	3,901 ±147	17.05 ±0.5	18.92 ±0.7
22°	SW	3	16.4 ±0.1	0.23 ±0.01	1,640 ±73	2,015 ±82	8.08 ±0.2	9.95 ±0.4
	FM	3	16.5 ±0.2	0.34 ±0.02	3,662 ±129	4,016 ±197	17.94 ±0.7	19.66 ±0.7
	CO	3	16.4 ±0.2	0.33 ±0.02	3,776 ±158	4,336 ±155	18.37 ±0.5	21.03 ±0.8

To convert from µl O_2 animal^{-1} day^{-1} to calories animal^{-1} day^{-1} the followed oxy-calorific coefficients were used: 1 µl O_2 is equivalent to 0.00492 calories for SW, 0.00484 for FM and 0.00483 for CO diet.

Table 5.14. Summary of statistical data acquired from regression analysis ($Ln\ R = a + b \times Ln\ W$) of oxygen consumption ($\mu l\ O_2\ animal^{-1}\ day^{-1}$) against dry weight in grams (W) from the Main group.

°C	W (g)	N	a	b	r^2	P (>)	P (<)
15°	0.12	9	8.60	0.78	0.97	0.0001*	
	0.12	9	8.74	0.79	0.70		
18°	0.43	9	9.26	1.09	0.97	0.0001*	0.0001**
	0.43	9	9.41	1.07	0.95		
22°	0.52	9	9.21	0.90	0.98	0.0001*	
	0.52	9	9.36	0.85	0.99		

Data in the first row are day and the second row are night measurements. ANOVA between groups on day and night under the same temperature* and with significant difference between temperature treatments **.

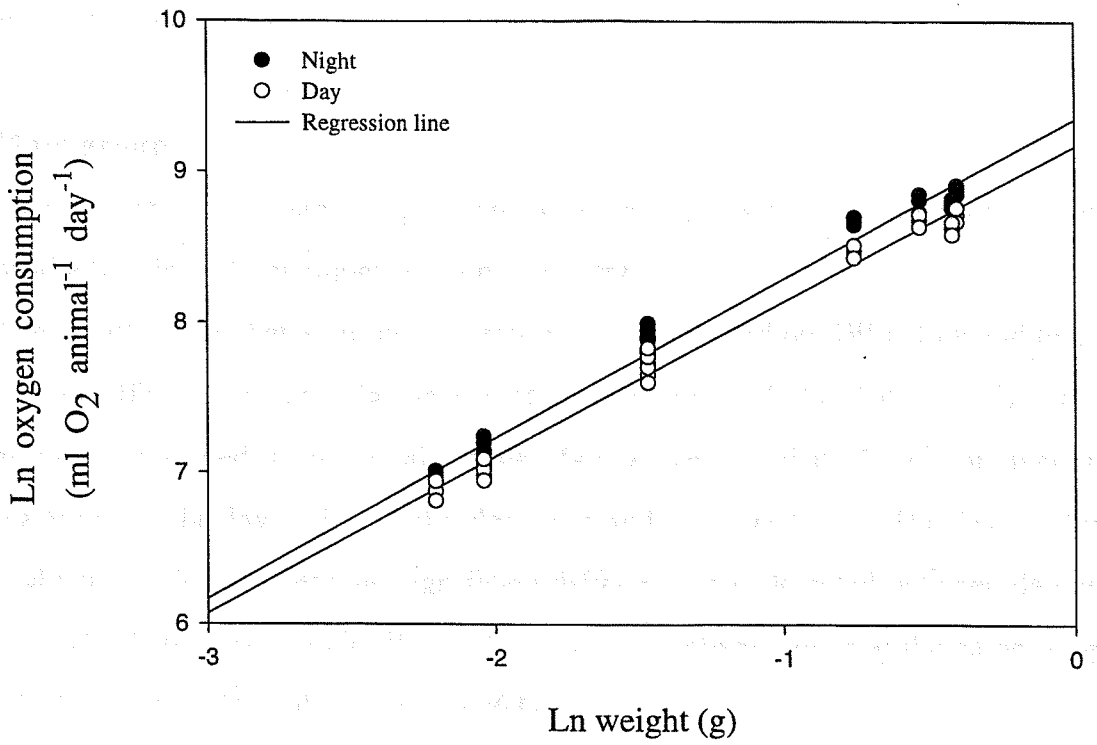


Figure 5.7. Natural logarithm (Ln) of oxygen consumption in µl animal^{-1} day^{-1} against natural logarithm dry body weigth in grams of *Haliotis tuberculata* of Main group.

5.3.7. Excretion

Excretory products based on the total organic nitrogen and NH3-N excretion, ammonia account between 40 to 90% for some marine gastropods (Mace and Ansell, 1982; Bayne and Newell, 1983; Barakai and Griffiths, 1987).

To convert from µg-atoms NH_4 animal^{-1} day^{-1} to calories animal^{-1} day^{-1} the following factor was used: 1 µg-atom NH_4 is equivalent to 0.0689 calories (Brafield and Solomon, 1972).

Main group

Each value in the table represents a three replicates determination of ammonia production by ~ 42 abalones in each treatment.

The results show that ammonia excretion by abalone fed on SW is low and temperature has an effect by increasing the excretion rates up to 67% (Table 5.15). The rate of ammonia excreted from animals of the Main group reared at 15° was an average of 4.6 µg-atoms NH_4 day^{-1} during the day-time and 5.0 µg-atoms NH_4 day^{-1} during the night-time. Nevertheless, no significant difference was detected between day and night rates (K-W test, $H = 36.9$, df = 5, $P \leq 0.1$) and between diets at the same temperature (K-W test, $H = 10.5$, df = 5, $P = 0.063$).

When animals were tested at 18° and 22°C the rate of ammonia excretion increased considerably over values at 15°C, increasing from 64 to 196% giving an average of 129%. Where an animal with an average dry weight of 0.67 g spend 0.95 and 1.01 calories as ammonia production reared at 22°C and fed on FM or CO diet respectively (Table 5.15).

The regression results from the main group show variable values of the constant *b* ranged from -0.38 to 0.61. (Tables 5.16; Figures 5.7).

Table 5.15. Data obtained using all batches of those juveniles *H. tuberculata* reared in the experiment for growth (Main group) from each treatment after six months of culture (September). Mean values and standard error in ±.

°C	Diets	N	Shell Length (mm)	Dry Weight (g)	Ammonia produced in Day	Ammonia produced in Night	Energy lost as ammonia in Day	Energy lost as ammonia in Night
					µg-atoms NH_4 animal^{-1} day^{-1}	µg-atoms NH_4 animal^{-1} day^{-1}	Calories animal^{-1} day^{-1}	Calories animal^{-1} day^{-1}
15°	SW	42	12.2 ±0.3	0.11 ±0.01	4.79 ±0.38	4.94 ±0.28	0.33 ±0.03	0.34 ±0.02
	FM	39	13.9 ±0.4	0.13 ±0.02	4.65 ±0.29	5.52 ±0.30	0.32 ±0.02	0.38 ±0.02
	CO	40	13.4 ±0.4	0.13 ±0.02	4.34 ±0.40	4.65 ±0.15	0.30 ±0.03	0.32 ±0.01
18°	SW	42	15.2 ±0.6	0.23 ±0.03	7.85 ±0.27	8.13 ±0.43	0.54 ±0.02	0.56 ±0.03
	FM	42	21.9 ±0.9	0.59 ±0.05	13.79 ±0.71	14.64 ±0.83	0.95 ±0.05	1.01 ±0.06
	CO	40	20.5 ±0.8	0.47 ±0.03	11.47 ±0.57	12.1 ±0.69	0.79 ±0.04	0.83 ±0.05
22°	SW	41	16.7 ±0.8	0.23 ±0.02	7.56 ±0.39	7.84 ±0.27	0.52 ±0.03	0.54 ±0.02
	FM	43	22.6 ±0.9	0.67 ±0.05	13.36 ±0.73	13.79 ±0.86	0.92 ±0.05	0.95 ±0.06
	CO	41	21.9 ±1.0	0.66 ±0.03	11.92 ±0.72	14.66 ±1.15	0.82 ±0.05	1.01 ±0.08

To convert from µg-atoms NH_4 animal^{-1} day^{-1} to calories animal^{-1} day^{-1} the following factor was used: 1 µg-atom NH_4 is equivalent to 0.0689 calories.

Table 5.16. Summary of statistical data acquired from regression analysis ($Ln\ U = a + b \times Ln\ W$) of ammonia production (μg-atoms NH_4 animal $^{-1}$ day $^{-1}$) against dry weight in grams (W) from the Main group.

°C	W (g)	N	a	b	r^2	P	P
15°	0.12	9	0.71	-0.38	0.15	= 0.03*	
	0.12	9	1.93	0.15	0.02		
18°	0.43	9	2.90	0.58	0.96	= 0.63*	< 0.0001**
	0.43	9	3.01	0.61	0.97		
22°	0.52	9	2.73	0.49	0.95	= 0.16*	
	0.52	9	2.88	0.56	0.98		

Data in the first row are day and the second row are night measurements. ANOVA between groups on day and night under the same temperature* and between temperature treatments **.

Subgroup

Animals used in this experiment were similar in size (shell length and body weight) and number (n). In these specimens, there is a significant effect of diet and temperature on the rates of excretion between individuals of *H. tuberculata* (K-W test, $H = 15.7$, df = 5, $P \leq 0.001$) (Table 5.17).

Animals fed on formulated diets appear to excrete more ammonia than those fed on mixed fresh seaweed at any of the temperature treatments ranging from 35 to 191%. Ammonia excretion was an average of 113% higher when abalone were held at 18° and 22°. The energy utilized by the low density (n = 3) individuals with mean body dry weight of 0.33 g was greater (Table 5.16) than for those reared at higher density (around 40) of individuals with mean body dry weight of 0.66 g (22°C, fed CO: Table 5.15). The effect of body size on rates of ammonia excretion was determined from data of the day and night excretion at the same temperature and also between groups of each temperature treatment as shown in Tables 5.17 and Figure 5.8.

Table 5.17. Data obtained using juvenile *H. tuberculata* with same size (Subgroup) reared in the experiment for growth from each treatment after six months of culture (September). Mean values and standard error in ±.

°C	Diets	n	Shell Length (mm)	Dry Weight (g)	Ammonia produced in Day	Ammonia produced in Night	Energy lost as ammonia in Day	Energy lost as ammonia in Night
					μg-atoms NH_4 animal^{-1} day^{-1}	μg-atoms NH_4 animal^{-1} day^{-1}	calories animal^{-1} day^{-1}	Calories animal^{-1} day^{-1}
15°	SW	3	16.2 ±0.4	0.20 ±0.02	5.53 ±0.58	4.66 ±0.56	0.38 ±0.04	0.32 ±0.04
	FM	3	16.2 ±0.4	0.21 ±0.01	6.69 ±0.73	7.71 ±0.71	0.46 ±0.05	0.53 ±0.05
	CO	3	16.7 ±0.4	0.23 ±0.01	5.96 ±1.02	6.99 ±0.58	0.41 ±0.07	0.48 ±0.04
18°	SW	3	16.3 ±0.6	0.22 ±0.02	7.54 ±0.45	7.98 ±0.85	0.52 ±0.03	0.55 ±0.06
	FM	3	16.4 ±0.2	0.24 ±0.02	11.80 ±0.50	13.82 ±0.60	0.81 ±0.04	0.94 ±0.04
	CO	3	16.7 ±0.5	0.21 ±0.02	12.09 ±0.73	14.67 ±0.75	0.84 ±0.05	1.08 ±0.05
22°	SW	3	16.4 ±0.9	0.23 ±0.04	9.44 ±0.47	10.22 ±0.73	0.65 ±0.03	0.70 ±0.05
	FM	3	16.5 ±0.8	0.34 ±0.02	15.98 ±0.89	18.88 ±0.44	1.12 ±0.06	1.33 ±0.03
	CO	3	16.4 ±0.7	0.33 ±0.02	16.07 ±1.05	20.31 ±1.07	1.15 ±0.08	1.39 ±0.07

To convert from μg-atoms NH_4 animal^{-1} day^{-1} to calories animal^{-1} day^{-1} the following factor was used: 1 μg-atom NH_4 is equivalent to 0.0689 calories.

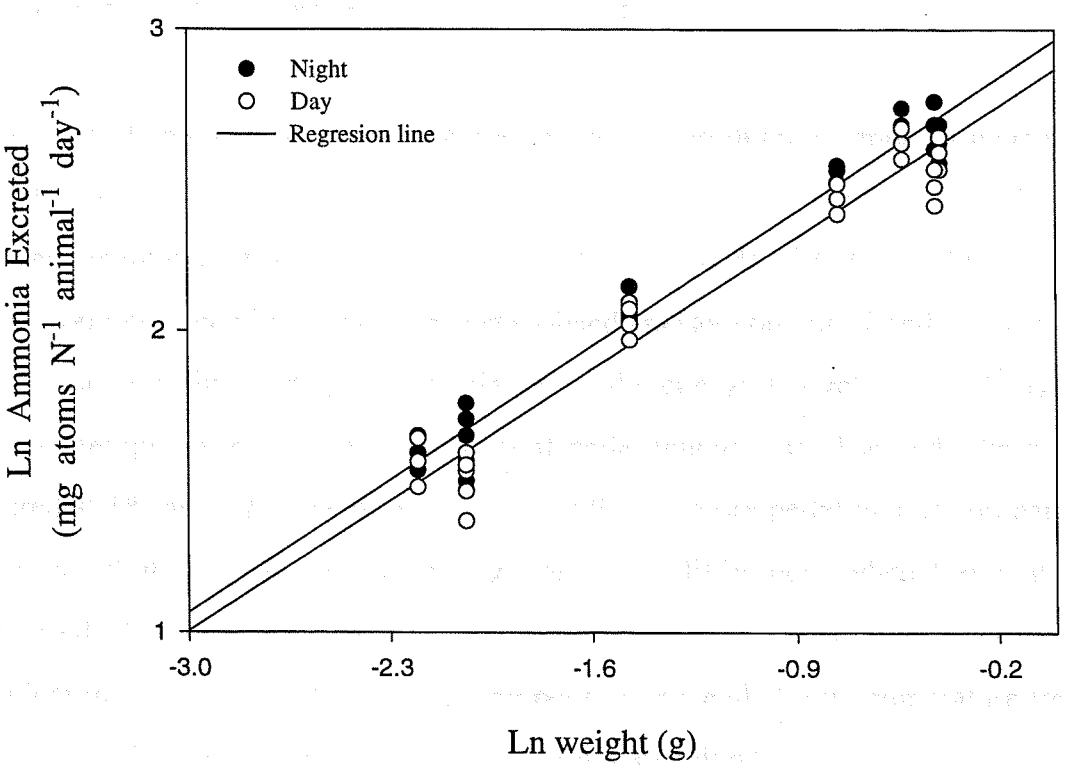


Figure 5.8. Natural logarithm (Ln) of ammonia excreted in µg atoms N^{-1} $animal^{-1}$ day^{-1} against natural logarithm dry body weigth in grams of *Haliotis tuberculata* from Main group.

5.3.8. Pedal mucus production

The quantification of pedal mucus from juveniles used in laboratory conditions was difficult to obtain. Animals under stress produce high quantities of mucus, especially when they are transferred from one place to another. Nevertheless, animals used in this study remained relatively undisturbed to minimise stress. When animals were obviously stressed more production was obtained.

Table 5.18 shows that at each temperature, mucus production tends to increase with animal size. On the other hand, no differences in energy content of pedal mucus between treatments were found (K-W test, $H = 3.53$, df = 8, $P = 0.896$). Thus, the values were combined to obtain the metabolised energy content of pedal mucus (2,338 ± 97 cal g^{-1}), to be used in the calculation of the energy budget losses. A significant effect of temperatures on the production of pedal mucus was observed where animals cultured at 18° and 22°C spend an average of 92.7% more pedal mucus compared with those reared at 15°C and also showing significant differences when formulated diets were used (ANOVA, $F_{4,18} = 14.9$, $P < 0.0001$). Energy expenditure of pedal mucus for adherence and locomotion at high temperature exceeded low temperature treatment by up to 153% when abalone were fed on formulated diets.

When analysis was performed using the same size animals from each treatment, they revealed no differences between diets at each determined temperature (Table 5.19). It was noticed that when an analysis was performed using a small group of animals of the same size, the production is less than when using the larger group of animals, despite the value obtained being reported per animal. The energy expended through pedal mucus production was between 0.51 to 2.3% of the total energy budget, depending on the animal size.

Table 5.18. Mucus production and energy utilised by juvenile *Haliotis tuberculata* from Main group.

°C	Diets	n	Shell Length (mm)	Body Weight (g)	Mucus production (g) x 10^{-3}	Energy utilised (calories)
15°	SW	42	12.2 ±0.3	0.11 ±0.01	0.359 ±0.010	0.84 ±0.04
	FM	39	13.9 ±0.3	0.13 ±0.01	0.372 ±0.008	0.87 ±0.03
	CO	40	13.4 ±0.2	0.13 ±0.01	0.389 ±0.012	0.91 ±0.04
18°	SW	42	15.2 ±0.4	0.23 ±0.01	0.599 ±0.014	1.4 ±0.05
	FM		21.9 ±0.5	0.59 ±0.05	0.642 ±0.016	1.5 ±0.07
	CO	40	20.5 ±0.5	0.47 ±0.03	0.684 ±0.013	1.6 ±0.04
22°	SW	41	16.7 ±0.3	0.23 ±0.01	0.770 ±0.014	1.8 ±0.05
	FM	43	22.6 ±0.3	0.67 ±0.05	0.941 ±0.023	2.2 ±0.08
	CO	41	21.9 ±0.3	0.66 ±0.03	0.983 ±0.021	2.3 ±0.07

One gram of mucus is equivalent to 2,338 ± 97 calories. Values of mucus production and energy utilised are given in calories animal $^{-1}$ day $^{-1}$.

Table 5.19. Mucus production and energy utilised by juvenile *Haliotis tuberculata* from the Subgroup.

°C	Diets	n	Shell Length (mm)	Body Weight (g)	Mucus production (g) x 10^{-3}	Energy utilised (calories)
15°	SW	3	16.2 ±0.4	0.20 ±0.02	0.218 ±0.005	0.51 ±0.02
	FM	3	16.2 ±0.4	0.21 ±0.01	0.226 ±0.011	0.53 ±0.04
	CO	3	16.7 ±0.4	0.23 ±0.01	0.231 ±0.014	0.54 ±0.05
18°	SW	3	16.3 ±0.6	0.22 ±0.02	0.286 ±0.008	0.67 ±0.03
	FM	3	16.4 ±0.2	0.24 ±0.02	0.299 ±0.003	0.70 ±0.01
	CO	3	16.7 ±0.5	0.21 ±0.02	0.316 ±0.015	0.74 ±0.05
22°	SW	3	16.4 ±0.9	0.23 ±0.04	0.364 ±0.006	0.85 ±0.02
	FM	3	16.5 ±0.8	0.34 ±0.02	0.394 ±0.009	0.92 ±0.03
	CO	3	16.4 ±0.7	0.33 ±0.02	0.389 ±0.013	0.91 ±0.04

One gram of mucus is equivalent to 2,338 ± 97 calories. Values of mucus production and energy utilised are given in calories animal $^{-1}$ day $^{-1}$.

5.4. DISCUSSION

In this study most of the measurements of energy budget parameters were made on a large group of organisms from each treatment, called the Main group. Also, the parameters were obtained in a small number of individuals (three) with approximately the same length and weight from each treatment, called the Subgroup.

To date there are three other energy budgets assessments for *Haliotis* species, one by Peck *et al.* (1987) for *H. tuberculata*, the second by Barkai and Griffiths (1988) for *H. midae* and the latest by Donovan and Carefoot (1998) for Haliotis *kamtschatkana*. Our data cannot be compared with those for *H. midae* and *H. kamtschatkana* because the size of the animals were much bigger than those for this study (ranging from 13 to 175 g body weight) and also these abalones were wild adults. Nevertheless, when comparison are made between Peck's results and those in this study, the energy utilised in some of the parameters are similar.

5.4.1. Growth

The energy used for growth is the proportion of ingested energy, which is converted to body tissue energy. Variation in the growth of the abalone *H. tuberculata* during this study showed some correlation with variation in the concentration of protein, lipid and energy content in the different diets. The sudden changes or decrease in growth rate and in energy expenditure probably occurred in response to the dietary treatment. The present study should therefore be considered against a background of growth-related changes in body composition and energy expenditure.

Growth rate studies are necessary to estimate the sustainable production of commercially important species. Table 5.20 shows average growth rates of *Haliotis*

species when fed on single or mixed algae (macroalgae) and formulated diets when abalone was cultured at different temperatures. In this study, growth rate was consistent with those previously observed for abalone species fed on rich diets (formulated) (Britz, 1996; Capinpin and Corre, 1996; Harada, 1992; Sakata and Ina, 1992; Viana *et al.*, 1993, 1996; Corazani and Illanes, 1998; López *et al.*, 1998, chapter 4 in this thesis) and cultured at high temperatures (above that of their natural environment). This proved to be the case for each abalone species or culture conditions. Dixon (1992) and Britz *et al.* (1997) have determined growth rates of *H. midae* using formulated diets at different temperatures of culture. They concluded that abalone growth increased significantly with temperature.

When culture temperature was at 15°C, it was noted that *H. tuberculata*, fed on any of the three diets, did not develop any gonad and growth in shell length and body weight were constant and steady over the seven-month experimental period. The higher growth rates of abalone cultured at 15°C was for formulated diets with an average of 48.22 ± 3.05 μm day^{-1} $animal^{-1}$ and 2.3 ± 0.1 mg day^{-1} $animal^{-1}$ during the entire experiment. Thus, differences on growth rates between SW and formulated diets were 24% higher in abalone reared at 15°C.

In the same context, water temperature is one of the most important environmental factors influencing metabolic rate and energy expenditure. The growth rates of *H. tuberculata* have been shown to be much faster when cultured at a temperature of 18° (Peck, 1989; Shpigel *et al.*, 1996a, b). Moreover, according to several authors (Mercer *et al.*, 1993; Mai *et al.*, 1995), *H. tuberculata* fed on natural, mixed and fortified diets and cultured at temperatures of 12° to 15°C produced acceptable growth rates of shell length and body weight. We found low growth rates on abalone cultured at 15°C compared with those cultured at 18° and 22°C.

H. Tuberculata provided with a FM diet and cultured at 18º and 22ºC produced slightly superior growth rates by 120 and 90 day respectively, in terms of shell length and body weight compared with abalone fed on CO diet and cultured at the same temperature (134.8 and 127.9 μm day^{-1}, respectively).

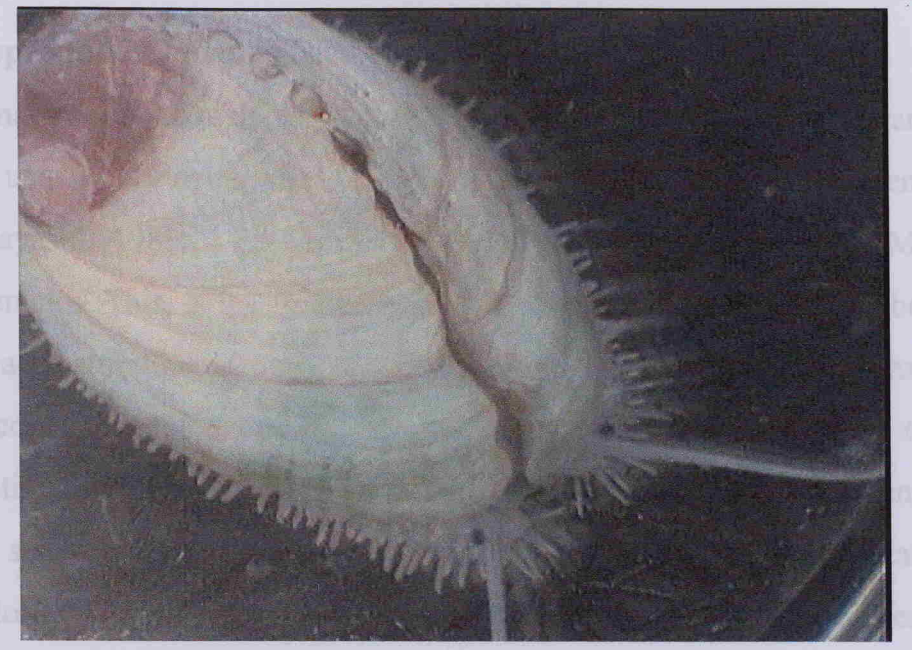
Table 5.20. Major publications on average growth rates of *Haliotis* species using various diets and temperatures of culture. Days are duration of the experimental trial. Temperature of culture (°C). * Data not available.

Author	Diet	*Haliotis* species	Days	°C Culture	Growth rates µm day^{-1}	mg day^{-1}
Peck, 1987	fresh algae	*H. tuberculata*	*	15 ±1°	44	12
Peck, 1989	fresh algae	*H. tuberculata*	27-40	22.4 ±1°	44	3.95
Viana *et al.*, 1993	fresh algae / formulated	*H. fulgens*	90	21.5 ±1°	25 / 100	37 / 14.5
Mercer *et al.*, 1993	fresh algae	*H. tuberculata*	~230	15 ±1°	94	72.8
	fresh algae	*H. discus hannai*	~230	15 ±1°	88	39.8
Mercer and Donlon, 1993	fresh algae	*H. tuberculata*	~365	15 ±1°	88	40
Mai *et al.*, 1995	formulated	*H. tuberculata*	100	14 ±1°	*	14.4
	formulated	*H. discus hannai*	100	14 ±1°	*	20.9
Britz, 1996	formulated	*H. midae*	95	19 ±2°	93	25
Capinpin and Corre, 1996	fresh algae / formulated	*H. asinina*	0-90	29 ±1.3°	137 / 163	70 / 90
Viana *et al.*, 1996	dry-fresh algae / formulated	*H. fulgens*	175	22 ±2°	1.5 / 57	*
Corazani and Illanes, 1998	fresh algae / formulated	*H. rufescens*	~ 270	15.5 ±2.5°	40 / 45	9.14 / 23.4
	fresh algae / formulated	*H. discus hannai*	~ 270	15.5 ±2.5°	37 / 62	34 / 51
Shpigel *et al.*, 1996b	fresh algae	*H. tuberculata*	~240	22.8 ±3.8°	106	*
Lopez *et al.*, 19981	dry-algae / formulated	*H. tuberculata*	105	22 ±0.5°	* / 123	* / 3.1

The abalone commercial diet (CO) was used as a control. Nevertheless, on the commercial diet shell deformation was observed in 89 % of the population after four months feeding, and it continued until the end of the experimental period (Figure 5.9). Moreover, the shell of these organisms was thin and fragile in most cases.

The nutritional value of a diet is of major importance and this is especially so far the content of essential nutrients (amino acids, fatty acids, mineral elements and vitamins) necessary to give a proper balance between energy and growth. Formulated diets for use during ongrowing of abalone to marketable size is crucial to achieve a successful commercial production. However, the diet must contain the correct balance of nutrients to ensure efficient utilization by the organism in culture. Unfortunately the diet CO was not tested by the manufactures for enough time (maybe more than 4 or 6 months) to ensure the formulation contained the required dietary nutrients for ongrowing commercial abalone. Fishmeal provides abalone with a high quality of protein and a balance of amino acids and fatty acids adequate to produce fast growth rates.

A



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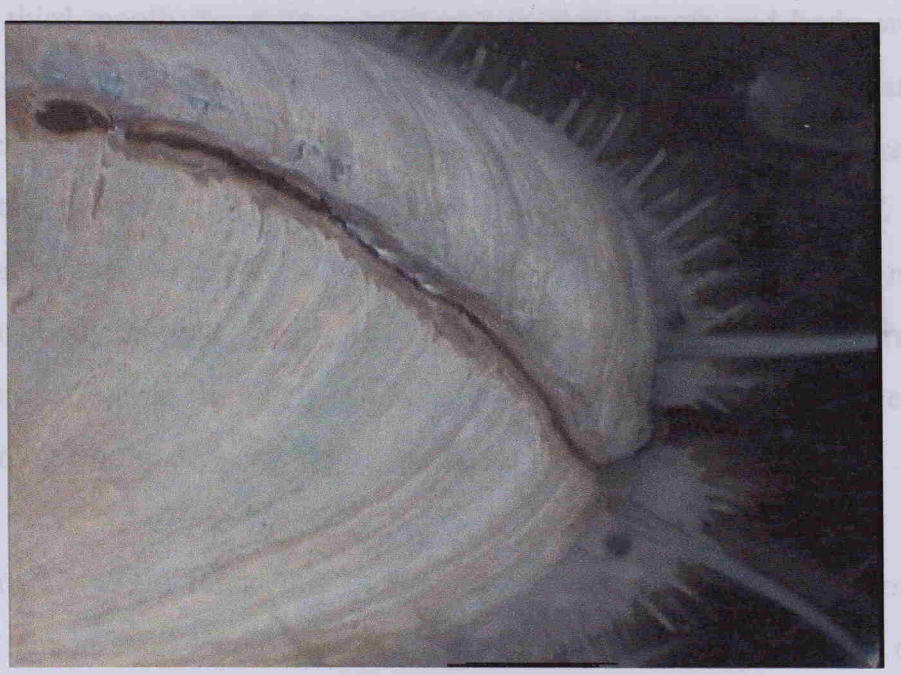

Figure 5.9. Shell deformation of the juvenile *Haliotis tuberculata* fed on abalone commercial diet. A.- abalone after four months feeding. B.- abalone after seven months feeding.

5.4.2. Reproductive investment

Gonad maturation in male *H. tuberculata* has been reported to be earlier than in females, usually occurring after the second year at 25 to 40 mm shell length (Girard, 1972; Hayashi, 1980a). Similar results were obtained in this study. Males mature before females. This, is the first time showing, that abalone species has been found to start gonad maturation as small as 1.09 cm shell length and at 10 months old. Gonad energy content for females was higher than for males. Therefore, to asses the reproductive output (*Pr*) used in the energy budget equation the gonad energy content for each sex was used. Organisms cultured at 15º and 18ºC preferentially allocated energy to growth, while abalone cultured at 22ºC assigned more energy to be reproduction.

After the third month the daily growth rate in shell length and body weight of the animals cultured at 22°C decreased (Figures 5.3 and 5.4) and at the same time the gonads developed (Figure 5.5). On the juveniles cultured at 18°C the decreased was a month later, however, only in body weight. In the present study *H. tuberculata* spawned at an average size of 1.78 ± 0.54 cm (equivalent to an age of 13 months). The decline in body weight might also be associated with a more straightforward temperature effect on energy partitioning with elevated temperature leading to an increase in metabolic demand and a subsequent reduction in growth.

Mercer *et al.*, (1993) found that the average size of sexual maturity for ormer was about 3.2 cm in shell length. The same author reported a reduction in daily growth rates during sexual maturation and spawning of *H. iris* fed on good quality algal diets. Furthermore, Capinpin and Corre (1996) obtained similar results where rich diets such has *Gracilariopsis heteroclada* and an artificial diet promoted fast growth rates and

progress of gonad maturation while low quality diets produced poor growth rates and failed to promote gonad development. To date, no study on *Haliotis* species have reported spawning of year-old abalone *H. tuberculata*. Abalone spawning at such a small size is a severe commercial disadvantage as somatic growth energy is being diverted to reproduction.

A review of the literature has indicated that whilst energy metabolism has been investigated extensively in abalone species, there is considerable variation between studies of the conditions under which measurements are made, even when the same term is used. Differences occur in both the temperature at which measurements are made, and the conditioning prior to measurement. Energy metabolism is also influenced by the previous nutrition of the animal. In our study, factors of culture such as seawater temperature, diet, salinity and light were maintained the same since animals were three months old until approximately seven months old. Thus, animals were not stressed by changing these conditions when components of the energy budget were assessed.

In order to assess the effect of diets and temperature on the energy budget and growth rates of *H. tuberculata*, daily energy expenditure of physiological parameters, such as respiration, excretion and mucus, were examined on animals that were born and reared under laboratory conditions. Such data may be subsequently applied to culture systems.

5.4.3. Respiration

The daily respiration energy expenditure in both groups (Main group and Subgroup) increased with temperature and diet also affected these rates. Animals fed on formulated diets spent more energy through respiration than those fed on seaweed,

even at the same temperature. Those results show that temperature and diet have an effect on oxygen consumption rates. Energy used for respiration was higher during the night time (16-34% at 15°C, 12-16% at 18°C and -1.9-17% at 22°C), but it was not significantly different from that spent for organism cultured at 18° and 22°C and fed on formulated diets. Thus an abalone with mean shell length 16.5 cm and mean body dry weight of 0.34 g spent 18.8 cal day^{-1} during the day time when been fed on fish meal diet and cultured at 22°C. In other words the same animal spent 32% of the energy absorbed through respiration.

The rate of oxygen consumption and the respective exponent values from this study were strongly dependent upon both body size and temperature. The respiratory exponent of 0.78 to 0.79 (Table 5.14) are quite comparable with those found for *H. tuberculata* when fed on seaweed and cultured at 15 ±1° with values of 0.76 (Peck *et al.*, 1987). Furthermore, Donovan and Carefoot (1996) found comparable data on the exponent of oxygen consumption for *H. kamtschatkana* which ranged from 0.74 to 0.78. However, the respiratory exponents found in this study, with values above 1.0 for animals cultured at higher temperatures, are slightly higher than those reported for *H. midae* by Barakai and Griffiths (1987) with values ranging from 0.83 to 0.94 at 14° and 19°C, and also for grazing gastropods (mean values of 0.67) (Bayne and Newell, 1983). Oxygen consumption for *H. tuberculata* from this study was considerably elevated by higher temperatures up to 22°C.

In the same context, elevated physiological rates resulting from handling and experimentation stress and subsequent confinement are attributed to give no uniform level of respiration and excretion rates. This effect is illustrated by the fact that different studies with the same species have reported results in which the value is different by at least four times.

5.4.4. Excretion

Nitrogen excretion is probably influenced by protein quality of the food offered. Ammonia is assumed to be the dominant end product of protein catabolism in molluscs (Bayne and Newell, 1983). Therefore, the rates measured in this work may be used to calculate significant parts of the energy expended by *H. tuberculata* during its trial of the metabolism and nitrogen excretion process during the growth stage.

Ammonia excretion rate, as with the respiration rate, did not present variation between day and night time, but is affected by temperature, diets and animal size. Ammonia excretion by abalone fed on SW diet was low, however, temperature has an effect by increasing the excretion rate up to 67%. Moreover, when abalone were tested at 18° and 22°C the rate of ammonia increased considerably, giving an average of 129%. Despite ammonia excretion increasing with temperature and diet the values of the exponent *b* were low (-0.38 to 0.61). The exponent -0.38 was obtained with a smaller average dry body weight (0.12 ±0.01 g dry weight) compared with the exponent 0.61 (0.43 ±0.2 g dry weight) (Table 5.16). At least an order of magnitude range of the animals dry weight was used to analysed body size effects on metabolic rates, as a very limited range of size is frequently responsible for wide variations in *b* values.

When these results are compared with those obtained by Peck *et al.* (1997) for the same abalone species (0.66) the results obtained are clearly lower. The diet and temperature of culture were however different between his experiments and those used in this study. On the other hand, Barakai and Griffiths (1987) reported an exponent of 0.39 at a 14°C culture, and this is low compared to those for other marine gastropods.

The relationship of oxygen consumed to nitrogen excreted (atomic relation O:N) may

be used as a qualitative index of the substrates: protein, lipid, or carbohydrate which are oxidised for energy obtained by animals. Values around 15 to 30 indicated a protein-dominant metabolised product. On the other hand, when the relation O:N were higher than 30:1, indicated a lipid-dominant metabolism (Mayzaud and Conover, 1988). The O:N ratio ranging from 7.21 to 13.2 indicated that *H. tuberculata* fed on SW and cultured at 15°C primarily catabolised protein.

The highest values were for those organisms fed on formulated diets and cultured at 22°C indicating that probably abalone was catabolising any of the three components: protein, carbohydrate and lipid, because the value of O:N ratio increased with the proportion of lipid and carbohydrate being utilised (Clarke, 1990). Thus, our results show that temperature and diets may have effects on the biochemical substrate metabolised by *H. tuberculata* (Table 5.20). O:N ratio appears to be higher in herbivores than in carnivores, probably reflecting the nitrogen-rich diet of carnivores. Thus, carnivores had higher respiration and excretion rates than herbivores, with lower O:N rations (Stickle and Bayne, 1982).

Table 5.21. Relation O:N of consumed oxygen to nitrogen excreted of *Haliotis tuberculata* from Main group and Subgroup.

		Main Group				Sub group			
°C	Diets	N	Shell Length (mm)	Dry Weight (g)	O:N	n	Shell Length (mm)	Dry Weight (g)	O:N
15°	SW	42	12.2 ±0.3	0.11 ±0.01	7.21	3	16.2 ±0.4	0.20 ±0.02	12.52
	FM	39	13.9 ±0.4	0.13 ±0.02	16.24	3	16.2 ±0.4	0.21 ±0.01	14.65
	CO	40	13.4 ±0.4	0.13 ±0.02	18.75	3	16.7 ±0.4	0.23 ±0.01	18.44
18°	SW	42	15.2 ±0.6	0.23 ±0.03	9.02	3	16.3 ±0.6	0.22 ±0.02	11.66
	FM	42	21.9 ±0.9	0.59 ±0.05	21.22	3	16.4 ±0.2	0.24 ±0.02	19.59
	CO	40	20.5 ±0.8	0.47 ±0.03	21.10	3	16.7 ±0.5	0.21 ±0.02	18.41
22°	SW	41	16.7 ±0.8	0.23 ±0.02	11.41	3	16.4 ±0.9	0.23 ±0.04	13.20
	FM	43	22.6 ±0.9	0.67 ±0.05	34.42	3	16.5 ±0.8	0.34 ±0.02	15.61
	CO	41	21.9 ±1.0	0.66 ±0.03	33.89	3	16.4 ±0.7	0.33 ±0.02	15.37

5.4.5. Pedal mucus production

The energy expended through pedal mucus production by *H. tuberculata* from this study was from 0.51 to 2.3% of the total assimilated energy. Evidence for effects of temperature on secretion of mucus is rare. Our data show significant effects of temperature in mucus secretion, with increase at high temperatures. The same behaviour was found in the whelk *Buccinum undatum*, averaging 12.4 mg h^{-1} at 8.2°C, 13.1 mg h^{-1} at 10.5°C and 19.5 mg h^{-1} at 15°C (Kideys and Hartnoll, 1991).

Estimations for "inactive" abalone under laboratory conditions, where food is available and space limited maybe different to that obtained from the field conditions, where animals need to be more "active", foraging for food. Donovan and Carefoot (1998) estimated daily secretions of mucus of *H. kamtschatkana* in the absence of activity by assuming that the abalone adheres to the substratum once per day and then remains still. However, it is possible that more energy would be expended on activity, especially in areas where food is scarce or predation intense. Thus the values above indicated for *H. kamtschatkana* would be much higher.

Peck *et al.* (1987) found that for an abalone *H. tuberculata* of 13.7 mm shell length and 0.12 g body dry weight spends an average of 2.4 cal per day, when abalone were fed on *Ulva lactuca* and cultured at 15°C, where mucus accounting for ~23% of consumed energy. In the same context, Davies *et al.* (1990) found ~23% of consumption was spent in mucus production by *Patella vulgata*. Nevertheless, animals of the same size from the present study fed on SW and cultured at the same temperature shown an average three fold reduction compared to that observed by Peck *et al.* (1987). He demonstrated the importance role of mucus in energy budget for those organisms, which use mucus, such as *H. tuberculata*. It is possible that an assessment of the energy utilised for an individual of the same species can present very different results. For these reasons, it is important to determine the energy partitioning for a particular

organism under specific conditions. Therefore, to get a measure of a specific parameter it is necessary that organisms of the same sex, age, and body size be compared under the same experimental conditions.

5.4.6. Energy budget

In aquaculture it is important to identify the environmental and nutritional conditions in order to minimise the utilisation of the energy by the organism in culture. Thus the survival and efficient development of abalone depends mainly on the storage, distribution and use of the energy assimilated. The balance on the energy utilisation can be positive or negative. When an animal utilised extra energy sources different to that from food consume, it is possible that their own reserves (tissue components) are being utilised.

Animals fed on formulated diets and cultured at 18°C shown up to 62% invested in growth from the total energy assimilated. These animals more efficiently channelled ingested energy. In that case *H. tuberculata* possess physiological mechanisms to utilise energy more efficiently when fed on formulated diets and cultured at 18°C compared with those fed on the same diets but cultured at 22°C. This is probably a refection of increased metabolic demand with increasing temperature.

As expected, all values on the energy budget obtained for abalone reared at 15°, 18° and 22°C were different. When *H. tuberculata* was cultured at low temperature (15°) the percent of the energy used was comparable between diets with those cultured at higher temperatures. Abalone reared 18° and fed on formulated diets utilised 94% energy consumed, with most of this energy going toward growth and respiration. On the other hand, individuals cultured at 22°C invested 22% less energy in growth and almost two and half times more energy in reproduction than the organisms cultured at 18°. In other words, organisms cultured at 18° spend more energy in growth and less in

reproduction than the abalone reared al 22°C. Peck, *et al.* (1987) obtained similar energetic partitioning to that in abalones from this study cultured at 15° and fed on fresh seaweed; reporting values of 83% absorption, 15.5% egestion and excretion, 20% respiration, 34% growth, but mucus was three fold higher for *H. tuberculata.*

Depending on species a 50 g dry weight abalone is reported to spend 88% (*H. tuberculata*), 74.5% (*H. midae*) and 104% (*H. kamtschatkana*) from the total energy consumed by Peck, *et al.* (1987), Barakai and Griffiths (1988) and Donovan and Carefoot (1998), respectively. However, abalone from this study cultured under the same conditions (15°C and fed on fresh seaweed), but only an average body weight of 0.22 g, used an average of 59% of the energy assimilated. Tables 5.22 and 5.23 shows the differences between the energy partitioning assimilated and the total energy utilised by young abalone *H. tuberculata* in the present study fed on different nutritional diets and cultured at different temperatures. These differences are probably a result of an underestimation on the different parameters of the energy budget assessed for abalone from this study. Although, the assessment of each of the different parameters of the energy budget was carefully analysed both in a group or individual context, the percent of the energy utilised was variable (from 60 to 94%).

In the recent years there has been a rapid increase in the number of research groups improving abalone farming and developing formulated diets. There is however, a general lack of information concerning the dietary nutritional requirements of abalone under practical farming conditions, the digestion and assimilation response of the abalone when fed on diet different to that of the natural one. All these questions are important an understanding the energy budget of cultured *Haliotis* species using artificial environmental conditions.

The importance of environmental effects on metabolism and physiology has been

demonstrated in aquaculture management (Brett and Groves, 1979). Temperature has been considered one of the most important environmental factors that controls food utilisation at all levels and all stages of growth in aquatic poikilothermic animals (Lovell, 1989). The data obtained in this study clearly shows that temperature and energetically rich diets have a significant influence on growth rates and early gonad development. Thus, the main goal of the present information is to assist nutritionists in developing a formulated diet for *H. tuberculata,* and to increase the scientific knowledge of the associated physiological responses at different temperatures of culture.

Table 5.22. The Energy budget (calories per animal per day) of Ingestion (*I*), Egestion (*E*), Absorption (*Ab*), Somatic growth (*Pg*), Reproductive investment (*Pr*), Respiration (*R*), Ammonia excretion (*U*) and Mucus production (*M*). Data obtained using all (Main group) juveniles *H. tuberculata* reared in the experiment for growth from each treatment. Mean values and standard error in ±.

°C	Diets	Shell Length (mm)	Body Weight (g)	*I*	*E*	*Ab*	*Pg*	*Pr*	*R*	*U*	*M*	Energy Utilised (%)
15°	SW	12.2 ±0.3	0.11 ±0.01	14.2 ±0.5	2.4 ±0.3	11.8 ±0.2	2.7 ±0.2	null	2.4 ±0.3	0.33 ±0.03	0.84 ±0.02	53
	FM	13.9 ±0.4	0.13 ±0.02	23.2 ±1.2	2.8 ±0.3	20.4 ±0.6	5.5 ±0.3	null	5.5 ±0.2	0.34 ±0.02	0.87 ±0.02	60
	CO	13.4 ±0.4	0.13 ±0.02	23.6 ±2.9	3.0 ±0.5	20.8 ±1.9	5.1 ±0.5	null	5.8 ±0.4	0.31 ±0.03	0.91 ±0.02	58
18°	SW	15.2 ±0.6	0.23 ±0.03	24.0 ±3.2	2.7 ±0.1	21.3 ±2.8	6.1 ±0.6	1.0 ±0.02	4.9 ±0.5	0.54 ±0.02	1.4 ±0.01	64
	FM	21.9 ±0.6	0.59 ±0.05	76.7 ±3.1	8.8 ±0.6	68.0 ±2.2	22.5 ±0.9	3.1 ±0.05	21.1 ±1.1	0.99 ±0.05	1.5 ±0.01	71
	CO	20.5 ±0.9	0.47 ±0.03	97.0 ±4.2	8.7 ±0.5	88.9 ±3.1	18.5 ±0.7	2.5 ±0.03	17.3 ±0.9	0.82 ±0.04	1.6 ±0.02	46
22°	SW	16.7 ±0.8	0.23 ±0.02	32.2 ±2.7	3.7 ±0.2	28.5 ±1.8	7.9 ±0.4	1.2 ±0.01	5.9 ±0.4	0.52 ±0.03	2.0 ±0.02	61
	FM	22.6 ±0.9	0.67 ±0.05	90.7 ±4.4	7.5 ±0.4	82.7 ±3.7	13.6 ±0.6	3.6 ±0.04	32.3 ±1.3	0.94 ±0.05	2.2 ±0.03	62
	CO	21.9 ±1.0	0.66 ±0.03	123 ±7.1	7.6 ±0.6	115 ±4.2	14.5 ±0.6	3.5 ±0.06	30.9 ±1.1	0.91 ±0.05	2.3 ±0.02	45

Null are those organisms that did not present gonad development. Seaweed diet (SW), Fishmeal diet (FM) and Commercial diet (CO), at 15°, 18° and 22°C of culture. Absorption (*Ab*) = Ingestion (*I*) + Egestion (*E*). The energy utilized as: $I - E = Ab - (Pg + Pr + R + U + M)$.

Table 5.23. The Energy budget (calories per animal per day) of Ingestion (*I*), Egestion (*E*), Absorption (*Ab*), Somatic growth (*Pg*), Respiration (*R*), Ammonia excretion (*U*), Reproductive investment (*Pr*) and Mucus production (*M*). Data obtained using same size (Subgroup) juveniles *H. tuberculata* reared in the experiment for growth from each treatment. Mean values and standard error ±

°C	Diet	Shell Length (mm)	Body Weight (g)	*I*	*E*	*Ab*	*Pg*	*Pr*	*R*	*U*	*M*	Energy Utilised (%)
15°	SW	16.2 ±0.08	0.20 ±0.01	25.0 ±0.5	3.02 ±0.4	22.1 ±1.1	7.75 ±0.7	null	4.4 ±0.3	0.35 ±0.02	0.51 ±0.02	60
	FM	16.2 ±0.1	0.21 ±0.01	29.4 ±1.2	4.21 ±1.0	25.2 ±0.7	10.63 ±0.7	null	7.2 ±0.5	0.49 ±0.05	0.53 ±0.04	74
	CO	16.7 ±0.1	0.23 ±0.01	32.1 ±2.9	6.80 ±1.1	25.3 ±2.1	9.65 ±0.8	null	8.1 ±0.7	0.44 ±0.07	0.54 ±0.05	73
18°	SW	16.3 ±0.3	0.22 ±0.01	33.9 ±3.2	3.99 ±0.5	29.9 ±1.7	11.29 ±0.4	0.68 ±0.04	6.2 ±0.6	0.53 ±0.03	0.67 ±0.03	64
	FM	16.4 ±0.2	0.24 ±0.02	70.8 ±3.1	7.89 ±0.8	62.9 ±2.3	39.42 ±2.1	1.15 ±0.06	17.1 ±1.0	0.87 ±0.04	0.70 ±0.01	94
	CO	16.7 ±0.3	0.21 ±0.02	69.4 ±4.2	11.21 ±0.6	58.2 ±3.0	34.44 ±1.8	1.01 ±0.08	17.9 ±1.2	0.97 ±0.05	0.74 ±0.05	93
22°	SW	16.4 ±0.1	0.23 ±0.01	45.8 ±2.7	5.90 ±0.2	40.0 ±2.0	17.05 ±0.5	0.87 ±0.03	9.0 ±0.6	0.68 ±0.03	0.85 ±0.02	71
	FM	16.5 ±0.2	0.34 ±0.02	68.5 ±4.4	9.03 ±1.2	59.5 ±2.9	25.21 ±1.0	2.28 ±0.09	18.8 ±1.1	1.20 ±0.06	0.92 ±0.03	82
	CO	16.4 ±0.2	0.33 ±0.02	75.8 ±7.1	10.89 ±1.9	65.9 ±2.6	31.43 ±1.2	2.22 ±0.11	19.8 ±1.2	1.29 ±0.08	0.91 ±0.04	84

Null are those organisms that did not present gonad development. Seaweed diet (SW), Fishmeal diet (FM) and Commercial diet (CO), at 15°, 18° and 22°C of culture. Three organisms in each treatment. Absorption (*Ab*) = Ingestion (*I*) + Egestion (*E*). The energy utilized as: I - E = *Ab* - (*Pg* + *Pr* + *R* + *U* + *M*).

SUMMARY OF THE ACHIEVEMENTS

1) Data from this study shows that for annual production, the most natural way to condition the gametes from ripe abalone was using slight increments of seawater temperature throughout the day.

2) The relationship between the number of eggs spawned and the body weight of abalone was significant. Nevertheless, the relationship between the number of eggs spawned and the shell length was low. Number of sperm was not significant in both body weight and shell length. It was possibly due to the small size range of adult *H. tuberculata* used in the present study.

3) Until 1996 *Tetraselmis suecica* was the main source of food for early juvenile *H. tuberculata* in the rearing stage (one to twelve weeks old) on glass plates. Nevertheless, data from this study shows that *T. suecica*, as a sole food, is not sufficient for successful development and growth of early juvenile abalone. However, the use of marine diatoms such as *Skeletonema costatum, Navicula ramosissima* and *Cylindrotheca closterium* were easily ingested and assimilated depending on the size of the animal and conditions of culture. Food quality is important, but also maintaining the proper concentration of the food is critical to reach good growth rates and survival in the culture of early juvenile abalone.

4) Juvenile *H. tuberculata*, when fed on formulated diets for more than three months show that the growth rates are variable. Although the development of a formulated diet has been identified as crucial to the success of the abalone aquaculture industry, it is necessary to recognize other aspects of the diet other than ability to promote growth. These include its digestible energy and the amount of energy available for reproduction

and maintenance. Abalone from this study was fed on energetically rich diets (FM and CO). However, the total assimilation of the energy was low. However, due to the energy rich diet basic maintenance costs were met while leaving additional energy available for other processes. Such as growth and reproduction or stored as glycogen in the foot, or excreted as waste.

5) Faster-growing animals reach maturity at a smaller size and early age (McShane and Naylor, 1995). This study demonstrates that when juvenile abalone *Haliotis tuberculata* were fed on energetically rich diets (protein, lipid and carbohydrate) and cultured at 18º and 22ºC a great part of the energy was channeled to premature gonad production.

6) The abalone commercial diet (CO) used in this study produced shell deformation in 89% of the population after four months of feeding. The information of these studies have important implications when considering the nutritional requirements of cultured abalone when fed on formulated diets, which may contain not only the energy, but also the necessary micronutients, which are required to produce good growth rates and also healthy animals.

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APPENDIX 1. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on seaweed (SW) diet and reared at 15°C.

Number of organism	March	April	May	June	July	August	September	October
1	4.28	5.91	6.34	10.83	9.42	10.39	16.06	10.13
2	4.61	8.15	8.87	8.06	8.96	9.74	12.59	13.92
3	4.96	6.27	7.49	6.91	11.41	12.05	8.94	15.11
4	5.05	4.87	9.36	8.66	7.87	11.28	10.58	12.35
5	4.73	5.22	7.60	7.90	9.75	9.76	9.69	11.28
6	4.76	5.31	6.76	6.87	8.22	13.59	12.47	12.58
7	5.62	4.40	7.85	8.75	9.16	12.74	17.04	13.97
8	5.89	7.28	8.19	8.06	11.38	14.42	15.82	18.41
9	5.37	5.76	7.43	10.48	12.93	13.28	10.28	17.05
10	5.44	6.43	6.24	9.44	12.51	9.90	12.94	11.29
11	4.78	4.87	9.46	7.11	10.25	8.84	11.37	17.78
12	4.29	5.28	7.49	7.99	9.04	10.43	10.93	12.52
13	4.96	6.09	6.73	8.22	10.33	8.25	14.18	16.22
14	5.81	6.39	8.28	10.97	11.91	11.96	11.72	12.99
15	4.88	6.28	7.55	7.24	8.50	9.68	11.24	16.22
16	5.27	5.51	6.94	8.76	10.43	11.35	12.19	14.28
17	5.49	5.56	7.49	10.28	9.35	9.70	12.51	9.59
18	5.56	6.81	6.39	7.96	8.92	10.33	10.22	16.04
19	5.72	5.24	6.77	11.34	8.14	8.52	13.44	11.45
20	4.19	7.65	5.93	8.59	7.29	11.97	12.57	12.18
21	4.65	5.89	9.22	7.73	13.47	9.28	10.21	16.37
22	5.90	6.33	6.89	9.14	10.31	13.62	11.40	10.69
23	5.22	7.52	7.52	7.62	8.25	11.83	15.56	14.22
24	4.48	5.79	6.64	8.43	8.99	9.55	10.35	16.96
25	4.83	5.96	7.96	6.96	7.94	10.34	14.20	11.19
26	5.79	5.72	10.18	9.55	10.38	8.17	14.12	14.06
27	4.95	7.01	6.14	6.99	9.64	11.18	10.68	11.43
28	5.02	7.03	7.99	10.17	8.01	9.59	15.72	9.52
29	5.88	7.18	6.20	8.51	8.84	14.12	8.86	13.70
30	5.51	6.42	5.11	9.69	11.62	8.78	9.78	12.87
31	4.64	6.26	5.82	6.87	8.36	15.22	15.33	13.74
32	4.33	6.33	7.44	9.45	10.18	13.86	11.66	19.43
33	4.75	7.12	8.26	7.92	11.44	8.69	10.91	11.18
34	5.52	5.78	5.91	11.58	9.28	14.91	9.89	12.21
35	5.38	6.58	6.79	8.28	12.53	10.01	13.77	15.88
36	4.66	5.67	8.05	9.12	8.24	9.87	9.96	10.99
37	4.61	6.19	7.33	7.40	10.12	9.24	14.20	11.87
38	5.10	5.43	6.81	7.33	9.48	10.94	10.53	11.30
39	5.92	6.94	8.87	11.43	7.59	11.88	13.49	12.83
40	5.48	5.80	6.94	7.82	8.47	10.31	10.24	10.58
41	5.36	6.44	7.59	8.29	12.22	9.77	13.78	
42	4.87	5.66	8.09	7.05	8.48	10.75	11.16	
43	4.24	7.38	6.06					
44	5.38	5.69						
45	4.19	6.55						
Mean	5.07	6.18	7.37	8.61	9.75	10.95	12.20	13.41
SE	0.08	0.12	0.17	0.21	0.25	0.29	0.33	0.40

APPENDIX 2. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on seaweed (SW) diet and reared at 18°C.

Number of organism	March	April	May	June	July	August	September	October
1	4.21	8.55	7.52	13.51	13.28	17.16	20.53	15.32
2	4.53	6.24	8.58	14.06	15.17	13.57	15.70	17.86
3	4.77	5.71	10.93	13.22	9.95	18.26	18.56	21.53
4	5.02	8.06	7.77	9.96	12.21	12.98	13.78	19.28
5	4.18	6.98	10.90	8.84	12.33	18.22	15.14	18.14
6	4.71	5.88	10.41	7.66	14.18	13.89	11.92	15.47
7	5.84	4.97	8.54	11.25	11.90	14.04	15.95	18.78
8	5.66	7.76	7.98	10.61	12.36	10.91	12.66	17.01
9	4.75	5.65	6.88	9.05	11.72	14.08	13.79	16.89
10	5.31	5.81	10.81	7.70	11.69	16.55	16.10	18.36
11	5.85	6.03	6.55	12.93	16.24	14.41	19.51	12.92
12	4.22	6.92	8.32	10.11	12.19	10.96	12.64	16.12
13	4.93	6.73	7.35	9.62	10.79	12.04	14.04	22.35
14	4.69	5.49	10.14	10.79	8.85	14.32	17.33	15.58
15	5.77	6.69	8.99	12.96	11.44	13.35	15.26	16.11
16	5.81	6.77	7.29	8.77	11.06	14.12	20.76	17.53
17	5.90	6.13	8.35	9.59	15.93	10.33	17.11	16.29
18	4.88	8.18	10.27	8.88	12.78	13.24	11.19	17.91
19	4.96	6.46	7.72	12.33	12.55	12.69	19.96	14.26
20	5.07	5.54	6.35	10.11	10.66	11.22	12.81	15.44
21	5.89	6.31	8.66	10.74	16.01	14.09	15.32	13.28
22	4.48	6.93	10.73	9.65	13.11	11.78	18.85	16.79
23	4.55	6.57	7.12	7.58	10.84	12.42	14.28	18.15
24	5.34	7.33	8.25	9.21	14.89	10.96	16.69	16.21
25	5.16	5.93	10.24	8.75	11.33	13.44	14.90	21.91
26	4.22	5.17	8.85	11.51	12.59	12.48	15.94	19.68
27	4.37	6.22	7.42	6.96	14.42	15.19	16.22	17.73
28	5.23	5.98	7.15	7.87	13.05	17.02	14.46	15.50
29	4.74	8.74	7.82	8.45	10.18	14.11	12.08	14.87
30	5.25	6.90	6.95	10.33	8.55	10.75	16.84	13.61
31	5.88	5.67	8.09	10.56	11.20	15.24	14.77	15.33
32	4.19	7.93	9.56	11.88	9.38	18.73	13.81	21.70
33	5.26	6.82	5.79	9.99	12.81	9.94	12.57	16.83
34	5.48	8.44	10.74	10.21	11.59	13.88	15.24	12.92
35	4.80	6.77	8.53	9.57	10.51	12.65	12.45	14.25
36	4.36	8.56	7.32	8.94	9.78	10.12	14.61	20.61
37	5.32	7.44	6.55	10.61	10.50	13.13	15.56	14.55
38	5.29	7.22	8.01	9.44	13.88	11.15	11.74	16.26
39	4.33	6.99	9.49	11.38	9.46	15.18	16.13	21.04
40	4.51	5.09	8.14	12.55	8.92	12.80	14.99	17.09
41	4.17	5.85	9.21	7.87	14.66	15.45	13.23	14.21
42	4.21	6.33	7.77	9.92	9.96	13.34	14.29	17.45
43	5.30	4.85	8.56	10.09	8.75			
44	4.44	5.24	8.68	12.36	11.38			
45	4.38	7.88	6.65	10.69				
Mean	4.94	6.62	8.40	10.20	11.93	13.58	15.23	16.98
SE	0.08	0.16	0.20	0.26	0.31	0.34	0.37	0.39

APPENDIX 3. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on seaweed (SW) diet and reared at 22°C.

Number of organism	March	April	May	June	July	August	September	October
1	4.72	6.49	8.59	11.24	14.67	14.94	19.29	16.24
2	4.19	8.22	11.55	12.31	12.36	18.24	17.83	21.79
3	5.07	6.44	7.21	9.10	11.02	17.12	13.51	19.02
4	5.44	8.03	9.04	7.93	10.08	16.20	15.66	16.61
5	4.33	5.74	6.96	12.55	12.56	13.83	17.69	20.84
6	4.81	6.07	8.63	9.18	12.68	12.44	14.51	17.23
7	5.24	8.71	11.28	9.96	13.54	11.21	13.75	18.49
8	5.48	5.58	10.34	11.12	11.93	17.09	19.63	24.15
9	4.25	5.34	8.13	9.78	12.79	13.23	15.28	15.89
10	4.72	5.18	6.88	11.61	9.87	16.82	14.92	16.76
11	5.55	7.36	7.21	14.72	14.94	12.60	18.22	22.68
12	5.09	5.97	7.40	10.64	14.16	15.22	14.28	18.44
13	4.77	6.99	8.14	12.39	12.71	12.31	18.11	20.81
14	5.65	7.53	7.12	13.75	9.49	14.19	16.74	18.52
15	4.98	6.18	8.17	8.90	10.24	12.58	18.71	16.79
16	4.56	7.01	10.73	10.10	11.82	14.21	13.95	15.26
17	4.74	6.22	6.82	12.29	12.40	11.75	14.82	22.15
18	4.48	7.99	8.05	9.24	11.63	17.38	14.59	20.88
19	5.15	6.19	10.42	8.86	11.64	11.22	15.77	16.22
20	5.31	6.11	7.94	11.18	13.82	15.15	16.78	19.94
21	5.90	6.85	8.47	8.92	14.58	13.91	14.12	15.72
22	4.82	7.34	9.15	12.79	10.86	12.96	19.63	20.68
23	5.17	6.62	8.58	7.94	15.25	15.29	14.84	21.35
24	4.59	8.24	9.24	10.22	10.67	17.85	16.61	17.28
25	4.51	5.73	7.13	12.33	12.97	12.62	15.86	15.69
26	4.77	7.12	9.49	10.06	14.79	11.89	20.89	24.21
27	5.60	6.65	8.51	8.28	16.91	14.56	20.05	17.98
28	5.86	8.48	6.97	11.65	14.28	13.43	16.10	22.06
29	5.30	5.27	8.26	9.99	16.81	18.39	15.24	15.96
30	5.63	6.49	9.42	8.92	10.88	15.44	16.31	18.09
31	4.67	7.69	8.53	8.66	9.17	14.61	14.53	16.83
32	5.52	7.09	6.96	9.91	15.01	11.28	21.68	17.56
33	4.21	5.41	7.01	10.54	14.19	18.10	18.96	16.69
34	4.19	6.20	8.22	9.21	10.92	12.63	16.34	22.35
35	5.13	5.88	9.57	11.50	12.33	16.25	14.18	19.86
36	4.69	5.97	7.44	9.01	10.92	15.69	17.86	17.62
37	5.78	6.73	10.11	10.46	9.49	14.95	19.42	17.46
38	4.59	7.26	7.09	10.35	15.02	13.71	15.58	15.70
39	5.22	5.48	7.00	11.69	12.71	11.93	21.09	14.98
40	5.23	7.29	9.68	12.48	14.36	19.47	12.93	18.82
41	4.88	4.96	8.07	10.19	10.74	17.55	16.52	20.66
42	4.72	7.52	10.08	11.32	13.82	14.81		
43	5.18	5.87	8.73	10.08				
44	4.29	6.35	9.22					
45	5.61	7.16	8.48					
Mean	4.99	6.64	8.49	10.54	12.64	14.64	16.65	18.69
SE	0.07	0.14	0.19	0.24	0.31	0.35	0.36	0.40

APPENDIX 4. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on the fishmeal (FM) diet and reared at 15°C.

Number of organism	March	April	May	June	July	August	September	October
1	5.18	5.97	7.41	10.12	9.87	10.98	10.38	11.42
2	4.90	6.44	8.13	8.97	9.85	14.67	15.54	17.62
3	5.75	6.89	7.92	10.36	10.66	10.75	11.41	12.22
4	4.82	6.77	8.58	9.63	9.71	9.88	14.32	15.79
5	4.71	5.72	7.05	9.51	10.83	13.56	13.52	14.49
6	4.50	6.18	9.49	9.62	11.43	12.07	15.26	16.96
7	4.75	6.45	8.25	7.29	10.72	10.44	11.51	12.47
8	5.78	5.88	8.92	7.90	11.88	12.24	12.71	13.60
9	5.23	7.45	7.66	9.31	10.05	9.83	15.88	17.75
10	5.46	6.34	7.58	10.15	9.41	13.13	16.55	18.06
11	4.69	6.28	6.92	11.53	11.52	15.92	12.96	13.85
12	5.88	6.66	6.57	8.15	10.74	11.66	13.77	14.56
13	4.96	5.14	8.04	9.09	11.62	11.57	12.84	13.74
14	4.83	6.93	8.66	8.56	12.35	11.86	16.91	18.90
15	4.80	6.18	7.61	8.60	9.89	14.22	12.20	13.18
16	4.92	6.36	8.18	11.05	9.17	10.50	13.43	14.52
17	5.33	6.33	8.76	10.40	9.11	15.11	11.70	12.99
18	5.21	6.15	6.65	7.55	10.67	10.36	15.45	17.31
19	4.95	6.75	7.21	10.20	9.42	12.46	16.76	18.55
20	4.99	7.44	7.19	6.97	12.74	13.06	12.78	14.55
21	5.22	6.33	7.62	8.93	12.16	9.82	11.52	12.34
22	5.66	5.52	9.09	9.47	10.78	12.49	10.64	11.22
23	5.39	6.99	6.76	9.55	10.62	10.92	15.37	16.45
24	5.45	5.59	7.18	8.84	9.43	13.00	15.59	17.02
25	4.88	6.11	7.04	11.24	8.77	13.15	14.28	16.11
26	4.83	6.12	7.62	9.81	10.91	10.64	17.47	18.97
27	4.96	6.22	6.48	8.41	11.55	12.10	13.35	14.64
28	4.65	6.01	7.43	11.33	10.56	13.83	14.78	15.23
29	4.84	5.85	8.28	8.42	10.44	11.80	14.33	16.31
30	5.74	7.82	7.67	8.99	13.09	10.22	13.81	15.86
31	4.99	6.23	7.64	7.85	10.99	11.55	12.93	14.96
32	5.05	6.13	8.46	10.78	11.52	14.77	15.35	16.73
33	5.12	6.08	7.85	10.11	12.98	10.54	14.10	15.40
34	4.92	6.58	7.50	11.17	13.10	13.77	12.44	13.58
35	4.90	6.42	8.44	8.98	11.59	13.40	11.84	12.93
36	5.23	6.91	7.98	7.65	12.78	10.61	17.36	19.37
37	5.66	6.12	7.87	9.84	10.31	14.04	13.59	15.62
38	5.32	6.77	7.68	8.88	11.04	12.97	14.77	16.92
39	4.94	6.78	6.79	10.06	9.82	13.88	11.06	12.68
40	5.22	6.52	6.02	8.78	8.95			
41	5.65	6.29	9.27					
42	4.96	6.02	9.22					
43	5.12	6.77						
44	4.59	5.23						
45	5.30	5.95						
Mean	5.12	6.35	7.78	9.35	10.83	12.25	13.86	15.25
SE	0.05	0.08	0.13	0.18	0.19	0.26	0.31	0.35

APPENDIX 5. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on the fishmeal (FM) diet and reared at 18°C.

Number of organism	March	April	May	June	July	August	September	October
1	5.4	6.9	10.1	13.2	17.9	14.4	16.1	27.9
2	4.8	9.1	8.9	11.3	16.4	17.9	21.3	24.7
3	4.4	7.2	11.2	13.2	18.6	19.4	18.9	26.7
4	5.2	6.3	13.3	17.3	20.0	22.1	24.0	21.5
5	4.6	8.4	7.7	13.9	16.5	24.7	18.0	22.7
6	5.8	7.2	12.5	11.1	17.2	16.2	20.9	27.7
7	5.1	6.9	10.3	13.0	16.4	19.3	26.9	25.6
8	5.3	5.8	11.2	13.1	15.9	23.5	21.8	26.3
9	5.0	8.2	8.2	14.0	15.6	21.8	23.4	27.4
10	5.2	7.4	9.9	12.9	20.8	17.8	21.8	23.0
11	4.8	7.1	9.6	12.2	15.1	19.0	26.2	24.8
12	5.4	5.9	10.1	13.3	15.0	20.1	22.4	25.3
13	5.9	6.2	10.1	13.0	17.4	21.2	24.9	19.6
14	5.2	6.9	9.8	13.2	18.1	18.7	19.6	22.9
15	5.8	7.4	9.9	11.7	12.3	20.4	17.2	29.2
16	4.7	7.1	13.2	12.9	21.1	14.4	20.7	22.7
17	5.0	8.4	9.9	13.2	16.8	18.8	21.7	24.6
18	5.1	7.0	8.7	15.4	16.9	17.9	24.3	22.9
19	5.4	7.6	9.4	14.3	15.9	22.1	16.2	20.2
20	4.2	6.8	8.2	12.5	18.2	15.9	28.2	27.0
21	5.3	5.8	7.8	14.0	13.4	24.8	20.6	26.1
22	5.2	5.9	9.2	13.3	16.9	19.8	22.9	20.8
23	5.9	6.7	10.3	16.0	21.6	24.3	24.5	17.8
24	5.6	7.0	9.9	16.8	16.7	19.9	21.4	23.0
25	5.6	8.9	8.3	13.5	19.2	15.5	18.1	20.3
26	5.3	7.1	11.3	12.7	16.7	19.4	24.1	29.1
27	5.4	7.2	10.7	13.0	15.3	21.3	23.7	22.8
28	5.1	8.0	9.9	9.9	22.0	18.6	19.7	26.8
29	5.1	6.9	11.5	13.3	13.0	22.3	20.0	19.0
30	4.8	7.5	12.1	14.6	20.5	24.7	20.2	28.5
31	5.2	7.2	10.2	12.3	17.3	19.1	26.0	23.8
32	4.5	6.7	9.3	13.6	17.0	14.8	23.0	25.4
33	4.5	6.0	10.0	17.2	18.9	23.6	27.3	24.0
34	4.8	8.2	9.4	13.2	16.4	19.7	20.7	22.0
35	5.1	7.2	7.7	12.7	21.2	15.0	24.2	18.4
36	4.9	7.4	10.2	9.8	16.0	22.4	17.0	21.7
37	5.1	7.0	9.8	14.9	16.5	18.1	24.9	19.5
38	5.4	9.1	10.4	9.9	17.2	17.9	26.2	22.8
39	5.6	7.2	9.8	13.4	13.2	20.1	21.4	23.8
40	5.2	6.2	9.9	13.0	12.6	18.9	23.1	26.5
41	5.9	9.1	8.2	13.2	19.6	21.7	20.4	17.9
42	5.1	7.8	7.7	12.3	14.9	18.0	16.7	26.7
43	5.0	6.5	10.1	12.9	12.8	20.9		
44	5.1	6.9	12.9	9.9	15.9	23.9		
45	5.9	7.0	10.5	13.7				
Mean	5.17	7.20	9.98	13.20	16.97	19.77	21.92	23.78
SE	0.06	0.13	0.21	0.25	0.37	0.43	0.49	0.48

APPENDIX 6. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on the fishmeal (FM) diet and reared at 22°C.

Number of organism	March	April	May	June	July	August	September	October
1	4.48	5.96	10.07	13.98	15.95	25.12	20.14	20.99
2	5.09	6.99	12.28	17.91	16.89	19.95	24.78	24.51
3	4.78	7.55	7.66	18.78	17.62	22.77	27.34	23.97
4	5.87	6.88	10.59	13.84	12.91	18.82	22.45	28.14
5	5.55	7.64	11.80	15.18	19.88	25.69	20.44	22.73
6	4.72	9.10	10.36	14.00	21.34	18.10	21.27	18.11
7	4.48	8.28	7.91	10.21	18.74	25.92	26.31	21.85
8	5.20	6.96	10.75	14.15	23.15	18.54	19.59	20.00
9	5.96	5.87	11.89	16.24	17.22	20.12	24.09	28.84
10	4.66	8.66	13.81	10.06	23.09	14.94	16.86	22.66
11	4.33	6.72	12.56	18.22	17.55	25.28	20.18	23.70
12	5.57	6.75	11.52	16.66	22.16	22.98	26.28	27.97
13	5.31	7.45	10.47	14.10	12.78	24.14	27.12	29.26
14	4.39	6.71	12.21	17.21	19.83	21.75	20.42	26.41
15	4.77	8.09	10.60	12.95	17.31	14.70	28.48	30.44
16	5.34	6.81	13.65	18.22	15.87	19.24	24.83	27.99
17	5.81	5.95	11.76	12.92	19.36	20.06	27.21	25.69
18	4.45	8.40	8.83	13.28	17.40	23.87	29.04	22.88
19	4.89	9.01	9.90	14.11	22.28	18.90	21.96	18.83
20	5.90	6.30	10.13	18.44	13.24	20.15	22.18	25.28
21	5.60	5.89	12.56	14.55	23.10	16.81	19.55	22.85
22	4.52	6.52	11.24	15.83	15.98	24.16	16.04	17.96
23	5.11	8.06	10.62	17.91	16.05	18.11	21.95	22.79
24	4.70	7.77	11.94	13.77	21.44	22.34	23.10	20.12
25	5.92	6.96	13.10	11.96	16.97	19.22	26.44	26.88
26	4.54	8.81	8.78	13.06	14.92	19.56	20.06	28.05
27	4.88	7.43	10.31	10.87	22.88	18.94	22.51	22.51
28	4.40	8.98	10.88	17.35	17.58	23.41	16.84	18.15
29	5.87	6.92	9.15	13.42	20.65	19.33	18.17	25.77
30	4.39	7.24	12.64	16.92	17.11	24.36	24.13	21.78
31	5.52	5.77	10.41	18.50	14.94	18.44	18.34	30.33
32	5.64	6.61	9.87	15.56	17.61	24.17	26.98	23.18
33	4.95	9.11	10.72	12.87	22.14	25.18	22.20	25.94
34	4.50	6.72	11.85	15.91	13.53	22.46	21.18	28.44
35	5.97	7.33	9.60	16.21	20.18	18.11	28.11	29.77
36	4.49	7.20	13.40	14.79	22.78	15.04	24.31	30.05
37	4.68	6.22	11.58	13.65	16.08	16.91	21.87	23.91
38	5.73	6.82	12.05	18.20	21.10	21.09	26.12	29.55
39	5.47	7.85	11.48	11.44	18.20	19.66	26.74	21.77
40	4.78	8.17	12.34	15.99	20.94	15.18	20.99	22.36
41	4.38	7.55	8.59	13.53	19.10	26.21	18.84	20.44
42	4.81	7.99	9.99	16.93	15.95	20.86	16.75	25.19
43	5.12	7.66	11.48	17.45	16.28	26.08	20.41	20.25
44	4.82	8.49	9.92	13.74	18.99	20.15		
45	5.09	6.11	10.93	15.33				
Mean	5.05	7.34	10.98	15.03	18.39	20.84	22.62	24.38
SE	0.08	0.14	0.22	0.35	0.45	0.50	0.54	0.56

APPENDIX 7. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on the commercial (CO) diet and reared at 15°C.

Number of organism	March	April	May	June	July	August	September	October
1	5.09	6.18	7.22	10.75	11.89	12.40	13.05	14.58
2	4.55	5.42	7.85	8.96	11.87	10.24	14.83	11.22
3	5.38	6.54	8.21	8.44	9.64	13.72	12.94	13.66
4	5.35	6.67	7.39	10.50	9.50	11.58	12.90	16.85
5	4.72	5.77	8.64	9.82	11.84	12.05	9.85	12.38
6	5.39	6.83	7.86	9.23	10.46	10.44	15.95	15.25
7	5.10	6.34	7.99	9.92	9.26	14.78	13.37	16.73
8	5.13	6.29	7.56	9.08	11.25	12.35	12.88	13.89
9	5.28	6.51	8.47	10.56	11.79	10.66	16.09	15.30
10	4.92	5.98	9.28	7.83	8.04	12.93	13.75	13.92
11	4.79	6.82	7.49	9.23	10.83	13.98	11.53	14.09
12	5.60	7.05	9.62	9.14	12.94	11.87	11.18	15.48
13	4.82	6.11	9.30	10.22	10.03	10.15	12.48	15.82
14	5.06	6.31	7.36	8.49	10.61	13.55	11.17	12.44
15	4.85	5.79	6.78	11.15	9.85	12.81	12.66	17.78
16	5.44	6.54	8.07	7.95	9.95	11.95	14.05	15.06
17	5.78	7.12	9.05	8.91	9.98	10.46	14.17	13.25
18	5.69	6.85	7.46	9.10	12.94	10.88	13.33	14.47
19	4.76	5.88	7.98	8.43	9.33	10.92	14.98	17.62
20	4.93	6.13	7.65	9.35	10.67	12.99	12.28	13.70
21	5.96	7.34	8.04	8.39	9.92	13.69	13.68	18.29
22	5.54	6.78	6.47	9.36	9.82	14.41	11.72	13.01
23	4.77	5.91	8.71	9.46	11.36	11.82	16.83	12.86
24	4.92	7.22	8.97	9.86	10.92	11.29	14.30	14.03
25	4.83	6.10	7.44	7.98	9.76	12.38	12.82	14.44
26	4.79	5.71	6.69	9.02	9.59	15.22	13.51	16.33
27	5.24	6.68	7.46	8.21	9.86	14.17	11.47	13.99
28	5.13	6.43	8.42	10.26	10.48	10.97	12.90	14.06
29	4.60	6.48	7.29	9.33	11.21	12.00	14.71	15.15
30	4.83	5.80	7.77	8.39	9.17	11.82	11.91	13.91
31	5.35	6.44	8.45	8.87	12.10	10.63	14.64	17.15
32	4.86	5.67	7.37	10.20	9.46	11.11	12.76	14.88
33	5.56	7.55	7.58	9.11	9.59	11.69	16.38	13.93
34	4.95	5.98	6.85	7.84	11.59	10.33	17.40	18.73
35	4.87	5.77	7.05	8.39	10.48	10.24	13.98	15.55
36	5.69	7.21	7.69	8.77	11.26	11.88	11.69	12.78
37	4.70	5.88	9.50	9.07	11.09	10.06	12.66	13.80
38	4.87	6.23	7.61	8.96	10.88	11.81	11.15	12.77
39	4.89	5.55	5.86	8.44	11.67	9.11	12.94	13.52
40	4.83	5.78	8.84	9.41	10.44	11.95	13.40	
41	5.72	6.99	7.77					
42	5.66	6.85	8.57					
43	5.75	7.06	6.89					
44	4.83	5.75						
45	5.72	7.20						
Mean	5.14	6.39	7.87	9.16	10.58	11.93	13.36	14.68
SE	0.06	0.08	0.13	0.13	0.17	0.23	0.26	0.28

APPENDIX 8. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on the commercial (CO) diet and reared at 18°C.

Number of organism	March	April	May	June	July	August	September	October
1	5.17	7.52	11.11	14.68	15.58	19.58	19.75	17.21
2	5.62	7.59	9.78	12.82	17.280	15.82	23.78	25.14
3	4.48	5.94	10.44	13.41	16.22	21.33	21.90	27.44
4	4.36	5.91	9.06	12.49	15.55	13.84	18.32	19.85
5	5.88	8.01	9.77	12.34	17.81	15.62	21.09	23.07
6	5.32	7.59	7.78	8.71	13.05	18.91	24.30	27.15
7	5.11	7.02	10.59	14.38	18.72	21.96	24.25	23.45
8	5.06	7.03	7.88	9.53	11.32	17.62	17.34	17.56
9	4.78	6.39	10.36	13.75	13.43	21.90	20.04	25.06
10	4.89	6.55	8.84	11.32	13.08	21.85	21.61	17.48
11	5.36	7.88	10.70	12.94	15.59	13.94	21.00	24.98
12	4.77	5.85	11.86	15.85	16.10	21.27	15.55	20.96
13	4.59	6.11	7.59	9.79	14.27	21.77	22.99	22.14
14	4.58	6.08	9.13	12.05	16.98	16.51	23.96	15.97
15	5.03	7.12	11.33	14.68	17.15	18.63	17.72	22.59
16	5.13	6.98	7.58	10.16	17.16	22.90	21.44	27.02
17	4.88	6.77	7.44	10.00	15.98	19.85	25.13	19.08
18	4.96	7.23	9.94	13.19	16.04	23.08	15.95	21.36
19	5.13	7.86	10.91	14.59	15.21	18.98	24.22	21.68
20	5.88	8.76	7.55	9.03	15.60	16.97	21.66	26.11
21	4.60	5.63	10.87	14.39	17.36	13.87	20.14	18.77
22	5.23	6.75	8.69	9.05	12.71	16.95	24.12	22.36
23	5.53	8.16	10.92	14.83	14.87	22.12	17.05	23.81
24	4.79	6.01	11.51	15.94	18.37	18.28	21.24	22.13
25	4.87	6.77	7.91	10.48	13.68	19.83	15.82	26.57
26	5.57	8.20	8.07	10.73	14.87	15.99	23.17	17.55
27	4.70	6.83	6.98	8.86	16.02	18.41	16.28	23.38
28	5.51	7.79	10.40	14.73	16.47	13.51	23.70	25.25
29	5.58	8.04	10.83	15.53	14.21	20.40	19.11	22.89
30	4.77	6.05	9.81	13.55	12.98	17.11	20.33	17.22
31	4.97	6.25	10.25	14.09	19.42	20.97	14.92	25.99
32	5.78	8.83	10.92	14.61	11.29	22.55	20.22	23.34
33	5.83	8.44	11.09	14.49	18.91	14.82	25.38	22.08
34	5.32	8.12	10.85	12.56	18.91	18.10	17.10	25.18
35	4.87	5.72	8.97	13.62	18.90	18.06	18.00	19.83
36	4.48	5.93	10.32	12.16	15.55	22.65	19.73	22.66
37	4.96	7.06	9.87	14.71	18.86	14.60	24.77	27.09
38	5.39	7.57	10.86	15.33	19.63	15.40	14.61	16.77
39	5.66	7.71	11.84	12.05	18.63	18.44	20.36	23.48
40	5.58	7.69	9.21	12.02	19.61	18.90	22.65	26.32
41	4.47	5.84	9.78	11.48	16.43	19.00		
42	5.89	7.88	8.97	12.35	20.45	15.15		
43	5.78	7.95	8.73					
44	5.17	7.55	9.85					
45	4.77	6.23						
Mean	5.13	7.09	9.71	12.70	16.20	18.51	20.52	22.45
SE	0.07	0.13	0.2	0.32	0.36	0.44	0.50	0.53

APPENDIX 9. Shell length in millimetres of the juvenile abalone *H. tuberculata* fed on the commercial (CO) diet and reared at 22°C.

Number of organism	March	April	May	June	July	August	September	October
1	5.84	6.91	11.44	13.69	19.53	23.20	23.41	24.62
2	4.49	8.51	10.45	15.09	19.97	20.53	24.80	26.85
3	5.08	7.22	13.25	11.98	19.71	16.93	21.55	23.38
4	5.59	7.88	9.55	16.75	20.05	23.65	22.27	24.01
5	4.57	6.75	10.05	14.36	17.02	22.77	23.36	25.13
6	4.88	8.79	10.80	14.15	17.85	21.34	22.66	24.77
7	5.51	7.55	9.45	9.88	17.29	22.78	22.97	25.20
8	5.33	8.22	9.52	15.94	18.39	16.63	21.88	23.56
9	5.01	7.36	10.87	13.95	17.48	16.84	24.49	26.98
10	4.85	6.88	11.51	14.66	20.87	21.81	20.40	22.07
11	4.70	7.22	12.33	15.99	19.11	20.89	24.05	25.82
12	5.11	7.58	12.53	12.04	18.28	18.85	25.44	27.70
13	4.56	7.52	9.75	16.80	15.72	19.64	19.98	22.64
14	4.48	6.96	9.77	14.44	18.79	22.80	22.06	23.23
15	4.66	7.53	9.88	14.50	15.17	22.04	22.80	24.92
16	4.92	7.45	8.98	14.73	17.83	21.90	23.22	25.05
17	5.85	8.06	11.86	15.30	15.37	20.07	21.01	22.74
18	5.85	6.96	12.15	17.33	18.91	18.79	23.78	25.09
19	5.21	7.11	10.44	16.07	17.22	22.23	22.74	24.53
20	4.86	7.21	10.24	15.25	16.77	18.88	17.83	19.78
21	5.57	6.94	10.78	13.27	20.59	21.44	25.00	26.46
22	4.92	7.94	11.59	15.52	18.79	17.99	19.70	19.70
23	4.78	5.98	7.82	16.87	15.44	20.05	18.62	19.88
24	5.10	6.91	8.57	12.40	20.33	16.62	21.11	22.73
25	5.36	6.39	10.98	14.70	16.83	21.31	26.07	28.35
26	4.88	7.94	10.18	10.25	16.20	22.17	18.61	20.40
27	5.33	5.84	10.73	13.44	18.01	13.91	20.75	22.11
28	5.25	6.72	7.44	15.06	17.58	22.74	23.44	25.68
29	4.55	7.58	10.93	14.69	17.44	15.21	17.95	18.93
30	4.92	6.98	11.22	12.13	19.88	18.92	15.16	16.83
31	4.82	6.71	10.56	15.34	16.52	20.22	24.81	26.87
32	5.78	7.81	10.28	12.37	18.16	20.78	19.71	21.22
33	5.06	7.76	10.23	14.58	15.36	18.85	18.76	20.44
34	5.58	6.59	10.77	13.87	18.78	16.88	20.50	22.04
35	5.39	7.22	12.36	15.83	12.92	20.69	24.33	26.28
36	4.88	7.43	9.78	14.51	16.94	19.70	16.88	18.03
37	4.49	7.25	9.44	13.21	17.60	19.23	22.37	24.20
38	4.82	7.18	10.99	12.25	13.11	20.95	20.73	21.66
39	4.79	6.88	10.66	13.06	17.83	18.43	25.54	26.44
40	5.67	7.09	9.36	16.71	21.05	21.20	24.98	23.85
41	5.97	5.35	10.36	16.97	16.55	19.38	20.33	22.17
42	4.89	8.55	12.55	13.18	19.56	21.35		
43	5.23	7.94	10.76	15.73	16.94			
44	4.44	6.78	10.84	13.98				
45	5.28	5.95						
Mean	5.09	7.23	10.55	14.38	17.76	20.01	21.85	23.47
SE	0.06	0.11	0.18	0.26	0.29	0.35	0.41	0.43

APPENDIX 10. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the seaweed (SW) diet and reared at 15°C during 210 days during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	17.3	62.4	29.9	144.2	80.4	125.7	449.2	228.4
2	18.5	18.0	48.1	61.4	55.3	112.5	81.8	241.2
3	26.8	21.5	51.7	38.5	92.3	377.2	141.5	494.2
4	29.2	43.3	30.7	80.1	131.0	269.1	243.7	367.0
5	24.0	26.7	49.0	57.9	208.8	73.0	172.9	485.8
6	29.3	33.6	38.8	95.3	136.0	169.9	358.6	182.9
7	28.8	20.1	50.3	118.4	265.3	206.1	344.9	505.3
8	19.1	28.7	24.8	50.0	62.9	91.5	437.0	138.7
9	24.8	43.2	85.0	86.5	107.9	105.8	115.3	144.8
10	17.8	32.0	121.3	53.2	60.6	374.2	436.5	101.0
11	25.2	34.2	30.9	40.9	78.9	64.0	178.9	878.2
12	21.2	29.1	49.7	172.1	162.1	407.2	347.0	440.7
13	26.3	40.9	59.2	49.3	63.2	120.0	139.0	198.1
14	21.6	25.4	57.1	178.2	97.0	196.7	122.6	245.9
15	16.1	23.8	37.6	63.9	84.6	128.5	164.5	169.9
16	19.4	28.8	36.5	39.4	169.3	121.9	249.1	324.0
17	21.5	22.8	101.8	76.4	120.7	288.5	591.0	748.6
18	20.1	17.3	50.7	59.1	118.9	125.4	115.4	560.0
19	26.8	29.8	67.4	135.0	88.3	134.0	138.0	250.8
20	15.2	31.7	28.9	64.3	73.2	64.6	262.8	94.2
21	28.9	25.0	37.2	148.7	90.7	210.5	270.1	341.7
22	24.8	38.5	38.5	47.4	62.3	104.2	429.0	562.3
23	27.2	50.5	63.0	80.3	47.0	131.3	121.1	207.0
24	23.1	31.9	26.6	54.9	132.7	58.9	129.5	292.1
25	29.0	26.3	38.5	118.2	64.5	157.4	72.9	221.0
26	25.3	42.9	80.1	122.1	239.8	430.8	139.2	147.7
27	16.6	43.8	40.8	73.2	124.9	369.2	130.4	221.2
28	19.6	28.3	29.3	35.5	205.2	144.1	384.3	126.3
29	18.3	27.5	49.5	54.4	80.7	112.6	278.4	304.9
30	25.8	33.1	78.7	44.9	91.1	213.1	236.0	406.9
31	20.9	31.5	100.6	59.2	64.8	160.1	109.1	242.9
32	25.9	19.1	53.7	171.5	161.0	241.1	452.4	175.9
33	22.2	22.4	62.3	75.5	242.2	117.3	196.1	585.0
34	22.6	33.2	49.5	77.4	236.8	121.6	163.5	491.5
35	25.4	22.0	34.5	42.4	92.9	128.8	221.8	236.5
36	14.9	26.9	36.0	120.5	127.4	59.5	117.6	320.8
37	21.3	52.1	36.1	116.3	87.5	351.9	276.5	270.0
38	24.2	28.5	61.8	60.0	58.2	222.3	105.9	301.8
39	29.4	44.3	27.2	65.3	169.7	69.7	172.0	159.2
40	20.0	34.5	21.4	85.8	116.5	125.2	381.0	138.7
41	26.4	33.0	29.1	48.2	81.3	88.7	98.4	
42	19.5	29.2	48.9	42.3	52.4	132.4	294.9	
43	30.0	21.0	68.6					
44	26.1	49.8						
45	15.9	34.9						
Mean	22.9	32.1	50.3	81.2	116.3	174.0	235.0	313.8
SE	0.7	1.5	3.4	60.1	9.0	15.8	19.8	28.4

APPENDIX 11. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the seaweed (SW) diet and reared at 18°C during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	19.3	69.4	64.4	310.8	248.3	552.5	1048.0	438.6
2	21.2	28.2	148.3	70.2	427.9	281.1	744.2	864.2
3	29.4	40.7	57.7	160.4	211.1	604.2	296.5	562.3
4	24.1	27.6	36.9	141.6	365.5	327.5	448.2	568.7
5	28.4	30.9	46.3	52.1	187.3	142.4	239.1	241.8
6	29.3	24.8	83.8	115.9	77.9	218.6	471.4	580.0
7	24.8	35.5	57.4	214.8	153.2	378.1	432.2	744.3
8	25.6	34.0	44.1	89.7	239.5	260.2	551.9	507.5
9	22.5	32.9	64.2	140.4	486.0	239.0	497.5	1285.0
10	15.2	28.7	128.9	122.5	139.8	425.1	409.3	312.4
11	26.7	37.3	61.8	93.5	250.4	364.1	242.8	421.9
12	20.3	68.6	148.0	236.0	121.8	133.9	388.9	571.2
13	25.3	50.2	34.3	52.9	74.5	120.0	201.0	370.0
14	19.2	19.1	62.3	108.0	110.7	244.3	274.4	574.5
15	15.2	55.3	94.7	149.3	164.3	243.1	453.2	642.1
16	16.3	18.3	141.2	353.2	118.5	612.9	847.4	1249.0
17	25.0	24.9	57.3	125.5	215.7	321.7	244.3	445.7
18	28.8	39.8	72.2	83.0	194.6	387.3	371.4	702.9
19	27.7	37.0	51.8	119.3	161.3	157.8	1122.0	485.3
20	16.5	32.6	69.0	248.5	447.3	339.0	609.2	1395.0
21	29.1	63.8	128.3	79.4	245.2	127.9	179.3	578.5
22	23.4	22.3	157.5	140.8	128.6	169.4	442.0	869.3
23	24.1	35.4	79.2	74.3	243.0	351.0	369.6	595.4
24	21.8	20.5	43.0	188.4	386.2	258.2	552.2	449.2
25	27.0	29.0	41.0	42.2	160.3	279.2	306.4	406.5
26	22.2	40.5	28.0	59.0	95.1	536.4	261.5	1212.0
27	26.1	58.3	45.1	103.1	241.4	441.0	439.0	248.9
28	25.4	70.3	61.0	83.4	122.2	329.1	464.5	388.2
29	20.9	44.1	101.3	151.6	79.1	222.4	371.9	491.2
30	21.2	34.8	66.0	229.4	228.1	345.3	457.1	722.9
31	15.6	35.1	50.1	295.1	182.4	538.0	198.4	841.1
32	19.5	61.1	133.2	50.5	479.6	261.5	335.2	475.8
33	23.8	27.4	38.3	249.3	205.1	194.0	578.2	496.0
34	28.3	51.4	166.6	148.1	132.1	174.0	942.0	604.3
35	20.9	35.9	67.9	78.6	401.9	225.1	234.1	359.5
36	30.1	41.2	107.4	117.1	154.3	761.9	778.0	438.3
37	26.5	47.6	34.6	112.9	247.0	117.2	351.2	266.0
38	21.4	33.3	74.3	109.4	188.7	266.3	412.3	371.9
39	17.5	81.7	48.9	43.8	115.6	152.8	466.9	1009.0
40	29.8	26.6	56.3	70.6	119.4	426.6	218.5	1155.0
41	25.9	37.3	101.5	129.8	334.3	459.0	435.0	569.0
42	22.8	41.6	73.0	190.1	107.9	269.7	420.8	453.0
43	16.6	22.4	57.1	121.0	81.8			
44	25.0	30.2	77.3	175.5	396.5			
45	20.6	23.4	35.9	120.4				
Mean	23.2	38.9	75.5	136.7	215.3	315.7	454.9	619.4
SE	0.6	2.3	5.6	11.0	17.4	23.4	34.6	44.8

APPENDIX 12. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the seaweed (SW) diet and reared at 22°C during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	20.2	36.9	71.2	154.2	397.0	383.1	854.2	568.2
2	14.9	28.7	43.1	86.4	153.7	551.5	449.8	724.5
3	24.2	29.5	168.9	88.1	121.5	158.9	371.5	1801.0
4	26.5	50.1	67.3	210.4	234.1	349.5	579.1	485.2
5	19.1	30.4	38.5	124.4	209.1	247.7	327.9	1382.0
6	21.4	31.0	62.8	203.0	246.9	326.6	342.3	559.5
7	25.7	49.9	136.9	83.3	97.2	250.2	878.4	430.1
8	27.1	63.2	94.2	234.5	179.5	382.7	388.1	579.1
9	16.8	33.2	42.0	63.6	449.2	207.0	953.4	1844.0
10	20.6	69.5	65.3	133.2	242.9	629.5	507.6	928.6
11	29.2	41.3	89.3	85.0	381.8	498.4	522.6	593.4
12	27.9	28.4	66.4	133.9	140.0	210.8	691.0	462.7
13	30.1	43.4	43.0	230.9	420.9	412.3	443.1	729.0
14	28.7	19.0	61.1	158.2	251.4	849.1	271.0	995.4
15	26.2	29.1	106.2	128.1	228.3	231.7	599.5	448.3
16	19.1	35.2	76.3	228.2	248.3	558.4	404.2	1259.0
17	209	61.0	37.2	122.8	112.8	266.3	421.7	563.6
18	18.6	30.6	44.9	160.9	374.5	550.0	396.5	1109.0
19	28.7	75.3	63.6	394.6	125.9	442.9	739.4	721.7
20	28.9	22.7	159.8	137.3	188.6	230.2	774.1	1314.0
21	30.1	32.2	87.4	80.5	169.0	358.8	368.9	951.9
22	20.9	41.9	76.0	85.3	384.2	196.0	579.0	478.5
23	26.1	32.6	100.4	60.3	489.4	254.1	918.1	1069.0
24	19.1	59.3	41.8	123.0	139.7	301.7	415.3	461.2
25	18.6	40.3	63.4	76.7	425.0	759.1	389.6	645.1
26	22.3	26.0	94.9	120.5	241.1	397.4	856.7	579.5
27	26.7	38.9	49.2	161.2	380.7	408.2	525.3	1344.0
28	28.6	43.3	38.4	130.6	122.4	581.7	1207.0	592.9
29	26.2	24.7	126.0	160.1	237.4	631.0	294.2	841.2
30	28.6	43.8	81.3	222.1	246.2	482.6	571.5	1112.0
31	20.9	61.6	179.2	63.8	420.8	325.7	873.8	585.0
32	27.8	31.3	90.2	151.9	237.6	261.1	349.8	736.3
33	14.4	20.7	36.5	120.5	101.8	575.5	724.7	1094.0
34	14.6	44.7	121.7	269.5	135.9	160.0	393.0	426.0
35	28.3	41.9	45.3	81.7	128.8	428.8	480.0	1206.0
36	20.5	40.4	39.5	157.1	399.8	590.3	590.2	1283.0
37	29.4	43.3	58.9	131.3	377.0	389.1	455.4	494.2
38	21.4	23.2	53.9	211.5	490.4	401.4	342.6	699.3
39	24.9	35.1	69.3	165.8	85.7	165.4	856.2	643.0
40	25.6	50.2	74.3	123.2	224.1	258.7	922.0	553.8
41	21.0	22.2	40.1	82.1	140.3	409.9	563.0	401.3
42	19.6	32.3	42.9	80.9	269.4	330.6		
43	29.7	30.8	112.7	125.0				
44	18.3	57.5	104.2					
45	28.7	33.2	78.4					
Mean	23.7	39.1	76.5	142.9	253.6	390.6	575.4	821.8
SE	0.7	2.0	5.4	10.1	18.7	25.5	35.2	57.3

APPENDIX 13. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the fishmeal (FM) diet and reared at 15°C during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	21.8	37.4	53.3	108.8	118.7	130.5	146.3	183.9
2	28.9	31.5	55.0	49.9	141.9	268.8	439.8	577.4
3	18.2	32.7	41.4	94.2	202.5	184.9	272.2	490.8
4	18.8	40.8	73.2	117.4	101.5	181.4	241.6	346.0
5	19.9	39.1	64.6	65.0	178.8	145.2	378.4	746.2
6	24.1	30.9	43.2	72.8	226.0	142.0	569.3	566.2
7	25.4	31.6	54.0	136.3	117.3	235.7	220.0	509.4
8	21.6	33.4	86.6	110.0	141.4	156.8	193.0	840.3
9	22.3	27.2	61.1	78.4	145.5	260.2	440.1	394.2
10	20.9	56.4	59.8	159.9	133.9	125.3	365.2	439.6
11	23.2	32.1	56.0	70.2	151.9	369.0	580.4	321.2
12	25.4	29.8	90.2	59.3	246.5	375.2	371.2	268.7
13	23.8	21.7	29.2	121.1	99.4	497.8	201.5	555.2
14	21.7	32.0	59.4	126.0	120.8	144.1	179.4	245.8
15	23.1	32.5	84.1	114.9	147.3	226.4	388.3	241.6
16	20.2	29.5	56.2	60.3	164.9	121.2	437.1	390.1
17	18.6	38.7	60.7	169.1	118.5	201.1	472.4	599.1
18	26.4	32.2	72.9	66.4	139.6	389.6	244.3	644.0
19	22.8	21.5	43.4	74.3	224.8	128.4	301.5	306.3
20	19.1	31.9	40.9	154.0	98.7	268.4	189.7	390.9
21	20.0	35.2	41.4	50.5	92.7	157.9	562.3	233.6
22	25.2	49.8	55.5	81.0	172.9	210.5	436.2	164.9
23	19.2	24.8	36.3	119.3	140.4	352.3	273.0	548.4
24	29.4	30.8	57.2	83.9	143.1	178.0	331.8	439.6
25	25.2	32.2	63.3	122.7	236.9	418.6	428.4	560.3
26	21.0	34.2	68.0	157.9	127.2	279.4	349.6	875.0
27	22.7	33.6	58.5	75.8	249.2	431.1	155.2	469.0
28	22.4	41.6	67.3	81.7	142.6	144.9	188.9	548.1
29	20.9	40.0	64.2	129.2	118.6	395.9	540.3	236.6
30	24.5	29.2	72.4	112.1	162.1	334.5	249.1	328.2
31	20.5	34.3	41.8	125.2	121.3	212.5	451.3	381.0
32	21.2	29.9	100.2	58.2	148.3	136.9	211.6	265.8
33	26.5	33.1	60.9	101.5	89.8	260.8	148.0	582.9
34	29.1	27.9	35.4	162.8	81.9	117.2	306.8	742.4
35	21.9	45.3	57.0	73.0	240.1	195.1	235.0	402.6
36	21.3	32.9	37.5	56.8	177.2	162.4	369.6	250.3
37	25.8	32.3	42.4	141.1	257.0	165.0	225.0	488.2
38	20.8	35.0	53.9	53.7	169.4	266.8	519.4	492.0
39	27.4	40.9	56.4	112.4	152.8	251.5	318.6	433.4
40	22.3	23.9	59.1	79.4	116.0			
41	22.0	30.8	41.3					
42	22.2	38.5	91.3					
43	24.1	33.0						
44	26.6	29.2						
45	18.2	37.1						
Mean	22.8	33.7	58.2	99.7	154.0	239.1	331.6	448.8
SE	0.4	1.0	2.5	5.5	7.6	16.3	20.6	28.2

APPENDIX 14. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the fishmeal (FM) diet and reared at 18°C during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	27.7	40.6	80.5	561.2	692.4	392.7	478.9	3421.0
2	18.3	96.1	54.2	287.0	784.0	693.5	1189.1	2905.0
3	17.6	44.2	122.7	294.0	562.7	884.6	805.2	2409.0
4	26.4	34.9	124.9	252.5	471.3	1292.2	1817.0	894.2
5	20.1	72.4	119.2	378.0	456.8	2059.1	639.4	1486.0
6	28.2	40.8	75.2	354.8	810.6	543.7	1129.0	1979.0
7	21.9	37.3	261.7	236.1	539.4	849.5	2191.0	1501.0
8	24.6	26.4	194.1	129.1	262.0	1674.8	1278.0	2939.0
9	22.7	62.7	140.3	248.1	1204.5	1262.0	1564.0	991.6
10	22.9	49.2	19.5	299.1	531.0	665.0	1271.0	589.1
11	21.8	43.5	196.3	218.5	1007.1	833.4	2744.0	2944.0
12	26.4	29.0	120.9	260.4	545.2	959.1	1398.0	1801.0
13	25.9	33.8	115.7	248.9	233.5	1207.0	2235.0	1223.0
14	28.1	38.9	120.1	122.3	402.5	795.0	867.1	1719.0
15	29.8	50.2	56.9	305.5	525.1	972.8	669.2	1953.0
16	19.5	43.5	255.2	269.2	431.5	369.8	1059.0	1394.0
17	21.3	68.4	129.5	325.9	588.8	822.5	1206.0	2702.0
18	23.6	40.3	187.3	154.3	1159.0	679.0	1892.0	2219.0
19	26.6	52.9	66.5	248.5	546.6	1384.6	572.1	955.0
20	14.6	38.7	101.8	300.5	464.2	498.7	2371.0	1468.0
21	25.5	29.7	106.9	185.0	854.9	2128.0	1105.0	840.3
22	24.1	31.0	64.6	292.2	872.5	883.3	1417.0	3120.0
23	29.0	36.5	92.2	222.1	243.2	1845.0	1880.0	1759.0
24	27.2	42.8	120.3	449.8	605.8	896.9	1223.0	1278.0
25	27.2	83.1	107.6	260.8	798.2	440.1	644.1	2904.0
26	24.4	45.2	52.5	398.5	529.2	868.2	1835.0	709.5
27	26.0	43.7	107.1	550.0	1220.8	1249.1	1773.0	3004.0
28	25.4	61.3	121.7	119.4	232.7	776.6	881.5	861.9
29	22.8	39.8	111.0	309.2	429.1	1401.5	928.2	1147.0
30	20.3	52.4	201.8	201.8	512.1	1879.0	949.3	3195.0
31	24.2	44.3	115.2	271.5	958.9	842.4	1866.0	3019.0
32	21.2	37.0	187.4	162.1	539.2	404.5	1490.0	1488.0
33	19.9	27.5	229.8	264.0	1118.4	1649.0	2855.0	2264.0
34	20.8	62.4	117.7	362.4	237.0	855.6	1336.0	1453.0
35	24.0	44.3	109.4	218.9	555.3	402.0	1715.0	3482.0
36	22.4	48.3	92.1	241.1	534.9	1417.0	502.0	2718.0
37	23.6	43.8	50.3	475.1	475.0	675.3	2348.0	966.0
38	27.1	87.2	67.2	241.0	1311.3	684.7	2563.0	3477.0
39	26.4	44.8	210.3	499.5	562.4	955.4	1192.0	1398.0
40	24.9	36.5	90.2	289.3	569.2	772.1	1508.0	2349.0
41	29.1	88.2	94.6	249.6	551.3	1269.0	998.7	816.1
42	23.5	60.0	120.7	318.8	874.3	647.8	559..2	1445.0
43	24.8	35.9	118.5	229.0	232.2	1070.0		
44	21.4	39.2	69.5	125.0	398.4	1801.5		
45	28.9	41.8	132.6	401.8				
Mean	24.0	47.8	120.7	285.1	623.5	1014.8	1406.5	1933.0
SE	0.5	2.5	8.2	15.7	42.9	70.6	127.3	136.0

APPENDIX 15. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the fishmeal (FM) diet and reared at 22°C during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	23.4	31.7	119.5	347.6	596.5	2475.1	969.4	1159.0
2	21.9	42.2	224.4	654.2	258.7	1469.8	1788.0	2017.0
3	29.7	58.8	54.5	729.4	984.3	687.4	2791.0	1278.0
4	24.9	40.5	139.6	341.0	1609.5	403.8	2954.0	3429.0
5	30.1	49.3	218.8	431.4	497.4	1207.6	967.0	3217.0
6	28.8	83.8	126.2	356.3	605.0	948.7	3324.0	2595.0
7	24.3	65.4	107.1	133.8	598.3	812.5	1839.0	2328.0
8	21.7	41.8	143.1	360.0	614.3	563.3	3018.0	1445.0
9	27.7	28.8	209.4	478.1	1328.2	654.1	1480.0	842.2
10	29.7	70.4	296.2	132.8	269.9	822.2	1351.0	668.4
11	22.5	39.0	248.2	685.2	1491.0	1839.3	601.6	2352.0
12	19.6	38.1	181.5	504.2	1135.9	1761.5	776.0	1392.0
13	29.4	46.3	139.5	354.9	1098.5	1405.0	1785.0	2766.0
14	29.0	41.4	209.0	549.0	450.2	877.5	579.1	3335.0
15	23.8	61.9	142.8	249.5	467.2	2588.4	812.8	1458.0
16	18.0	38.8	296.4	651.3	573.1	943.7	2193.0	2414.0
17	29.3	29.4	192.9	253.2	922.4	2428.0	985.3	959.2
18	26.5	70.0	81.3	249.2	569.5	744.3	2245.0	1479.0
19	29.1	85.4	117.9	364.4	442.9	1495.0	2917.0	1719.0
20	16.7	34.0	125.6	694.1	866.1	402.8	1169.0	2933.0
21	21.6	29.1	253.1	381.8	1351.4	802.6	861.5	2309.0
22	30.1	36.8	181.4	452.4	269.9	1766.1	488.3	1548.0
23	28.3	61.5	140.0	661.8	1210.0	956.0	1502.0	692.5
24	18.1	57.8	210.8	329.9	1507.0	1815.1	2369.0	1235.0
25	26.2	42.2	266.3	215.0	590.4	742.8	952.4	3418.0
26	19.0	83.2	78.8	249.5	428.8	2297.0	1395.0	1596.0
27	29.4	50.9	129.9	142.3	934.1	776.0	1166.0	3182.0
28	22.2	85.8	173.8	559.4	467.9	536.5	1844.0	2277.0
29	21.9	40.3	90.4	337.2	691.5	1136.9	2449.0	992.2
30	18.9	45.9	239.5	485.3	468.2	762.2	2845.0	1724.0
31	24.6	26.7	143.0	661.2	1189.1	2899.0	1391.0	1468.0
32	22.5	35.1	116.8	432..2	707.0	967.5	957.0	771.6
33	27.3	90.3	155.2	235.5	1571.5	2149.0	1805.0	3667.0
34	29.0	37.5	196.4	430.9	580.5	1829.0	536.0	2807.0
35	28.5	48.5	109.2	456.8	1617.4	1480.0	943.0	1415.0
36	24.5	46.3	273.0	375.2	569.8	849.0	744.6	959.7
37	20.9	32.6	192.4	307.4	1385.1	821.7	2602.0	2905.0
38	30.1	41.8	224.0	672.0	245.0	1644.0	1314.0	2577.0
39	18.1	60.2	169.2	185.6	620.1	781.2	3290.0	2490.0
40	20.9	65.7	216.8	454.8	408.4	420.4	1259.0	1699.0
41	28.3	50.8	89.4	306.9	1008.7	432.9	2518.0	2133.0
42	26.3	61.4	133.0	530.2	805.0	1107.0	1107.0	1241.0
43	20.0	55.2	162.4	640.0	829.2	2248.0	996.7	1085.0
44	18.2	72.9	127.7	315.0	510.1	968.8		
45	23.4	30.6	163.2	393.2				
Mean	24.5	50.8	169.1	416.2	803.3	1243.6	1625.1	1953.0
SE	0.6	2.6	90.	24.8	61.4	101.1	127.3	131.1

APPENDIX 16. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the commercial (CO) diet and reared at 15°C during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	17.2	31.5	42.2	85.8	197.9	139.2	353.8	391.6
2	25.9	23.2	65.3	114.3	123.1	209.0	246.3	332.5
3	21.1	36.4	58.9	93.8	141.9	161.5	107.3	257.2
4	22.5	39.5	39.1	59.0	205.4	210.9	372.4	581.8
5	22.8	28.7	89.7	72.1	108.6	139.3	265.0	448.3
6	21.9	42.9	38.5	95.5	136.4	396.7	312.3	466.2
7	23.4	34.3	85.0	60.1	93.8	245.3	243.6	267.9
8	22.4	35.5	68.1	68.2	61.1	164.5	427.4	512.0
9	21.2	40.3	38.8	133.9	122.3	196.2	218.7	328.5
10	26.4	41.9	43.1	83.9	130.0	189.0	245.1	398.5
11	22.7	28.6	63.9	127.7	124.7	197.1	573.3	375.7
12	25.9	30.5	45.2	88.0	135.2	80.2	194.3	328.1
13	27.1	27.2	48.0	96.5	122.1	369.4	251.6	210.7
14	23.1	26.0	27.3	136.7	184.0	237.6	268.1	571.1
15	28.9	43.1	54.7	118.5	144.2	425.8	502.2	316.7
16	18.6	37.4	52.5	90.8	242.9	249.8	339.5	444.1
17	19.7	32.1	56.0	133.3	124.7	164.3	165.5	621.2
18	20.5	36.0	87.5	56.1	93.5	161.9	201.4	407.9
19	28.5	30.1	53.9	74.1	259.8	259.6	353.2	368.6
20	28.7	43.6	100.1	154.9	101.5	199.4	215.8	281.9
21	27.3	27.3	59.6	78.1	140.5	435.1	184.6	663.6
22	20.2	38.2	36.3	87.9	133.2	184.0	281.5	248.6
23	18.5	38.9	53.2	73.5	109.7	166.2	165.3	388.2
24	18.3	34.3	65.3	96.1	115.7	157.0	231.5	201.8
25	27.2	49.8	58.2	54.7	122.0	117.2	387.1	292.5
26	16.9	29.0	52.3	71.2	114.7	121.7	521.4	432.0
27	28.9	29.0	38.0	70.0	124.5	352.3	262.5	378.3
28	18.6	32.5	56.9	140.5	163.2	122.8	251.4	332.6
29	19.4	38.3	70.0	69.1	120.6	369.3	479.2	259.6
30	26.9	26.2	58.4	87.8	92.5	181.0	262.8	270.6
31	22.7	26.7	71.3	86.4	132.8	203.6	232.1	595.0
32	20.0	34.2	53.2	124.4	139.5	221.1	169.7	330.9
33	23.6	27.9	72.7	71.2	94.1	148.4	259.1	381.0
34	21.9	31.6	40.3	97.5	130.8	157.3	158.4	385.8
35	20.9	38.2	53.3	122.3	119.8	266.1	379.3	581.4
36	26.4	45.1	50.5	86.6	96.64	172.9	481.1	802.9
37	20.5	34.9	59.1	70.2	208.0	431.5	378.4	441.3
38	30.1	27.6	49.9	85.8	181.1	111.5	355.0	239.6
39	26.7	43.3	58.6	84.3	152.9	187.2	228.9	466.4
40	21.4	28.2	82.5	72.1	130.3	192.8	161.3	
41	22.6	27.1	72.6					
42	21.2	25.9	42.4					
43	21.1	42.2	96.8					
44	20.4	43.3						
45	25.9	35.3						
Mean	23.0	34.3	58.3	91.8	136.9	217.4	291.8	400.3
SE	0.5	1.0	2.6	4.1	6.4	14.8	17.7	21.8

APPENDIX 17. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the commercial (CO) diet and reared at 18°C during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	19.2	51.1	86.8	392.4	249.7	1269.0	892.8	561.8
2	28.8	27.3	51.1	301.5	744.2	557.2	1294.0	2948.0
3	25.4	42.0	80.3	234.2	272.0	1285.0	1814.0	1495.0
4	23.9	52.7	87.5	217.5	560.9	822.4	953.2	2378.0
5	23.8	25.5	109.9	73.5	572.4	562.7	605.0	851.0
6	21.2	36.8	150.8	110.2	469.3	749.8	478.4	1207.0
7	25.6	64.9	49.5	271.9	485.0	302.6	1824.0	2699.0
8	26.4	29.9	153.1	218.6	433.5	1015.0	947.7	1321.0
9	21.4	37.0	178.9	109.5	739.1	1136.0	1183.0	1519.0
10	22.7	31.5	53.0	485.1	282.7	407.2	1514.0	2384.0
11	26.8	80.3	133.0	357.4	831.4	644.4	401.6	1562.0
12	21.0	28.4	126.5	406.2	420.9	833.7	1826.0	902.6
13	27.1	54.4	148.8	182.1	1109.3	422.1	629.7	939.7
14	22.8	57.2	196.6	243.3	498.9	802.2	1795.0	2978.0
15	23.3	52.5	107.4	178.0	604.1	1172.0	779.5	944.3
16	23.0	48.8	101.3	123.0	159.8	1282.0	1139.0	2691.0
17	20.9	26.4	140.9	130.8	251.1	936.5	1841.0	1678.0
18	22.2	43.1	200.1	261.5	437.4	1489.0	1150.0	1407.0
19	26.5	27.6	165.3	388.5	562.3	379.6	1784.0	2985
20	19.8	31.5	50.7	390.2	446.7	554.3	2296.0	1227.0
21	23.1	41.3	52.3	434.2	418.3	1318.0	489.2	805.2
22	24.3	39.4	75.9	80.0	375.8	659.3	883.4	2776.0
23	30.0	44.7	39.7	406.3	172.3	494.5	955.0	598.4
24	16.3	78.2	155.3	287.4	823.0	729.6	1496.0	2629.0
25	27.1	63.1	152.6	385.0	869.9	1482.0	564.3	1471.0
26	19.1	56.3	150.4	211.2	810.4	391.7	382.0	1366.0
27	26.9	32.2	83.9	430.8	870.6	431.2	1419.0	2243.0
28	20.2	61.9	137.3	209.4	512.7	831.2	671.2	294.6
29	27.3	28.4	110.4	385.9	442.8	922.7	1249.0	2298.0
30	28.9	54.4	104.5	229.7	569.5	464.3	432.3	604.2
31	27.9	59.1	160.2	448.6	430.0	1218.0	1480.0	2452.0
32	14.3	51.5	107.0	400.2	473.8	305.7	1277.0	573.3
33	22.5	33.8	141.5	87.2	389.0	441.1	1276.0	1134.0
34	20.9	34.0	64.1	92.4	579.2	1229.0	1849.0	1311.0
35	24.1	52.8	144.5	386.0	242.9	317.6	566.4	490.2
36	22.6	33.2	152.0	132.7	436.8	769.4	471.2	1397.0
37	27.6	43.9	58.3	138.5	491.1	596.1	1789.0	1783.0
38	21.0	37.5	153.0	396.5	523.5	1517.0	893.2	1459.0
39	22.3	61.2	66.3	220.1	266.4	910.6	1809.0	1622.0
40	29.2	68.5	148.4	310.1	857.9	555.2	1018.0	2735.0
41	28.1	43.2	109.3	215.9	832.7	1336.0		
42	21.6	58.4	94.2	224.8	812.4	752.3		
43	27.8	57.1	88.8					
44	15.7	27.8	80.8					
45	29.3	30.7						
Mean	23.8	45.4	113.7	266.6	531.7	816.6	1152.9	1625.5
SE	0.6	2.2	6.5	19.0	34.4	55.8	83.0	122.6

APPENDIX 18. Body wet weight in milligrams of the juvenile abalone *H. tuberculata* fed on the commercial (CO) diet and reared at 22°C during 210 days.

Number of Organism	March	April	May	June	July	August	September	October
1	19.9	36.4	117.2	586.3	947.5	947.9	1821.0	1147.0
2	22.2	57.2	139.8	415.4	559.3	568.2	1232.0	3195.0
3	23.2	81.3	221.3	365.0	590.8	1196.1	1528.0	3019.0
4	18.3	44.2	123.1	549.1	742.9	570.0	1452.0	1488.0
5	25.2	45.4	114.9	133.5	434.5	1183.5	2328.0	2264.0
6	29.1	47.1	141.8	355.1	763.5	869.5	1405.0	1453.0
7	28.0	40.6	57.4	331.5	524.4	1475.3	624.3	3482.0
8	28.7	60.2	219.0	370.6	751.3	814.7	876.1	2718.0
9	26.3	41.2	187.0	375.0	957.4	1305.1	1162.0	966.0
10	28.0	62.7	106.3	483.3	547.0	342.1	2226.0	3477.0
11	23.9	48.9	134.2	215.9	581.2	825.4	1022.0	1398.0
12	29.4	46.6	168.0	210.7	243.5	981.6	621.6	2349.0
13	26.7	42.1	74.3	580.7	1162.0	1009.4	986.0	1445.0
14	27.2	23.8	219.7	451.3	518.9	1225.0	1827.0	2057.0
15	26.1	40.5	139.3	112.0	912.6	1492.0	1519.0	1668.0
16	30.1	45.2	122.8	384.3	575.4	1439.0	1455.0	2273.0
17	25.1	39.7	109.9	551.7	704.9	575.1	1306.0	2902.0
18	18.2	51.4	209.1	373.1	584.0	847.4	1946.0	1310.0
19	26.2	49.9	98.3	380.9	1139.0	1285.0	1548.0	2397.0
20	29.4	61.3	96.5	430.4	644.2	994.1	2278.0	2004.0
21	22.8	42.3	217.4	572.5	774.2	825.8	759.8	915.8
22	25.6	46.1	65.3	449.3	481.3	1207.0	1618.3	1105.0
23	23.2	33.8	170.4	220.3	458.0	641.2	811.6	2575.0
24	25.5	29.1	120.9	418.0	1005.2	1420.0	593.0	634.2
25	24.7	40.8	180.7	200.8	589.4	444.7	1319.0	1724.0
26	28.9	41.3	189.0	369.1	762.8	990.5	1021.0	1345.0
27	23.5	61.2	98.6	380.8	592.1	918.0	966.4	2977.0
28	16.2	43.8	192.5	462.2	268.5	1174.3	1335.0	1814.0
29	17.7	41.7	131.9	321.0	589.2	1728.0	978.0	2224.0
30	26.1	81.3	140.2	370.1	849.2	1449.0	1812.0	3418.0
31	27.0	77.2	262.4	192.4	871.5	1285.0	937.0	1429.0
32	22.8	46.4	106.0	540.8	828.5	1318.0	1279.0	1478.0
33	26.9	40.2	142.5	472.0	430.1	1353.0	1177.0	2242.0
34	24.0	52.3	185.2	373.1	564.0	947.2	1796.0	1055.0
35	23.1	69.5	122.7	486.4	1018.0	549.5	1465.0	1283.0
36	25.8	40.8	151.5	190.0	402.3	1219.0	821.0	2528.0
37	20.3	46.0	194.3	364.3	686.0	878.4	436.8	840.0
38	21.7	29.2	69.3	485.2	590.1	575.4	2108.0	1208.0
39	27.1	37.9	135.8	328.8	731.8	852.4	992.0	1893.0
40	17.4	51.7	109.6	450.3	379.9	1144.3	1790.0	1275.0
41	23.3	55.5	182.0	385.0	568.0	801.7	2326.0	2749.0
42	27.9	50.8	164.1	361.4	659.7	872.2		
43	30.0	38.2	110.8	321.8	851.5			
44	28.7	58.3	77.1	360.5				
45	16.6	31.1						
Mean	24.6	47.8	143.6	380.3	670.6	1012.9	1353.0	1945.0
SE	0.6	1.9	7.3	17.86	32.7	50.3	79.5	124.0

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