

Nutrition and Broodstock Conditioning of the New Zealand
Pipi, *Paphies australis*

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A thesis submitted to
Auckland University of Technology
in partial fulfilment of the requirements for the degree of
Master of Applied Science (MAppSc)

2010

School of Applied Sciences

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ATTESTATION OF AUTHORSHIP

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning."

Signed:

Date:

ACKNOWLEDGEMENTS

I would like to acknowledge and extend my heartfelt gratitude to the following important persons who have made the completion of this thesis possible:

My respected supervisor, Dr Andrea C. Alfaro, for her detailed and constructive comments, and for her vital support throughout this work.

Aquaculture Biotechnology Group advisors, especially Professor John Brooks and Dr Colleen Higgins, for their advices and ideas.

All WS Chemistry and Biology Laboratories staff, for providing me the facilities in the laboratories and their help.

My colleagues, Annapoorna Maitrayee, Tim Young, and Adam Rusk, who have significantly supported my work. I want to let you all know how I have enjoyed working with you over the last two years. I wish you all success in the future and I am grateful for the opportunity to work with you.

All Earth and Oceanic Sciences Research Institute (EOSRI) and School of Applied Sciences members and staff.

My friends, particularly Nor Fazliyana, Syazwalaili, and Nurul Sakina. Their readiness to anything at anytime will never be forgotten. I feel very fortunate to have friends like them.

Most especially to my family: my parents, Mr. Mamat and Mrs. Fatimah Kamilah for giving me life in the first place, for unconditional support and encouragement to pursue my interests, even when the interests have kept us geographically separated. My brothers, Izmat Razwan, Nazri Shukran, and Shahir Nizam, who have always been my heroes and my sister in law, Nadia Iryani for her constant support.

And to God, who made all things possible.

ABSTRACT

The New Zealand pipi, *Paphies australis*, occurs widely around the coast of New Zealand and belonging to the family Mesodesmatidae. Pipi form a part of the recreational bivalve fishery, and there is also an annual restriction of the total commercial harvest. Previous investigations have examined the biological and ecological aspects of pipi in the wild. However, neither of them studied the nutritional requirements of pipi under hatchery conditions. This thesis discusses the feeding requirements of pipi collected from Waiwera Beach and Onehunga Harbour, during their growing and maturing stages. A series of studies on the cell clearance rates, feeding trials, proximate analysis, and broodstock conditioning of pipi clams were conducted over 15 months (September 2008 until December 2009) at the AUT Aquaculture Laboratory. In the cell clearance rates study, the rates for microalgal diets in descending order were *Thalassiosira pseudonana* > *Isochrysis galbana* > *Chaetoceros muelleri* > *Tetraselmis suecica* > *Pavlova lutheri*. It is inferred that pipi are capable of filtering a variety of microalgal species while at the same time being a selective filter feeder. The same microalgal species tested in the aforementioned study were fed to pipi of different size classes (spat, juveniles, and adults) in the feeding trials experiment. Three processed diets (baker's yeast, wheat flour, and corn flour) also were tested on the pipi clams. This study was carried out to test the effect of different diets on growth and survivality, and body composition of spat, juvenile, and adult pipi. There were positive relationships between the growth performance (length and weight) and dietary lipid content for spat and juveniles. For adult pipi, the carbohydrate content in the diets was positively related to pipi shell growth. However, proximate analyses of adult pipi at the end

of the experiment indicated an overall depletion of carbohydrates in the tissues, while proteins and lipids were accumulated. These results suggest that the gonadal development have initiated during the breeding season. Based on the results of the present study, it is suggested that baker's yeast would be a good substitute of spat and juvenile feeds, and wheat and corn flours would be good substitutes for adult feeds. In the conditioning experiment, broodstock pipi were conditioned with three different diets: exclusively *P. lutheri*, exclusively corn flour, and a mixed diet composed of *P. lutheri* and corn flour in 1:1 ration. On days 21 and 28, following the conditioning period, broodstock were induced to spawn by temperature shock treatment. Although both spawning induction trials failed to bring broodstock pipi into spawning condition, maturity was apparent for broodstock fed the mixed diet of *P. lutheri* and corn flour, indicating that *P.lutheri*-corn flour may be a better food source compared to *P. lutheri* or corn flour alone for pipi broodstock conditioning.

CHAPTER ONE
GENERAL INTRODUCTION

1.1. INTRODUCTION

Bivalves belong to the Class Bivalvia (or Lamellibranchia), Phylum Mollusca. Well-known bivalves include clams, mussels, scallops, oysters, and cockles, which are among the most common invertebrates found worldwide. Numbering about 30 000 species, the Class Bivalvia forms the second most diverse class of molluscs after gastropods. Bivalves are exclusively aquatic, requiring fresh or sea water for reproduction and respiration, and typically for feeding (Grzimek *et al.*, 2004). Bivalves frequently occur in large aggregations, forming shell beds in marine, estuarine, and freshwater habitats. Some bivalves are epifaunal, and attach themselves to a hard substrate (e.g., mussels, oysters) whereas others are infaunal, and burrow in sandy to muddy sediments (e.g., clams). Scallops are free-living and swim by rapidly clapping their valves and producing propulsive jets of water. Shell morphology typically reflects the nature of the habitat, with calm-water species often having more delicate and leaf-like shells compared to those subject to fast-flowing currents (Grzimek *et al.*, 2004). The majority of bivalves are filter feeders that feed on naturally occurring phytoplankton at the base of the food chain.

The molluscan bivalve exoskeleton consists of two calcareous valves. The valves are hinged dorsally by an elastic hinge ligament and the adductor muscles on each side hold the shell closed. When the adductor muscles relax, the ligament expands automatically and springs open the valves. Conversely, adductor muscle contraction rapidly closes the valves. The strong muscular foot is less muscular in the attached species (e.g., mussels and oysters) and is used to dig down into the sediment. Scallops lack a foot but are highly active

swimmers. Bivalves use siphons (tube-like structures in which water flows) for respiration, feeding, defecation, and/or reproduction (Campos & Veer, 2008). However, not all bivalves have siphons. Those that live on or above the substrate (e.g., scallops and oysters) do not need them. Only bivalves that burrow in sediment (e.g., clams) need to use these tube-like structures.

Bivalves have long been important for human consumption. They also have vital ecological roles in marine and freshwater ecosystems. In addition, their economic values include fisheries, ornament industry, and health products. Worldwide commercial bivalve species include the hard clam (*Mercenaria mercenaria*), the soft-shelled clam (*Mya arenaria*), the surf clam (*Spisula solidissima*), the ocean quahog clam (*Arctica islandica*), the grooved carpet-shell clam (*Ruditapes decussatus*), the Manila clam (*Ruditapes philippinarum*), the geoduck clam (*Panopea generosa*), the toheroa clam (*Paphies ventricosa*), the tuatua clam (*Paphies subtriangulata* and *Paphies donacina*), the pipi clam (*Paphies australis*), the blue mussel (*Mytilus edulis*), the green-lipped mussel (*Perna canaliculus*), the great scallop (*Pecten maximus*), the New Zealand scallop (*Pecten novaezealandiae*), the Lion's Paw scallop (*Lyropecten sobnodous*), the Pacific oyster (*Crassostrea gigas*), the black-lip pearl oyster (*Pinctada margaritifera*), the European flat oyster (*Ostrea edulis*), the Atlantic oyster (*Crassostrea virginica*), the Sydney rock oyster (*Saccostrea commercialis*), the blood cockle (*Anadara granosa*), and the New Zealand cockle (*Austrovenus stutchburyi*).

Bivalves (clams, mussels, scallops, and oysters) make up a significant proportion of the world's fisheries production. Total landings of bivalves from

capture fisheries and aquaculture operations increased from over 6 million metric tonnes to 13 488 000 tonnes during the period between 1991 and 2007 (FAO, 1993; 2009). During 2001-2007, bivalve landings from capture fisheries totalled only 13 217 000 tonnes, while landings from aquaculture recorded a total of 72 787 000 tonnes (Table 1). About 85% of the world's bivalve production between 2001 and 2007 was obtained from aquaculture operations (FAO, 2007).

Table 1: Production (in thousand tonnes) of bivalves worldwide from capture fisheries and aquaculture operations from 2001 to 2007 (FAO, 2009).

Species group		2001	2002	2003	2004	2005	2006	2007
Oysters	Capture fisheries	197	184	195	149	163	140	149
	Aquaculture	3787	3883	4016	4141	4155	4262	4401
Mussels	Capture fisheries	240	225	187	188	132	109	110
	Aquaculture	1375	1551	1618	1662	1707	1801	1631
Scallops	Capture fisheries	702	750	804	791	725	760	733
	Aquaculture	1102	1113	1102	1048	1144	1262	1463
Clams, cockles, arkshells	Capture fisheries	823	799	898	835	690	751	788
	Aquaculture	2800	3066	3372	3635	3678	3799	4213

In this thesis, the term “clam” refers to a group of bivalves which burrow in sediment. The Family Mesodesmatidae is represented by a few genera and species of marine clams, which include the genus *Paphies*. Four species of surf clams in this genus are found in New Zealand waters. These are the tuatua (*Paphies subtriangulata*), deepwater tuatua (*Paphies donacina*), pipi (*Paphies australis*), and toheroa (*Paphies ventricosa*) (Fig. 1). The toheroa and tuatua inhabit the intertidal zone, which is easily accessible at low tide and have been the basis of important hand-gathering fisheries (Anon, 2008). In comparison, deepwater tuatua are found in subtidal coastal waters (Leach *et al.*, 2001) while

pipi recruit both intertidal (Morton & Miller, 1973) and subtidal zones (Creese, 1988).

Toheroa are the largest clams of the family, and have shells larger than 150 mm in length (Hooker, 1995). The shells are thin and have a prominent gap at the margin of the valves. The tuatua, although very similar in shape to toheroa, are relatively smaller in size. Adult tuatua reach only approximately 90 mm in length. The shell is relatively thick and solid. Pipi shells are moderately large, solid white, and covered by a thin yellow organic coating, known as “periostracum”. All the New Zealand mesodesmatids are epicurean shellfish and therefore, the fishery is largely recreational. All the New Zealand mesodesmatids are potential candidates for aquaculture (McLeod & Wing, 2008).



Fig. 1: Comparative appearance of New Zealand mesodesmatids. (A) the toheroa, (B) the tuatua, and (C) the pipi.

1.1.1. Biology, population distribution, and ecology of pipi

The taxonomic hierarchy of pipi is as follows:

Kingdom: Animalia
Phylum: Mollusca
Class: Bivalvia
Order: Veneroida
Family: Mesodesmatidae
Genus: Paphies
Species: *P. australis* (Gmelin, 1790)

Pipi are found around mainland New Zealand and at the Chatham and Auckland Islands, and are distributed mainly in the North Island (Fig. 2). They inhabit mid to lower regions of protected beaches, especially those built of shell sand, and particularly near the mouth of harbours where salinity is lower than seawater. In the entrance to the harbour, juvenile pipi are abundant (Grace, 1966). Salinity is probably the key factor to their inward extension into harbours (Grace, 1966). In addition, settlement areas tend to be nearer harbour entrances, and adult beds are found seaward (Grace, 1972; Hooker 1997). Smaller pipi presumably could adapt to lower salinity environments.

Early studies between the 1960's and the mid 1980's have suggested that pipi are predominantly an intertidal species (Larcombe, 1968; Morton & Miller, 1968; Jones, 1983; Venus, 1984; Bradstock, 1985). As a result, most of the studies are based around intertidal pipi populations. Subtidal pipi have been regarded as temporary aggregations that have appeared from chance settlement events under favourable conditions (Venus, 1984; Dickie, 1986). However, the most recent studies on pipi have shown that they also may recruit in the sub-littoral

zone (to water depths of at least 7 m), forming extensive sub-tidal beds in harbour channels (Dickie, 1986; Hooker, 1995). Pipi can grow up to a maximum size of 83 mm in length and a height of 51 mm (Powell, 1979). Hooker (1995) showed that pipi at the Whangateau Harbour could grow up to 30 mm in over a year (16-17 months), and reached 50 mm after 3 years, with a slower growth rate thereafter.

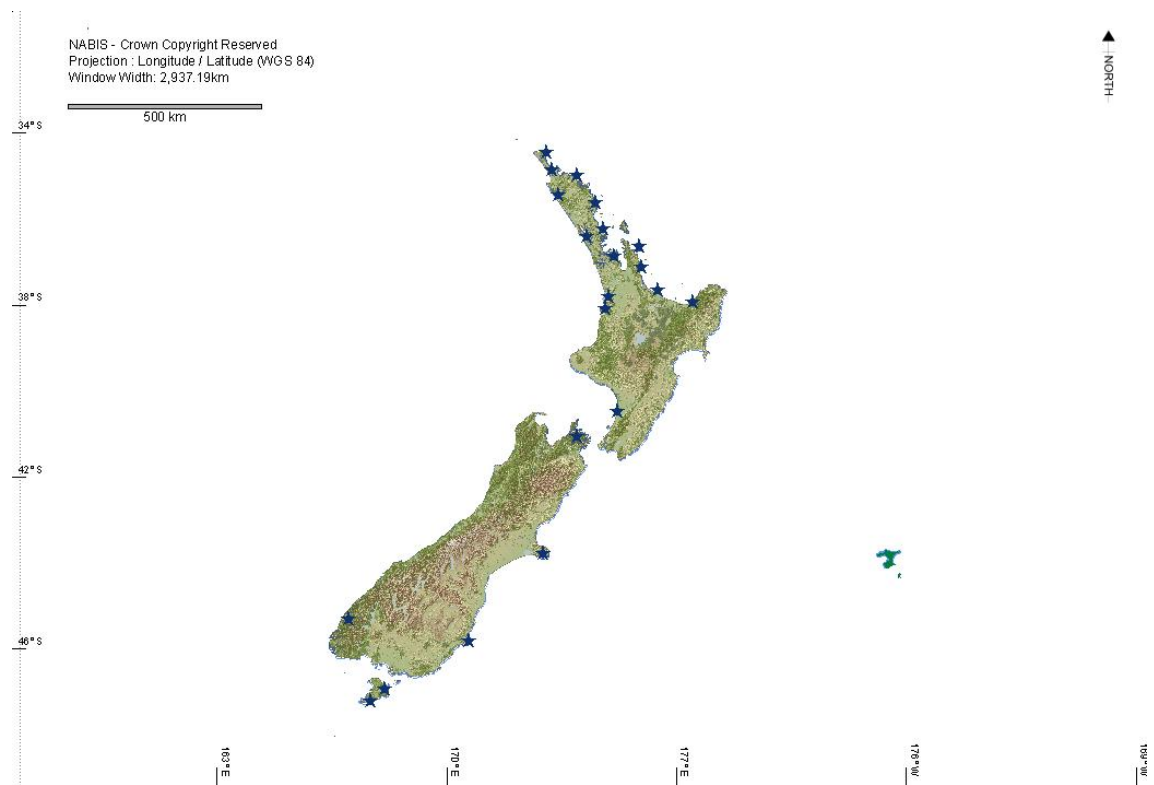


Fig. 2: Distribution of pipi around New Zealand (NABIS, 2004).

Less information is known about the optimum environmental conditions for pipi. However, it is assumed that pipi are tolerant to fluctuations in seawater temperature. In summer, New Zealand waters fluctuate between 17-22°C and in winter between 10-14°C (Walrond, 2009). New Zealand coastal waters tend to be colder in the south and warmer in the north. As spring arrives, the water gradually warms and reaches peak temperatures in late summer. Warmer

temperatures in northern New Zealand provide more favourable habitats for pipi than in the south. Observations in the AUT Aquaculture Laboratory revealed that pipi would die due to ammonia, nitrite, and nitrate accumulations (unpublished data). During the 3-month observation, ammonia levels fluctuated between 0 and 0.5 mg L⁻¹, and the highest concentrations recorded for nitrite and nitrate were 0.5 and 80 mg L⁻¹, respectively. A study by McLeod and Wing (2008) demonstrated that prolonged exposure (> 30 days) of pipi population to low salinity (< 10 ‰) significantly affected their survivorship. However, pipi could survive in low salinity up to at least 20 days if returned to normal seawater salinity (~ 34 ‰) (McLeod & Wing, 2008).

1.1.2. Nutrition of pipi

Pipi are active filter-feeders, straining particulate food from water pumped through the gills. Like most bivalves, pipi feed by filtering food particles from the surrounding aqueous environments. An incurrent siphon draws water in over the gills using ciliary currents and an excurrent siphon ejects the filtered water and wastes out (Fig. 3). After moving over the gills, food particles are trapped in the mucus around the gills, and then transported to the mouth, situated anterior to the gills. The mouth has two palps; outer and inner. The outer palp collects incoming food particles (which are then sorted by both palps), and the inner palp transfers acceptable food to the mouth. Rejected materials are expelled (by the outer palp) without having passed through the digestive tract, for immediate removal as pseudofaeces (Healy, 1998).

In the wild, filter-feeding bivalves (including pipi) feed on naturally occurring phytoplankton in the water column throughout their life cycle. Phytoplankton are microscopic single cell microalgae, which form the basis of life in the seas and the primary source of essential nutrients for most filter feeders including pipi. Filter feeding in bivalves also involves trapping of sediment and organic debris (Healy, 1998). When the tide rises, pipi open their valves to feed on plankton in the incoming water. At low tide, pipi close their valves, trapping enough water inside to support their needs until covered again by water at high tide.

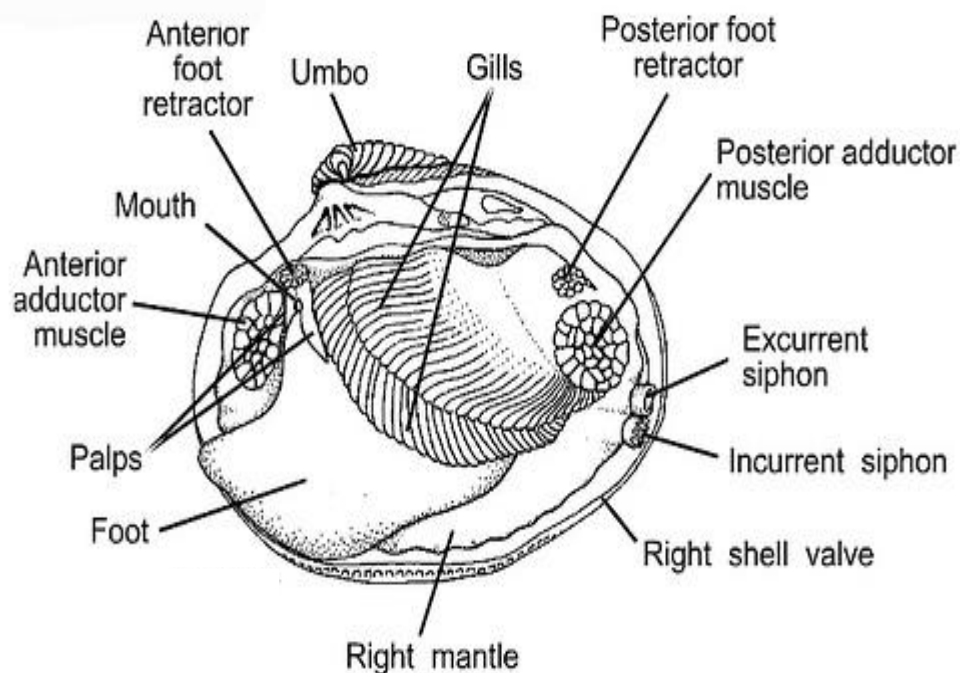


Fig. 3: General anatomy of a clam (Hosie, n.d.).

1.1.3. Reproduction and larval development of pipi

The understanding of reproduction is basic to life history studies, and may provide essential information for fisheries management and aquaculture practices. In fact, knowledge on reproduction and spawning is fundamental to

understand recruitment and population dynamics of any marine species (Shaw, 1962; Baron, 1992; William & Babcock, 2004).

P. australis is a dioecies species, where male and female reproductive organs are found in different individuals (Hooker, 1995). The process to form mature haploid gametes (gametogenesis) begins in autumn when the oocytes are in the early development stages and by late winter, pipi are sexually matured at about 40 mm shell length with ready-to-spawn gonads (Fig. 4). The spawning activity starts in early spring and continues through to late summer. Hooker (1995) investigated the reproductive cycle of pipi in Whangateau Harbour, northeastern New Zealand for two years (May 1991 to April 1993). He distinguished five developmental stages (early active, late active, mature, partially spawned and indeterminate) in pipi gonads based on microscopic examination of histological sections. The gametogenesis pattern was similar in both years.

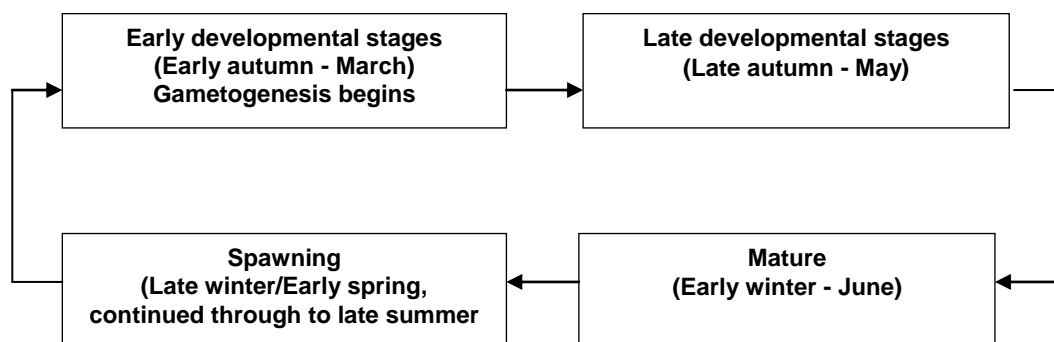


Fig. 4: Reproductive cycle of the pipi clam, *P. australis*.

Two major spawning seasons were observed, one in September/ October and the other in November/December. The sex ratio for pipi in Whangateau Harbour was 1:1, and there was no sign of hermaphroditism. In comparison to other *Paphies* species, the deepwater tuatua (*P. donacina*) clam has two spawning

seasons: a minor period in March and a major period in October/November (Greenway, 1981; Grant, 1994). The major spawning in the spring season is likely to be induced by changes in water temperature (Ohba, 1959; Holland & Chew, 1974; Mann, 1979b; Grant, 1994; Xie & Burnell, 1994). During spawning, sperm are released into the water column, which triggers females to release matured eggs (Helm & Bourne, 2004). Fertilisation takes place in the water column, when a single sperm penetrates the egg membrane. In the reproductive organs, germ cells undergo meiosis to produce haploid gametes (sperm and egg), which then diffuse during fertilisation to create new diploid embryos. The time taken for embryonic and larval development is species specific and temperature dependent (Helm & Bourne, 2004).

1.1.4. Potential for pipi aquaculture

Aquaculture is the cultivation of any salt or freshwater animal or plant, in natural (coastal waters, rivers, lakes) or controlled environments (tanks or enclosures). Commercial scale aquaculture in New Zealand began with an emphasis on bivalve species. It began in the late 1960's with the intertidal farming of oysters *S. commercialis* in the north of the North Island (Crimp, 2007). Pacific oysters (*C. gigas*) later became the dominant cultivated oyster species in New Zealand following their introduction into the country in the early 1970s. Mussels were firstly cultured in the late 1960's as a result of the collapse of two dredge fisheries in the Hauraki Gulf (in the North Island) and the Marlborough Sounds (in the South Island) (Dawber, 2004; Jeffs *et al.*, 1999). In 2006, the aquaculture industry in New Zealand developed a strategy to achieve a sustainable annual billion NZ dollar business by 2025 (Ministry of Fisheries, 2008). In 2007, action

was taken by the government to offer more support to the growing industry (FAO, 2010).

Hooker (1995) showed that pipi could be conditioned and induced to spawn by temperature manipulation in a hatchery. The adults were maintained in conditioning tank for 23 days at 22°C, and were fed daily with *Isochrysis galbana* and *Thalassiosira pseudonana*. At the end of the conditioning period, the adults were spawned using a combination of temperature shock (27°C) and a dilute sperm solution. The larvae were reared in larval tanks with the addition of *I. galbana*, *T. pseudonana*, *Pavlova lutheri*, and *Chaetoceros gracilis* microalgae. Spat were reared in a downweller arrangement before moved to an upweller after three months following the spawning. The successful induced spawning in the hatchery is an advantage to the future aquaculture prospects of pipi.

At present, the culture of marine bivalves in hatcheries depends on the availability of large quantities of microalgae. Many studies have been conducted to determine the best microalgal species that provide sufficient nutrients for optimal growth and survival of cultured bivalves (Provasoli, 1968; Walne, 1970; Brown *et al.*, 1991; Nell & O'Connor, 1991; Coutteau & Sorgeloos, 1992; Brown *et al.*, 1997; Lora-Vilchis *et al.*, 2004; Martinez-Fernandez *et al.*, 2006; Martinez-Fernandez & Southgate, 2007). More than fifty microalgal species have been tested as live food for bivalve molluscs, but less than twenty species have been used commercially in aquaculture (Brown, 1991; 2002). Some microalgal species which are regarded as being successful for bivalve cultures include *Isochrysis* sp. (strain T-ISO), *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros*

muelleri, *Chaetoceros calcitrans*, and *T. pseudonana* (strain 3H). These microalgae are usually fed to cultured bivalves (larval, juvenile, and broodstock stages) in combination of two or three species.

The *Isochrysis* spp. cells have no discrete cell wall, and only possess a plasma membrane covering (Zhu *et al.*, 1997). Cells are between 5 and 6 µm long, motile, and ellipsoidal in shape. They are generally solitary with a pair of flagella, more or less equal, showing at one end of a cell. *Pavlova* spp. are small golden/brown flagellates that are really similar to *Isochrysis*. The cells are ovate with approximately 6-8 µm in diameter. The green flagellate species, *Tetraselmis* cells are green, ovoid, free-swimming, 12-15 µm in diameter, and usually encountered as solitary. The body of the cells is surrounded by a theca (cell wall). *Chaetoceros* and *Thalassiosira* are diatoms. Diatoms are unicellular algae that make cell walls out of silica (hydrated silicon dioxide). *Chaetoceros* cells, with a diameter of 4-7 µm build chains of varying length. However, occasionally the cells are solitary. The cells are constructed like a box with a lid, and always carry outward brushes that point from the corners of the cell. *T. pseudonana* is encased in a hockey puck shaped shell comprised of a rigid cell wall, made mainly of silica. The average diameter of the cell is approximately 4-8 µm (Table 3).

Concentrations of algal cells in the cultures should be maintained at higher concentrations to provide enough food sources for reared bivalves. Therefore, microalgae are normally cultured in specialised maintenance media, for instance, Guillard's f/2 (Guillard, 1975), HESAW (Harrison *et al.*, 1980), or Walne's media (Walne, 1970). The f/2 medium has been used widely and is

suitable for growth of most microalgal species. The macronutrients in the f/2 medium include nitrate, phosphate, and silica. Inorganic micronutrients (trace metal compounds) include ferric chloride, EDTA salt, and a number of trace elements. Organic micronutrients consist of vitamins: thiamine (B₁), cyanocobalamin (B₁₂), and biotin (H). Silicate is specifically used for culturing diatoms which utilise this compound for production of an external shell. The composition and preparation of Guillard's f/2 is provided in Table 2.

Table 2: Composition and preparation of Guillard's f/2 medium.

F/2 Medium			Trace Metal Solution (TMS)		Vitamin Solution (VS)	
Compound	Stock per L	add in 1 L SW	Compound	Stock per L	Compound	Stock per L
NaNO ₃	75.0 g	1.0 mL	CuSO ₄ .5H ₂ O	10.0 g	Biotin	1.0 mg
NaH ₂ PO ₄ .H ₂ O	5.0 g	1.0 mL	ZnSO ₄ .7H ₂ O	22.0 g	B ₁₂	1.0 mg
Na ₂ SiO ₃ .9H ₂ O	30.0 g	1.0 mL	CoCl ₂ .6H ₂ O	10.0 g	B ₁	200.0 mg
TMS		1.0 mL	MnCl ₂ .4H ₂ O	180.0 g		
VS		0.5 mL	Na ₂ MoO ₄ .2H ₂ O	6.0 g		
			FeCl ₃ .6H ₂ O	3.2 g		
			Na ₂ EDTA.2H ₂ O	4.36 g		

Despite being the main food for bivalves, microalgae are time-consuming and expensive to produce under indoor facilities. Coutteau and Sorgeloos (1992) identified the mass production of microalgae as a major bottleneck in bivalve hatcheries and nurseries. The cost of microalgal production comprises up to 30-50% of the overall hatchery operational costs. Moreover, there is a great variability in the quantity and biochemical composition of the nutrients supplied, depending on the origin and culture conditions of the microalgae. Efforts have been made to improve the production and to find satisfactory alternatives that are more cost-effective than the conventional microalgal propagation. The operation of bivalve hatcheries would be greatly improved by total or even partial substitution of the live microalgae.

Therefore, there has been a considerable amount of research to find suitable substitutes for microalgae to feed commercial bivalve species. The studies

focused on the use of substitute diets for larvae, spat, and broodstock of bivalves. Tested substitutes include preserved microalgae (Brown & Robert, 2002; Knuckey *et al.*, 2006; Ponis *et al.*, 2008), yeasts (Epifanio, 1979; Coutteau *et al.*, 1994; Brown *et al.*, 1996), plant-based meals (Haven, 1965; Gillespie *et al.*, 1966; Ingle, 1967; Dunathan *et al.*, 1969; Albentosa *et al.*, 1989; Albentosa *et al.*, 1999; Pirini *et al.*, 2007), and micro-encapsulated diets (Laing, 1987; Kreeger & Langdon, 1994; Knauer & Southgate, 1997; Nevejan *et al.*, 2007). Researchers have observed promising alternatives for replacing live microalgae in the diets of cultured bivalves. Among artificial diets, baker's yeast (*Saccharomyces cerevisiae*), corn flour and wheat flour are graded as good potential alternatives (Table 4).

The quality of bivalve eggs and larvae depend principally on the physiological condition of the broodstock. Broodstock conditioning is an important step in the operation of bivalve hatcheries. It permits the induction of gametogenesis outside the normal reproductive period and in animals experiencing gametogenesis, to accelerate the gamete development (Sastry, 1979; Helm & Bourne, 2004). The period of conditioning depends on the maturity of gametes. Many temperate and cold water climate bivalves will require 4 to 8 weeks of conditioning during late winter and early spring before they are ready to spawn (Helm & Bourne, 2004).

The maturation of gametes is controlled by both endogenous and exogenous factors. Among the latter factors, temperature and nutrients are the most influential (Mann, 1979a; Muranaka & Lannan, 1984; Ruiz *et al.*, 1992; Heasman *et al.*, 1996; Berntsson *et al.*, 1997; Liu *et al.*, 2008). Excellent food is

needed, primarily for energy reserves to develop gametes. Inadequacy of energy during the conditioning process not only affects the spawning success, but also results in lower fecundity. It is unclear which microalgae are preferred by pipi during the conditioning period. Based on microalgal species availability, Hooker (1995) combined two microalgal species – *I. galbana* and *T. pseudonana* – to feed the conditioning broodstock. However, no investigations have been conducted to identify the preferred food for pipi under culturing conditions.

High water temperature during conditioning has greatly affected the spawning success in the clams, *R. philippinarum* (Delgado & Perez-Camacho, 2007) and *R. decussatus* (Matias *et al.*, 2008; Ojea, *et al.*, 2008) and *C. gigas* (Muranaka & Lannan, 1984; Chavez-Villalba *et al.*, 2002). Increases in water temperature commonly favour formation of the yolk of an egg (vitellogenesis). Indeed, an increase in water temperature during the pre-vitellogenic period may increase metabolism and stimulate the use of energy reserves for subsequent vitellogenesis. A successful conditioning period ensures a better prospect to thrive spawning. Spawning is induced by applying stimulus/stimuli on the broodstock with an aim to trigger the release of gametes (both sperm and eggs).

1.1.5. Objectives of the thesis

The pipi forms a part of the recreational bivalve fishery, and there also is a limited commercial harvest of this species. Despite the widespread distribution in both the North and South Island coastal waters and popularity of pipi, there is little information available on them. The limited studies on pipi have focused mainly on biological and ecological aspects including the reproductive cycle, spawning induction, larval growth and settlement, growth dynamics (Hooker, 1995), demographics (Hooker, 1995; Hewitt *et al.*, 1997; McLeod & Wing, 2008), and burrowing behaviour (Hull *et al.*, 1998).

Pipi are potential aquaculture candidates and have been successfully reproduced in the hatchery (Hooker, 1995). The major aspects in the operation of a bivalve hatchery are feeding and propagating the animals. There is clearly a lack of information on the nutritional demands of pipi during the growing stages and reproductive maturity. This thesis attempts to fill a gap in the feeding aspects of pipi, particularly on comparisons between different diets and how feeding may affect maturity in pipi under culturing conditions.

This thesis has three main objectives:

1. To determine the optimal diet for pipi from a range of microalgal and substitute diets.

2. To evaluate the nutritional benefits of each diet for adult pipi based on moisture, ash, protein, carbohydrate, and lipid contents of the diets and adult tissues.
3. To condition broodstock pipi with different diets (based on Objectives 1 and 2) prior to spawning under laboratory conditions.

Table 3: Summarises the biochemical composition of selected microalgae (TP, total protein; TL, total lipid; TC, total carbohydrate).

Algal species	Attributes		Percentage of dry weight			Comments	References
	Size (µm)	Shape/content	TP	TL	TC		
<i>Isochrysis galbana</i> (T-ISO strain)	5-6	<ul style="list-style-type: none"> Small, brown flagellate High in DHA Commonly used in bivalve hatcheries 	22.0	19.9	6.1	<ul style="list-style-type: none"> <i>Ruditapes decussatus</i> spat had greatest growth solely fed this micro-alga. FDW* 14.09 mg ind.⁻¹, IDW* 1.58 mg ind.⁻¹ in 35 days. Performed the best growth rate when fed solely to <i>R. philippinarum</i> spat without additional wheatgerm flour. Best to be fed to <i>Pinctada maxima</i> larvae. <i>M. mercenaria</i> juveniles displayed the best growth and survival rates with <i>Isochrysis</i>. <i>Isochrysis</i> and <i>Thalassiosira</i> were fed to <i>P. australis</i> broodstock during the conditioning period. 	<ul style="list-style-type: none"> Albentosa <i>et al.</i> (1999) Albentosa <i>et al.</i> (2002) Minaur (1969) Pales Espinosa & Allam (1996) Hooker (1997)
<i>Pavlova lutheri</i>	6-8	<ul style="list-style-type: none"> Rich in EPA and DHA Widely used in the hatchery culture of bivalves 	29.0	12.0	8.9	<ul style="list-style-type: none"> A mixture diet consisting of <i>Pavlova</i>, <i>Isochrysis</i>, <i>Thalassiosira</i>, and <i>Chaetoceros</i> were offered to <i>P. australis</i> spat. Spat of <i>Lyropecten (Nodipecten) subnodosus</i> grew the most with <i>Pavlova</i>-<i>Chaetoceros</i> diet. Cells were hoarded and preserved before being fed to <i>C. gigas</i> larvae. No significant differences in growth/survival with the fresh-fed larvae. Poor growth when the micro-alga was monospecifically fed to <i>P. maxima</i> spat. 	<ul style="list-style-type: none"> Hooker (1997) Ortiz <i>et al.</i> (2009) Ponis <i>et al.</i> (2008) Taylor <i>et al.</i> (1997)
<i>Tetraselmis suecica</i>	12-15	<ul style="list-style-type: none"> Theca cell wall, motile green alga Lacking in 22:6 fatty acids 	31.0	10.0	12.0	<ul style="list-style-type: none"> Poorly ingested and not digested by adult scallops <i>Pecten fumatus</i>. Not recommended as a monospecific diet to <i>C. gigas</i> larvae due to lower growth and higher mortalities. 	<ul style="list-style-type: none"> Heasman <i>et al.</i> (2000) Robert <i>et al.</i> (2001)
<i>Thalassiosira pseudonana</i>	4-8	<ul style="list-style-type: none"> Unicellular, hockey puck shape Connected by a single filament to form a chain 	34	19.4	8.8	<ul style="list-style-type: none"> <i>C. gigas</i> Thunberg; Alga grown under different steady-state conditions and the one grown under high light had more fatty acids 14:0 and 16:0. Supported optimum growth in Pacific oyster spat. 	<ul style="list-style-type: none"> Thompson & Harrison (1992)

<i>Chaetoceros calcitrans</i>	6-7	<ul style="list-style-type: none"> • Siliceous cells • Chains of varying lengths; cells are constructed like a box with a lid • Carries outward brushes that point from the corners of the cells 	34.0	16.0	6.0	<ul style="list-style-type: none"> • High mortality (62%) in silver-lip pearl oyster (<i>P. maxima</i>) spat. • Used as a monospecific diet to condition <i>Crassostrea belcheri</i> broodstock. • Fed to <i>Artemia franciscana</i>. 	<ul style="list-style-type: none"> • Taylor <i>et al.</i> (1997) • Chotipuntu (2005) • Khoi <i>et al.</i> (2006)
<i>Skeletonema costatum</i>	2-21	<ul style="list-style-type: none"> • Filamentous diatom 	25.0	10.0	4.6	<ul style="list-style-type: none"> • Used in <i>P. maximus</i> larval rearing. 	<ul style="list-style-type: none"> • Segueineau <i>et al.</i> (1996)
<i>Chaetoceros muelleri</i>	4-5	<ul style="list-style-type: none"> • Chains of varying lengths; siliceous cells are constructed like a box with a lid • Carries outward brushes, pointing from the corners of the cells 	12.0	7.4	2.7	<ul style="list-style-type: none"> • Greatest increase in shell length, wet weight and AFDW* of <i>P. maxima</i> spat. • Rated very highly as a diet for Sydney rock oyster (<i>S. commercialis</i>). 	<ul style="list-style-type: none"> • Taylor <i>et al.</i> (1997) • O'Connor <i>et al.</i> (1992)

Table 4: The development of artificial diets in various bivalve species (TP, total protein; TL, total lipid; TC, total carbohydrate).

Diets	TP	TL	TC	Bivalves species	Comments	References
Cereal flours	14 23	10 22	76 54.8	<ul style="list-style-type: none"> <i>R. decussatus</i> spat (2.10 ± 0.20 mm) <i>R. philippinarum</i> spat <i>R. decussatus</i> spat <i>C. coteziensis</i> spat 	<ul style="list-style-type: none"> 50% microalgal replacement with wheatgerm flour provided similar growth to that fed with 100% <i>Isochrysis</i>. Wheatgerm flour as complement (< 50%) to microalgae Cornmeal replacement (50%) diet showed the same growth rate in organic matter as those fed with phytoplankton alone. It is practicable to use cornstarch (< 50%) as a complement to live phytoplankton in the diet. 	<ul style="list-style-type: none"> Albentosa <i>et al.</i> (1999) Albentosa <i>et al.</i> (2002) Perez-Camacho <i>et al.</i> (1998) Mazon-Suategui <i>et al.</i> (2008)
Refrigerated microalgal cells				<ul style="list-style-type: none"> <i>M. mercenaria</i> juveniles (1-2 mm) <i>M. mercenaria</i> juveniles <i>M. mercenaria</i> juveniles 	<ul style="list-style-type: none"> <i>I. galbana</i>; damaged and aggregated empty cells after 1 week. After 10 weeks, only 10-20% cells presented intact walls. 58.7±1.8 % mortality when fed with refrigerated <i>Tetraselmis striata</i>; higher following the unfed controls (74.1±3.2 %). After 10 weeks, only 1-2 % of <i>P. lutheri</i> cells presented intact chloroplasts and cell walls. Cumulative mortality of the animals was 48.3±3.2 %. 	<ul style="list-style-type: none"> Pales Espinosa & Allam (2006) Pales Espinosa & Allam (2006) Pales Espinosa & Allam (2006)
Yeast				<ul style="list-style-type: none"> <i>M. mercenaria</i>, <i>Mytilus edulis</i>, and <i>Argopecten irradians</i> juveniles <i>Crassostrea virginica</i> <i>S. commercialis</i> 	<ul style="list-style-type: none"> Spray-dried <i>Candida utilis</i> had successfully substituted 50% of the live microalgae requirement. Poorly digested by the juveniles. Added in the fattening diet for adults. 	<ul style="list-style-type: none"> Epifanio (1979) Epifanio (1979) Nell (1985)
Formulated diet				<ul style="list-style-type: none"> <i>Mytilus galloprovincialis</i> <i>Mytilus edulis</i> 	<ul style="list-style-type: none"> MySpat (INVE Technologies, Belgium) fed animals grew as fast as the animals that received 75% microalgae. MyStock formulated diet 	<ul style="list-style-type: none"> Nevejan <i>et al.</i> (2007) Nevejan <i>et al.</i> (2008)

CHAPTER TWO
CELL CLEARANCE RATES IN *Paphies australis*

2.1. INTRODUCTION

The volume of water completely cleared of suspended particles per unit time is called the clearance or filtration rate. The filtration rate, or F , of filter-feeding bivalves can be measured by monitoring decreases in particle concentration, in a known volume, over short time periods, around the bivalve (Mohlenberg & Riisgard, 1979; Shumway *et al.*, 1985; Riisgard, 1988). Filtration rates have been studied for *Paphies donacina* (Marsden, 1999), *Mercenaria mercenaria* (Coughlan & Ansell, 1964; Riisgard, 1988), *Venerupis pullastra* (Foster-Smith, 1975; Mohlenberg & Riisgard, 1979), *Mytilus edulis* (Jorgensen, 1949; Foster-Smith, 1975; Hildreth & Crisp, 1976; Mohlenberg & Riisgard, 1979; Riisgard & Mohlenberg, 1979; Kiorboe & Mohlenberg, 1981; Riisgard & Randlov, 1981; Famme *et al.*, 1986; Jones *et al.*, 1992; Nielsen *et al.*, 1993; Cranford & Hill, 1999), *Perna perna* (Berry & Schleyer, 1983), *Crassostrea virginica* (Galtsoff, 1928; Jorgensen, 1949; Riisgard, 1988), *Argopecten irradians* (Riisgard, 1988), *Arctica islandica* (Mohlenberg & Riisgard, 1979), *Geukensia demissa* (Riisgard, 1988), *Spisula solidissima* (Riisgard, 1988), *Potamocorbula amurensis* (Foster-Smith, 1975), *Cerastoderma edule* (Foster-Smith, 1975), and *Ciona intestinalis* (Jorgensen & Goldberg, 1953).

Several methods have been used in the studies of filtration rates of bivalves. These different methods include direct measurement (Famme *et al.*, 1986, Jorgensen, 1988; Davenport & Woolmington, 1982; Redpath & Davenport, 1988), flow-through chamber (Bayne *et al.*, 1976; Hildreth & Crisp, 1976; Riisgard, 1977), suction (Mohlenberg & Riisgard, 1979; Kiorboe & Mohlenberg, 1981; Famme *et al.*, 1986), clearance (Jorgensen, 1975b; Griffiths, 1980;

Riisgard *et al.*, 1980; Riisgard, 1981; Berry & Schleyer, 1983; Famme *et al.*, 1986; Jorgensen & Riisgard, 1988; Jorgensen *et al.*, 1990; Clausen & Riisgard, 1996; Marsden, 1999; Dupuy *et al.*, 2000), photoaquarium (Winter, 1973, Riisgard & Mohlenberg, 1979), steady-state (Riisgard & Randlov, 1981), video observation (Nielsen *et al.*, 1993; Riisgard *et al.*, 1996; Riisgard & Larsen, 2000), replacement (Coughlan & Ansel, 1964), thermistor (Foster-Smith, 1976; LaBarbera & Vogel, 1976; Vogel, 1994), bio-deposit (Hawkins *et al.*, 1996), and impeller (Jones *et al.*, 1992).

2.1.1. Clearance method

In many studies, a large number of filtration measurements have been performed using the clearance method. This method measures volume of water cleared of suspended particles per unit of time (Dame, 1996). Experimental animals are kept in an enclosed system, with well-mixed seawater and microalgal cells. The clearance rate is measured as function of time using the formula:

$$F = (V/t) \ln (C_0/C_t)$$

where V = volume of water, t = time, and C_0 and C_t = particle concentrations at time 0 and t . The method also has been proved to be reliable in many filtration studies of bivalves (Jorgensen, 1975b; Griffiths, 1980; Riisgard *et al.*, 1980; Riisgard, 1981; Berry & Schleyer, 1983; Famme *et al.*, 1986; Jorgensen & Riisgard, 1988; Jorgensen *et al.*, 1990; Clausen & Riisgard, 1996; Marsden, 1999; Dupuy *et al.*, 2000). In a study on the brown mussel, *P. perna* by means

of the clearance method, the F values as a function of W (dry tissue weight) was $F = 8.85 W^{0.66}$ and a function of L (shell length) was $F = 0.0027 L^{1.86}$ (Berry & Schleyer, 1983). In *Coromytilus meridionalis* (Griffiths, 1980), *C. virginica*, and *G. demissa* (Riisgard, 1988), the rates were $5.37 W^{0.60}$, $6.79 W^{0.73}$, and $6.15 W^{0.83}$, respectively.

2.1.2. Filtration in bivalves

Filter-feeding bivalves continuously remove particles from the water column and dilute the suspension (Dame, 1996). Bivalves rely on particulate matter of sizes ranging from 1 μm to 40-100 μm , including phytoplankton, nano-zooplankton, bacteria, and detritus (Dame, 1996). It is suggested that the consumption and assimilation of particles is related to particle abundance (Kreeger et al., 1988; Langdon & Newell, 1990). Size and quality of the particles also determine the filtration and feeding in bivalves. Particles that are too large are not utilised as food (they will be rejected as pseudofaeces) and particles that are too small may pass through the filter. Ingestion capability is related to the size of bivalves, where smaller animals (spat and juveniles) have different preferences or selections of foods based on the cell sizes compared to adult bivalves (Gallager, 1988). His study showed that clam larvae (*Mercenaria mercenaria*) could dynamically ingest food as small as 0.5 μm as for cyanobacterium, *Synechococcus* sp. during the first day of their lives. On the second day, the larvae (larvae mean length 100 μm) were fed a mixture diet of 50:50 *Synechococcus* sp. and *Isochrysis galbana*. The larvae ingested 48 cyanobacterium cells for every *I. galbana* cell. The mean ratio was further dropped to 3:1 in the 10-day-old larvae (mean length 234 μm), indicating a

relationship between food and larval size. In *Placopecten magellicanus*, *M. edulis*, and *Mya arenaria*, 55% of the wild collected veligers were found to contain cells of 5-15 μm in their stomach. Only 3% of the veligers retained cells between 15 and 25 μm . These findings provide information on the optimal sizes of microalgae that are suitable for feeding bivalves, especially during their initial life stages.

Digestibility of several species such as *Chlorella autotrophica*, *Dunaliella primolecta*, and *Nannochloropsis atomus* is difficult due to the presence of thick cell walls. Although these microalgal species are ingested, they are not essentially digested as shown in the case of *C. virginica* (Babinchak & Ukeles, 1979). Based on the epifluorescence microscopy observations, the authors showed that larvae of *C. virginica* were incapable to digest those microalgal species mentioned earlier, which resulted in poor larval growth. Apart from the 'thick wall' barrier, diatoms with long siliceous spines (e.g., *Phaeodactylum* sp.) may be an irritant to bivalves, which causes closure of the shells (Helm & Bourne, 2004). Deficiency of appropriate digestive enzymes is the current hypothesis to explain the indigestibility.

2.1.3. Objective of the study

At present, no information is available on feed evaluation of pipi reared under laboratory conditions. To understand feeding capacity in hatchery conditions, information is needed on filtration ability of pipi. Therefore, objective of this study was to determine the cell clearance rates of pipi fed with different microalgal species.

2.2. MATERIALS AND METHODS

2.2.1. Pipi and microalgal samples

Pipi were collected in October 2009 from Waiwera Beach (north-eastern Auckland). Individuals (31.0-44.0 mm in width and 19.0-21.0 mm in height) were acclimated for two days upon arrival in the AUT Aquaculture Laboratory. Pipi were unfed during the acclimating period and kept in a recirculating seawater system equipped with biological filters, at $17 \pm 1^\circ\text{C}$.

The microalgal diets were grown in Guillard f/2 medium (Guillard, 1975). The microalgae were cultured in 1 L flasks with 24-h illumination by fluorescent tube lights and aerated. On the day of the experiment, individual pipi was set into separate 400 mL beakers. The experiment was carried out in triplicate for each diet (microalgae). The initial cell concentrations were counted for each microalgal diet (*Isochrysis galbana*, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros muelleri*, and *Thalassiosira pseudonana*) using a Neubauer haemocytometer. At every 30, 60, 180 and 360-minute intervals, 10 mL of seawater from each beaker were pipetted and cell concentrations were counted using a Nauebuer haemocytometer. Filtration rates were calculated as:

$$F = V/t \times \ln (C_0/C_t)$$

where V is the volume of water, t is time and C_0 and C_t are algal cell concentrations at times 0 and t , respectively.

2.2.2. Size of microalgae

Microalgal sizes used in this study were: 12-15 μm (*T. suecica*), 6-8 μm (*P. lutheri*), *I. galbana* (5-6 μm), 4-8 μm (*T. pseudonana*), and 4-5 μm (*C. muelleri*).

2.2.3. Statistical analyses

SPSS software (version 15.0) was used to analyse the experimental results. The measured filtration rates were assigned for two-way ANOVA (Repeated Measures) tests, at a minimum significance level of $p < 0.05$. To determine the differences between treatments, Independent t-tests were performed as described in Statistics with SPSS handbook (Binnie, 2008). An $\alpha = 0.05$ was used as the level of significance.

2.3. RESULTS

The clearance rates for pipi feeding on the experimental microalgae decreased with time (Figs. 5 & 6). The rates decreased immediately after the first 30 minutes in all food treatments except for *P. lutheri*, which was constant throughout the experimental period. During the first 30 minutes, the highest clearance rate was seen in *T. pseudonana* (0.81 ± 0.05 L hour⁻¹), while the slowest rate was recorded with *P. lutheri* (0.19 ± 0.04 L hour⁻¹). There were significant differences in the clearance rates of pipi fed different microalgal species within the first 30 minutes. Clearance rates between *I. galbana*, *T. suecica*, and *C. muelleri* were not significantly different (t-tests, $p > 0.05$). However, the rates were statistically different from *P. lutheri* and *T. pseudonana* (t-tests, $p < 0.05$). After the first 30 minutes, *P. lutheri* concentration dropped from 295 300 to 107 300, and *T. pseudonana* cells decreased from 391 700 to 285 300 within the same period (Fig. 5). The clearance rates were similar between all microalgal species during the 60-, 180-, and 360-minute intervals ($p > 0.05$). The average clearance rates for all microalgal species in descending order were *T. pseudonana* (0.28 L hour⁻¹) $>$ *I. galbana* (0.24 L hour⁻¹) $>$ *C. muelleri* (0.19 L hour⁻¹) $>$ *T. suecica* (0.19 L hour⁻¹) $>$ *P. lutheri* (0.10 L hour⁻¹). Overall, clearance rates decreased with decreasing algal concentrations. Such a direct relationship was especially evident between clearance rate and cell concentration, and this effect was consistent for all microalgae.

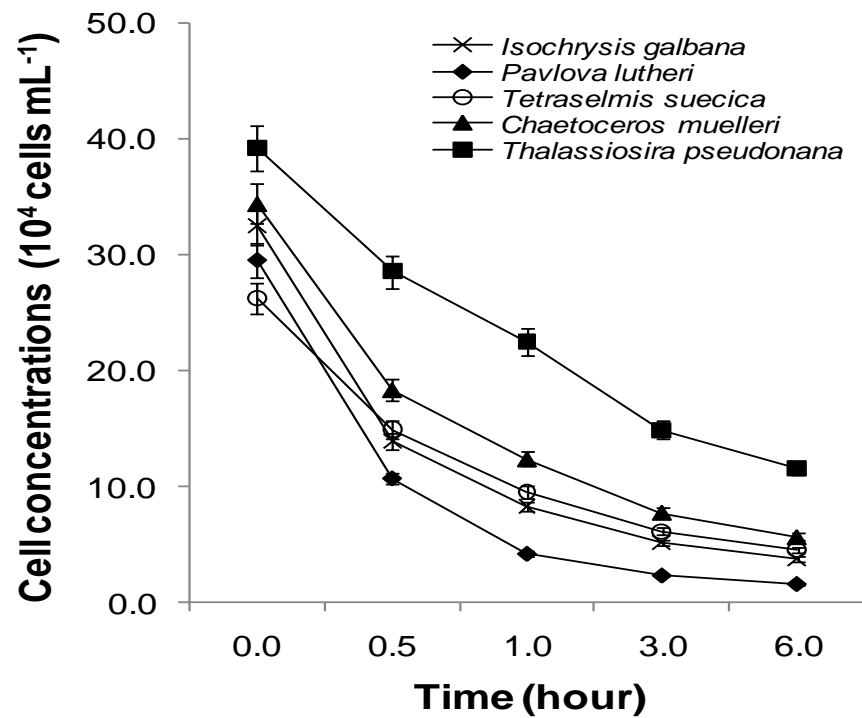


Fig. 5: Algal cell concentrations for five microalgal species used in the experiment. Numbers of cells were not standardised due to some difficulties.

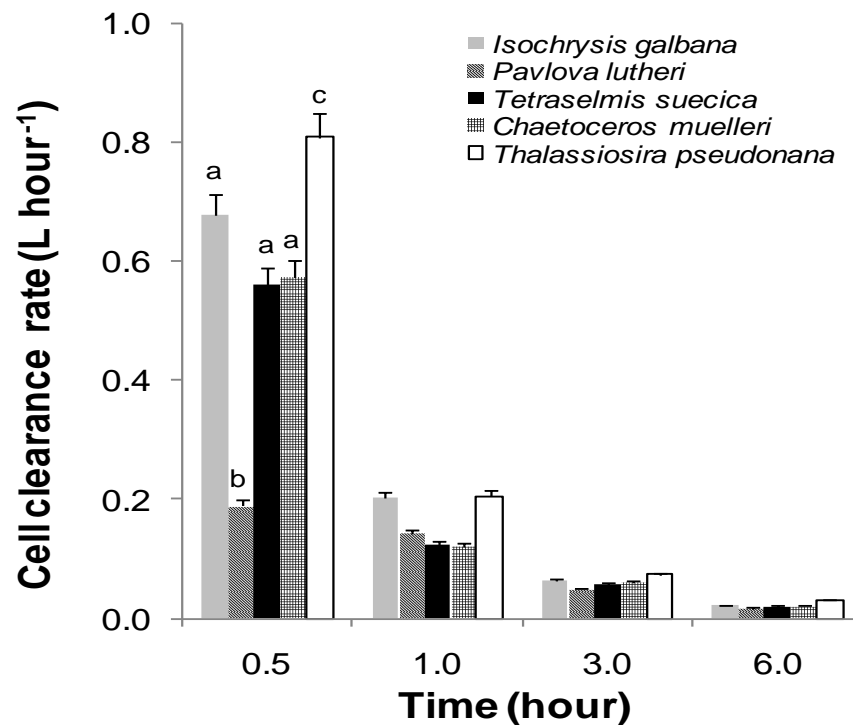


Fig.6: Cell clearance rates in *P. australis* fed five microalgal species (*Isochrysis galbana*, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros muelleri*, and *Thalassiosira pseudonana*). The clearance rates were determined at every 30, 60, 180 and 360-minute intervals.

2.4. DISCUSSION

The cell clearance rates of all experimental microalgae peaked during the first 30 minutes. The generally high consumption rate at the start of the experiment may be related to the initial encounter with food particles after a short period of starvation (Riisgard & Randlov, 1981). Differences between groups were observed only during the first 30-minute interval (Fig. 6). *T. pseudonana* was consistently the fastest filtered microalga, with a peak at the first 30-minute interval ($0.81 \pm 0.05 \text{ L hour}^{-1}$). The lowest clearance rates were with *P. lutheri* (highest at the 360-minute interval: $0.19 \pm 0.04 \text{ L hour}^{-1}$ and lowest at the 30-minute interval: $0.02 \pm 0.01 \text{ L hour}^{-1}$). These results indicate that pipi may be a selective filter feeder. However, pipi were capable of filtering all the experimental microalgae.

Various criteria have been have been proposed as the basis for particle selection in bivalves. They include particle size (Mohlenberg & Riisgard, 1978; Riisgard, 1988; Langdon & Newell, 1990), shape (Bayne *et al.*, 1977), aggregation (Waite *et al.*, 1995), motility and density (Brillant & MacDonald, 2000), chemical cues (Ward & MacDonald, 1996; Bacon *et al.*, 1998; Defosse & Hawkins, 1997; Nakamura, 2001).

2.4.1. Particle selection in bivalves

In the blue mussel, *Mytilus edulis*, *Cerastoderma edule*, and *Venerupis pullastra*, the rates of particle ingestion were found to be roughly proportional to the size of particles (Foster-Smith, 1975). A number of studies showed that

filtration and retention efficiency increased for larger particles. (Wright *et al.*, 1982; Riisgard, 1988; Sobral & Widdows, 2000). Wright *et al.* (1982) experimented with *Geukensia demissa*, found that the filtration efficiency of bacterioplankton (0.2-2.0 μm) was 42% lower than that of phytoplankton > 2.0 μm . In the clam *Ruditapes decussatus*, particles smaller than 3 μm in diameter were retained with low efficiency. On the contrary, algal cells in the size range 3-8 μm were efficiently retained (70% to 100% retention) (Sobral & Widdows, 2000). The studies mentioned previously concluded that filtration retention efficiency increased with increasing particle size. In the present study, because the particle size of microalgae varied between 4-15 μm , the overall ranking of cell clearance rates for the diets (*T. pseudonana* > *I. galbana* > *C. muelleri* > *T. suecica* > *P. lutheri*) did not follow the ranking of particle size: *T. suecica* (12-15 μm) > *P. lutheri* (6-8 μm) > *I. galbana* (5-6 μm) > *T. pseudonana* (4-8 μm) > *C. muelleri* (4-5 μm). Therefore, selection of particles in pipi may depend on other quantitative and/or qualitative characteristics.

Bacon *et al.* (1998) studied filtration in *Mercenaria mercenaria* using particles between 4 and 6 μm in diameter. The study concluded that size-dependent selection was probably not a significant factor, and selection by *M. mercenaria* was based on organic content, or possibly, other qualitative particle characteristics. The high filtration rates obtained for the diatom species (*T. pseudonana* and *C. muelleri*) could be explained by shape of the microalgae. Cells of *T. pseudonana* and *C. muelleri* are united in chains of varying lengths by fine filamentous threads (Round *et al.*, 1990). The cell structure may be responsible for their relatively high consumption rates (i.e., ingestion of multiple cells at once), which left less particles in water. Microalgae *I. galbana*, *T.*

suecica, and *P. lutheri*) used in this experiment were basically solitary cells, which would have explained the lower filtration rates for the flagellates.

During the experiment, all pipi had valves which were completely opened when microalgae were initially added to the beakers. As the time passed, the gap between valves decreased that denoted the decreasing filtration activity. Similarly, in mussel *Mytilus edulis*, the highest rates of water pumping were found in fully open mussels with expanded mantle edges and siphons, and the reduction of valve gape and retraction of mantle and siphon signifying the declining pumping rates (Jorgensen *et al.*, 1988). Partial or complete closure of the valves show reducing or discontinuity of filtering activities (Famme *et al.*, 1986).

Although bivalves show selectivity to different types of particulate matter, the mechanism used to differentiate between particles remains undefined. However, several authors (Kiorboe & Mohlenberg, 1981; Ward & Targett, 1989; Pales Espinosa *et al.*, 2007) have suggested that bivalves are capable of using chemical cues to discern among particles. Indeed, chemical communication is widely distributed in the marine surroundings and marine organisms interact with each other using chemical signals (Hay, 1996). Microalgae produce (Hodgson *et al.*, 1991) and excrete (Schmidt & Hansen, 2001; Fistarol *et al.*, 2005; Uronen *et al.*, 2005) metabolites (including nitrogenous substances, polysaccharides, amino acids, fatty acids and vitamins) during their life cycle. These metabolites can be re-categorised by bivalves and have significant effects on their feeding behaviour (Tillmann & John, 2002; Leising *et al.*, 2005). It is assumed that chemo-selection in bivalves is based on microalgal exudates

(Shumway *et al.*, 1985; Baldwin, 1995). Recently, extracellular metabolites were found to be involved in microalgal selection in oyster species (*Crassostrea gigas* and *Crassostrea virginica*). Pigi in this study showed selectivity to the tested microalgal species, which filtered diatoms at a faster rate than the flagellates. The two diatoms, *C. muelleri* and *T. pseudonana* may have produced chemical signals that affected the feeding selectivity. However, this claim needs further investigation.

It is concluded that pigi are capable of filtering a variety of microalgal species regardless of particle size. However, the fact that *C. muelleri* and *T. pseudonana* are long chain diatom species may be responsible for their relatively high consumption rate (ingestion of multiple cells at once). In addition, chemical cues signalling also may have affected the selectivity among varying microalgal species.

CHAPTER THREE
EFFECTS OF MICROALGAL AND PROCESSED DIETS ON THE
GROWTH, SURVIVAL, AND BODY COMPOSITION OF THE NEW
ZEALAND PIPPI, *Paphies australis*

3.1. INTRODUCTION

Many studies have been conducted to compare the food value of several diets to consistently support rapid growth of bivalves cultured under hatchery conditions (Robinson, 1992; Coutteau *et al.*, 1993; Millican & Helm, 1994; Heasman *et al.*, 1996; Utting & Millican, 1998; Knauer & Southgate, 1999; Lora-Vilchis *et al.*, 2004; Martinez-Fernandez *et al.*, 2006; Pales Espinosa & Allam, 2006; Martinez-Fernandez & Southgate, 2007). These comparisons allow characterisation of better potential diets for growth. Identification of such characteristics will facilitate the development of optimal diets for bivalves (Whyte *et al.*, 1990; Wikfors *et al.*, 1992). The list of microalgal species used in marine research institutions and commercial hatcheries is still short and there is a continuous attempt to find new and better microalgal feeds or the substitutes (Coutteau & Sorgeloos, 1992).

Hooker (1995) was able to maintain adult pipi in a hatchery with a mixed diet of *Isochrysis galbana* (clone T-ISO) and *Thalassiosira pseudonana* (clone 3H) for 23 days of the conditioning period. The adults were then induced to spawn and the resulting larvae were cultured with the same microalgae used to condition the adults. In a second spawning trial, Hooker (1995) fed the larvae with the *Isochrysis-Thalassiosira* diet with the addition of *Pavlova lutheri* and *Chaetoceros gracilis* microalgae. These initial studies showed that pipi could be fed and spawned under hatchery conditions, but the best food composition for optimal growth and reproduction still needs further investigation.

3.3.1. Microalgae as primary food source for bivalves

Fifty species of microalgae have been tested on larvae and juveniles of the commercially cultured bivalves, but only 16 microalgal species are commonly used in bivalve hatcheries (Brown, 1991; O'Connor *et al.*, 1992; Baptist *et al.*, 1993). Common species include T-ISO, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros muelleri*, and *T. pseudonana*. These microalgae are usually fed together as a mixed diet to bivalves. A microalga must possess a number of fundamental attributes to be useful in aquaculture (Brown, 1991). A species must have an appropriate size for ingestion and digestion and a rapid growth rate. In addition, a suitable microalga should be easy to mass produce, stable to environmental (e.g., temperature, light, and nutrients) fluctuations in hatchery systems, nutritionally adequate for bivalve growths, and free from toxins that might be transmitted up the food chain (Brown, 1991).

Protein, lipid, carbohydrate and mineral levels in microalgae determine the quality of each species, as these compounds constitute up to 90-95% of the dry weight of an algal cell. The remaining percent (5-10%) is comprised of nucleic acids (Becker, 1986). Producing microalgae in bulk would help to reduce the cost of microalgal production. Although having good nutritional value, some microalgal species are not used in commercial hatcheries due to difficulties in producing large amounts of cells. For instance, *Chaetoceros calcitrans* forma *pumilum* is rarely grown in volumes over 20 litres. Some species, such as *Tetraselmis suecica* are 'sticky' and taint culture tank walls which then have reduced light through them (Helm, 1990).

3.3.2. Substitute diets to microalgae for bivalves

Yeast and cereal flours are potential substitute diets over microalgae for bivalve feeds. Substitutions of microalgae with processed diets, such as yeast and plant-based flours, have been studied for their effectiveness in several clam species, such as *Tapes philippinarum* (Caers *et al.*, 1999), *Ruditapes decussatus* (Albentosa *et al.*, 1999) and *Mercenaria mercenaria* (Epifanio, 1979).

Yeasts are unicellular eukaryotic microorganisms that are common in seawater (Bass *et al.*, 2007). Yeasts have shorter generation times and can be produced more efficiently and economically than photosynthetic microalgae. Yeast was first used as a diet in bivalve cultures by Epifanio (1979). He compared the growth rates of juveniles in four bivalves – *Argopecten irradians*, *Mercenaria mercenaria*, *Mytilus edulis*, and *Crassostrea virginica* – fed a mixed diet of *T. pseudonana* and various percentages of yeast (*Candida utilis*). When supplied with a 50% yeast-based diet, juvenile *A. irradians*, *M. mercenaria*, and *M. edulis* grew faster than those fed the diatom only. It was also observed that yeast diets could be supplied at up to an 80% substitution level of microalgae (Epifanio, 1979).

Other potential replacements for microalgae are plant-based flours. Trials with these compounds were first studied on the American oyster, *Crassostrea virginica*, in the 1960's (Haven, 1965; Gillespie *et al.*, 1966; Ingle, 1967; Dunathan *et al.*, 1969). In these studies, microalgae and cornstarch were mixed and fed to the broodstock during the conditioning period, which then were

successfully spawned. Mazon-Suastegui *et al.* (2008) investigated the interactive effects of diet and stocking density on growth and biochemical composition of *Crassostrea corteziensis* spat, over 21 days. They discovered that the highest contents of protein, carbohydrate, and lipid in spat was promoted by microalgae, regardless of density. Nonetheless, it was concluded that it was practicable to replace microalgae by 50% with cornstarch, without significantly deteriorating growth or biochemical composition of the spat.

Evaluations on growth and biochemical composition of animals are commonly used to reflect quality of the experimental diets (O'Connor *et al.*, 1992; Coutteau *et al.*, 1994; Albentosa *et al.*, 1999; Caers *et al.*, 1999; Woodcock & Benkendorff, 2008). It has been shown (Chapter Two) that pipi are capable of filtering various microalgal species, namely the green flagellate *Tetraselmis suecica* and several brown microalgae (i.e. *Chaetoceros muelleri*, *T. pseudonana* (3H), *I. galbana* (T-ISO), and *Pavlova lutheri*). However, further investigations are necessary to evaluate effects of these microalgae on growth and body composition of pipi.

3.3.3. Biochemical composition of bivalves

Carbohydrates are generally considered as the main energy resource in bivalves (de Zwaan & Zandee, 1972; Barber & Blake, 1981). Carbohydrates also play a role in balancing the utilisation of proteins and lipids for biosynthesis against their catabolism for energy production (Whyte *et al.*, 1989). In addition, carbohydrates are important for gamete development and maintaining the condition of adults during periods of food scarcity or in winter (de Zwaan &

Zandee, 1972; Gabbott, 1975). Lipids are used principally during gametogenesis and energy lost throughout spawning of adult female bivalves (Gabbott, 1983). Additionally, lipids also are favoured during winter when carbohydrate levels are scarce (Beukema & de Bruin, 1979). Proteins simultaneously provide energy during gonad maturation (Barber & Blake, 1981) and maintaining the energy level in periods of inadequate food supply and carbohydrate levels depletion (Gabbott & Bayne, 1973; Beninger & Lucas, 1984; Whyte *et al.*, 1990). Although protein can be catabolised for energy, its primary function is to supply nitrogen and essential amino acids (EAAs) for tissue biosynthesis (Brown *et al.*, 1989).

Both eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids are important for their regulation of membrane fluidity in bivalves and also essential for the growth (Chu & Webb, 1984). The importance of these fatty acids is displayed by better growth rates of oyster juveniles fed lipids of animal origin containing EPA and DHA. In comparison, juveniles fed lipids extracted from plants that lack both important fatty acids were of poorer quality than those fed lipids of the animal origin (Numaguchi & Nell, 1991; Knauer & Southgate, 1997). A study with *Ostrea edulis* broodstock showed the increased levels of fecundity, dry meat weight, and larval survival with EPA-rich *I. galbana* (T-ISO) compared to *Dunaliella tertiolecta*, which is deficient in EPA and DHA (Millican & Helm, 1994).

3.3.4. Objectives of the study

Although pipi were shown to be successfully grown with *I. galbana* (T-ISO), *T. pseudonana* (3H), *P. lutheri*, and *C. gracilis* (Hooker, 1995), further effects of these microalgae and other potential substitute diets on growth, survival, and body composition of pipi are still unknown. Therefore, aims of this study were:

1. To determine the optimal diet for pipi spat, juvenile, and adult, from a range of microalgal and processed diets over a period of 21 days.
2. To evaluate the nutritional benefits of each diet on the growth based on biochemical composition of the diets and soft tissue.

3.4. MATERIALS AND METHODS

3.4.1. Sample collection and experimental setup

Pipi were collected from the Waiwera Beach (northern Auckland) during low tide and kept in running seawater in the AUT Aquaculture Laboratory for 3 days to clear food from their stomachs and to acclimatise the animals to the new conditions. Three size classes were established and defined as: spat (0-10.0 mm in shell length), juveniles (15.0-25.0 mm in shell length), and adults (30.0-40.0 mm in shell length). Three replicate tests were carried out for each experimental diet, giving a total of 24 replicates for each size class. Each diet consisted of three replicates and each replicate contained 30, 20, and 10 clams of the spat, juveniles and adults, respectively.

Experimental diets were fed to spat, juveniles, and adults, at different daily rations: 2, 4, and 6% (dry food weight/live animal weight), respectively. Unfed animals were used in three replicate tanks as control. The experimental tanks were cleaned daily to remove solid waste products. The water salinity was maintained at 35 ppt and the temperature at 17-18°C for 21 days of the feeding trials.

3.4.2. Experimental diets

Eight different diets were tested for their performance on growth of pipi. Five microalgal species (T-ISO, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros*

muelleri, and *Thalassiosira pseudonana*), yeast, wheat flour and corn flour were fed to pipi according to the rations.

Microalgal strains were obtained from CSIRO Microalgae Research Centre, Australia. They were cultured with f/2 nutrients (Guillard, 1975) in 15 L carboys at 18°C, water salinity of 35 ppt, and under 12 L:12 D light cycle, prior to the feeding experiments. The baker's yeast granules were ground to a powder form and sieved through a 20-µm sieve. All the processed diets (baker's yeast, corn flour, and wheat flour) were suspended in seawater before being poured into the tanks to avoid lumping.

3.4.3. Pipi growth measurements

Initial maximum shell lengths and wet weights were determined at the beginning of the experiments. Each animal was marked individually with a number on both valves so that the individual growth increment could be obtained at the end of the culture period. The maximum shell length (anterior to the posterior margins) was measured with a Vernier caliper to the nearest 0.1 mm. Individual wet weights were measured by placing a monofilament on the underside of a balance (Fig. 8). The monofilament was then attached to a weighing tray, which was submerged into the experimental tanks. After zeroing the balance, each marked pipi was put on the tray without removing it from the water. This method ensured that each bivalve was maintained fully saturated with water, and only the tissue and shell were being weighed (i.e., no variations due to amount of water drained from each animal).

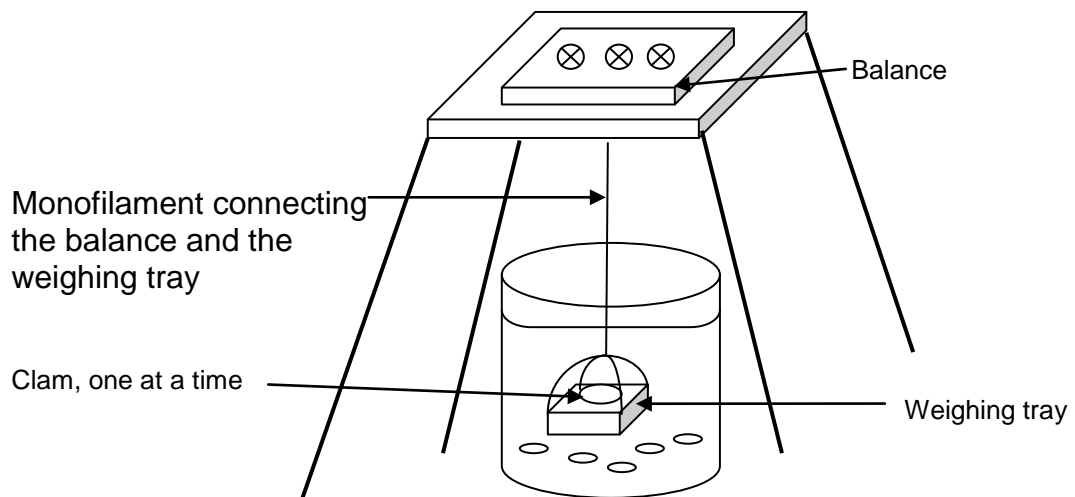


Fig. 8: System to measure wet weight of pipi while submerged in the water containers.

3.4.4. Dry weight determination of microalgal cells

For the determination of the dry weights of microalgae, 200 mL of the cultures were collected and cell densities were determined using a haemocytometer. Algal cells were filtered and collected through 0.45 μm pore-size fibre filters. The filters were then rinsed with 100 mL of 0.5 M ammonium formate to remove sea salt residue (Epifanio, 1979), and dried for 12 hours at 100°C. The dry weights of the algal cells were calculated as follows (see Table 5):

$$\text{Dry weight of algae (pg cell}^{-1}\text{): } \frac{[\text{Filter paper with algal cells}] - [\text{Filter paper}]}{\text{Number of cells}}$$

Table 5: Dry weights of the microalgal diets used in the culture of pipi.

Species	Dry weight (pg cell ⁻¹)
<i>Isochrysis galbana</i> (T-ISO)	31.64 \pm 0.01
<i>Pavlova lutheri</i>	110 \pm 0.02
<i>Tetraselmis suecica</i>	172.81 \pm 0.03
<i>Chaetoceros muelleri</i>	73.38 \pm 0.01
<i>Thalassiosira pseudonana</i> (3H)	29.67 \pm 0.02

3.4.5. Biochemical composition

Protein, carbohydrate, lipid, moisture, and ash content was determined for each diet and adult pipi tissues. Spat and juveniles provided insufficient materials to carry out the biochemical analyses; therefore, only the adult samples were analysed for evaluating the nutritional benefit of each diet on the growth and body content of pipi.

Before the start of the feeding trial, 30 adult pipi were frozen for the initial biochemical composition after 3 days acclimation. At day 21, following the growth measurements, the remaining adult pipi were sacrificed and the soft tissues were separated from the shell. The frozen tissues from the initial samples were thawed. The tissues (initial and tested samples) were carefully blotted with absorbent papers to remove excess wetness prior to oven drying. All the analyses were performed according to the Official Methods of Analysis of AOAC International (AOAC, 2005).

3.4.5.1. Total protein

Samples were analysed according to the Kjeldahl method. The method determines total nitrogen content and protein, as the nitrogen content of the sample multiplied by a conversion factor. A conversion factor of 6.25 is used for many applications including in the present study (AOAC, 2005). The method can be divided into three main steps: 1) digestion of the samples in sulphuric acid with a catalyst, which converts nitrogen into ammonia; 2) distillation of the

ammonia into a trapping acid solution; and 3) titration with hydrochloric acid to quantify the ammonia.

3.4.5.1.1. Digestion of samples

The purpose of the digestion was to break down the complex structure of protein and other forms of nitrogen and convert them into ammonia. The homogenous dry diet and animal tissue samples were placed in Kjeldahl tubes. Digestion was accomplished by boiling the homogenous dry diet and tissue samples (approximately 1.0 g) in 10 mL of concentrated sulphuric acid (98%) with the addition of 10 mL of 35% hydrogen peroxide solution. A mixture of 7.5 g of copper sulphate and potassium sulphate (9:1 ratio) catalyst was then added into each tube. The boiling was completed when the digested samples turned clear. Distilled water (50 mL) was then added into the tubes.

3.4.5.1.2. Distillation

This step involved separation of the ammonia from the digestion mixture. This was achieved by raising pH of the digestate with sodium hydroxide, which converts the ammonium ion into ammonia. The distillations were carried out with the semi-automatic steam distilling unit (UDK 126A, Velp Scientifica Ltd., Milano, Italy). Indicating boric acid solution (25 mL) was added to a 250 mL Erlenmeyer flask. The digestates were distilled with the addition of 45% sodium hydroxide, and the distillates were collected in the Erlenmeyer flasks containing boric acid. Then, distillation ceased when 150 mL distillate was collected in each flask. Ten drops of green methyl indicator were then added into each flask to indicate the acidity of solutions.

3.4.5.1.3. Titration with hydrochloric acid (HCl)

The purpose of the titration step was to quantify the amount of nitrogen present in the sample. The distillates were titrated with 0.05 M hydrochloric acid solution until they turned from green to red. The titrated volumes of hydrochloric acid were recorded. Total protein contents were calculated as follows:

$$\text{Total protein (\%)}: \frac{6.25 \times 14.01 \times (\text{mL titrant} - \text{mL blank})}{\text{Sample weight (g)}} \times \frac{(0.05 \text{ M HCl})}{1000} \times 100$$

3.4.5.2. Total lipid

A modified Bligh and Dyer method (1959) was used to extract and measure the total lipids. Samples (0.5 g) were put into 15 mL centrifuge tubes and then rehydrated with Millipore pure water. Then, a 3 mL mixture of methanol and chloroform (2:1) was added into each tube and carefully shaken to homogenise the contents.

Chloroform (1 mL) was added to the samples and the tubes were arranged in a tube rack. The tubes containing samples were sonicated for 10 minutes. Then, 1 mL of distilled water was added into each tube and further sonicated for 10 minutes.

After sonication, samples were centrifuged for 10 minutes at 2000 rpm. The samples showed two phases separated by a protein disc and sample bits. The tube contents were filtered on filter papers. The lower chloroform layer containing lipids was collected with a Pasteur pipette and transferred into a small pre-weighed glass tube. The glass tubes were re-weighed.

Tubes were then placed in a warm water bath (38-40°C) equipped with a nitrogen stream blow-down apparatus. The chloroform phase was evaporated to dryness under the stream of nitrogen. Tubes containing lipid extracts were re-weighed. Total lipids were determined as follows:

$$\text{Total lipid (\%)}: \frac{\text{Lipid extract (g)}}{\text{Lipids in chloroform solvent (g)}} \times 100$$

3.4.5.3. Total carbohydrate

Total carbohydrate content of each diet and adult tissue content was estimated by 'difference'. The sum of protein, lipid, and ash content was subtracted from 100 (Porter & Earl, 1990). Total carbohydrates were calculated using the formula:

$$\text{Total carbohydrate (\%)}: 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ lipid})$$

3.4.5.4. Moisture

Moisture content of the diet and tissue samples was determined by difference between the weights of the wet and oven dry samples. The tissue wet weights were measured using a balance (Shimadzu AUW 320, Kyoto, Japan) to the nearest 0.0001 g, before drying to a constant weight in an oven for about 24 hours at 60°C (Watvic oven). After drying, the samples were immediately placed in a desiccator to cool to room temperature for an hour, and then weighed. The percent moisture was calculated by multiplying the difference between the wet and dry samples by 100. After moisture content determinations, the dry tissues

were cut into small pieces and thoroughly mixed to ensure uniformity. The dry diet and tissue samples were used for the analyses of ash, total protein, total lipid, and total carbohydrate contents.

3.4.5.5. Ash

Ash content represents the quantity of the non-combustible material remaining in the samples after burning, which provides a measure of the total amount of minerals present within a sample (Fjell et al., 1996). Weighed dry samples (approximately 1.0 g) were placed into dried and pre-weighed porcelain crucibles. Samples were then burned in a furnace (SR-6000 McGregor Shimaden Co. Ltd., Japan) at 600°C for two hours and cooled to room temperature in a desiccator. Crucibles containing samples were then re-weighed. Weights before and after burning were recorded, and the differences were multiplied by 100 to get ash percent.

3.4.6. Statistical analyses

Data on the growth parameters (lengths and weights), mortality, and biochemical compositions (eight diets and soft tissues) were analysed with parametric statistics after all assumptions were met and transformations undertaken, when appropriate. The homogeneities of variances in different samples were assessed with Levene's tests. The proportions of shell lengths, live weights, mortality rates, and biochemical contents were transformed by arcsine square root transformation before subjecting to one-way analyses of variances (ANOVAs). Tukey's HSD *Post Hoc* tests (at $p < 0.05$) were conducted

to determine the differences between the means of all treatments. All analyses were performed with SPSS version 15.0 software.

3.5. RESULTS

3.5.1. Growth responses to different diets

Growth of spat, juvenile, and adult pipi varied considerably among diets. Increases in shell length (mm) and wet weight (mg) of spat, juvenile, and adult pipi fed different diets in relation to their initial lengths and weights are presented in Figure 9. In general, the wet weight of the starved pipi decreased during the starvation period. However, the shells still recorded minimum increments.

3.5.1.1. Growth of spat pipi

At the end of the 21-day experiment, spat that had the greatest length increase were those fed on *I. galbana* (1.21 ± 0.10 mm individual⁻¹). However, the increase was not significantly different to spat fed *P. lutheri* (0.91 ± 0.09 mm individual⁻¹), *C. muelleri* (0.99 ± 0.04 mm individual⁻¹), or yeast (1.00 ± 0.18 mm individual⁻¹) (Tukey test, $p > 0.05$). Significantly, slower growths were recorded in spat fed *T. suecica* (0.66 ± 0.08 mm individual⁻¹), *T. pseudonana* (0.83 ± 0.27 mm individual⁻¹), wheat flour (0.79 ± 0.05 mm individual⁻¹), and corn flour (0.72 ± 0.01 mm individual⁻¹) (Tukey test, $p > 0.05$). The unfed spat exhibited the smallest shell length increase with only 0.06 ± 0.01 mm per individual, which was significantly different to all fed groups (Tukey test, $p > 0.05$) (Fig. 9A).

In terms of wet weight, spat fed *P. lutheri* (125.14 ± 8.69 mg individual⁻¹) were significantly heavier than spat fed other diets or the unfed group ($p < 0.05$).

Spat fed yeast displayed a 92.33 ± 4.42 mg weight increase per individual, followed by those fed *I. galbana* (84.52 ± 12.37 mg individual⁻¹), wheat flour (74.94 ± 3.08 mg individual⁻¹), *C. muelleri* (73.64 ± 8.68 mg individual⁻¹), *T. suecica* (70.69 ± 1.77 mg individual⁻¹), corn flour (68.12 ± 18.58 mg individual⁻¹), *T. pseudonana* (65.30 ± 8.86 mg individual⁻¹), and the control group (-24.04 ± 1.30 mg individual⁻¹). It was noted that starvation resulted in significant weight losses during the three weeks of the culture period (Tukey test, $p < 0.05$) (Fig. 9B).

3.5.1.2. Growth of juvenile pipi

When a daily diet of 4% *I. galbana* was offered to juveniles, they exhibited the greatest length increase (1.72 ± 0.18 mm individual⁻¹) (Fig. 9C). However, the increase did not differ significantly from the increments obtained by juveniles fed *P. lutheri* (1.34 ± 0.16 mm individual⁻¹) and *T. suecica* (1.31 ± 0.44 mm individual⁻¹) (Tukey test, $p > 0.05$). Tukey tests showed that juveniles fed *T. pseudonana*, yeast, wheat flour, and corn flour were similar to those fed *C. muelleri* ($p > 0.05$). The lowest length increase for juvenile pipi was observed in the unfed group (0.033 ± 0.037 mm individual⁻¹).

The greatest wet weight increase in juvenile pipi was recorded in animals fed *P. lutheri* (177.94 ± 8.12 mg individual⁻¹), followed by *I. galbana* (163.27 ± 19.88 mg individual⁻¹), *C. muelleri* (154.63 ± 43.58 mg individual⁻¹), *T. pseudonana* (120.22 ± 8.49 mg individual⁻¹), corn flour (104.23 ± 7.16 mg individual⁻¹), *T. suecica* (96.72 ± 32.29 mg individual⁻¹), yeast (84.87 ± 5.78 mg individual⁻¹), and wheat flour (77.47 ± 17.29 mg individual⁻¹). Starved juveniles displayed a

significant decrease in terms of wet weight (-37.02 ± 7.22 mg individual⁻¹) (Tukey test, $p < 0.05$) (Fig. 9D).

3.5.1.3. Growth of adult pipi

For adult pipi, the greatest shell increase was on individuals fed corn flour. The increase was 0.79 ± 0.29 mm individual⁻¹, which was similar to adults fed *P. lutheri* (0.53 ± 0.09 mm individual⁻¹), yeast (0.51 ± 0.15 mm individual⁻¹), and wheat flour (0.59 ± 0.10 mm individual⁻¹) (Tukey test, $p < 0.05$). Adults fed with *I. galbana*, *T. suecica*, *C. muelleri*, and *T. pseudonana* displayed 0.33 ± 0.04 mm, 0.32 ± 0.13 mm, 0.29 ± 0.04 , and 0.26 ± 0.08 mm length increment per individual, respectively. The unfed adult pipi showed a minimal shell length increase (0.01 mm individual⁻¹) (Fig. 9E).

The greatest weight gain for adult pipi was on animals fed *P. lutheri* (167.74 ± 22.34 mg individual⁻¹). However, the increase was similar to those offered *C. muelleri*, yeast, and corn flour (Tukey test, $p > 0.05$). Adults maintained with *I. galbana*, *T. suecica*, *T. pseudonana*, and wheat flour showed 93.06 ± 15.46 mg, 118.63 ± 12.77 , 89 mg, 89.11 ± 17.80 mg, 80.69 ± 16.94 mg of wet gains per individual, respectively. The starved group was different to all fed groups (Tukey test, $p > 0.05$) (Fig. 9F).

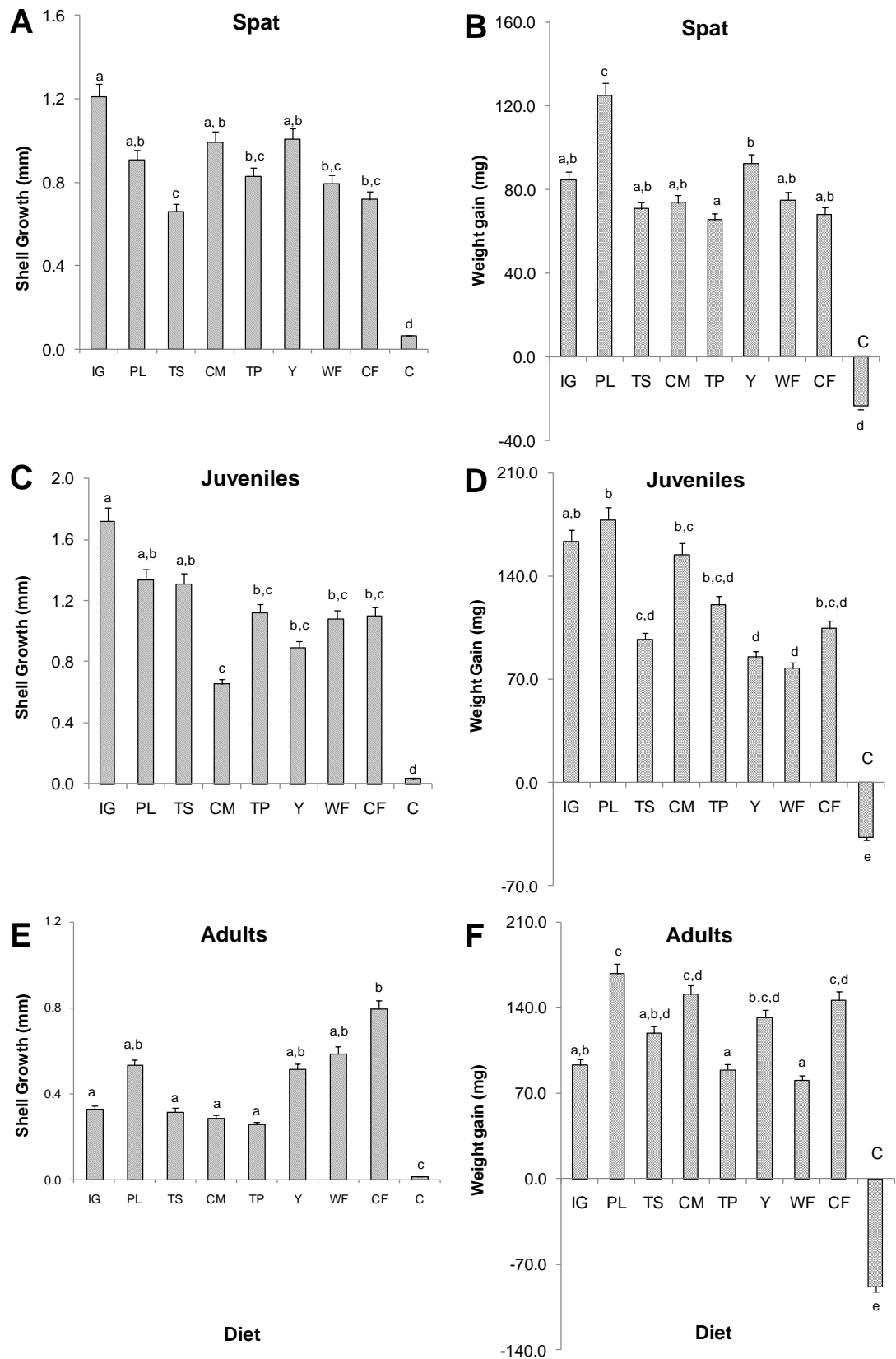


Fig. 9: Shell growth (mm) and weight gain (mg) for *P. australis* fed various diets for 21 days. 9A-B; spat, 9C-D; juveniles, 9E-F; adults. Abbreviations: IG; *I. galbana* (T-ISO), PL; *P. lutheri*, TS; *T. suecica*, CM; *C. muelleri*, TP; *T. pseudonana* (3H), Y; baker's yeast, WF; wheat flour, CF; corn flour, and C; control unfed group. The same letters over the error bars indicate non-statistical differences. Error bars represent 95% confidence interval of the mean.

3.5.2. Mortality

Relatively low mortality was observed after the 21-day experiment across all treatments for spat, juvenile, and adult pipi. Spat that were starved displayed the highest percent mortality at $22.22\% \pm 3.85$ SD, followed by spat fed yeast ($18.89\% \pm 3.85$ SD). Spat fed *I. galbana*, *T. suecica*, *C. muelleri*, *T. pseudonana*, and yeast treatments had pipi mortalities of $16.67\% \pm 3.33$ SD, $17.78\% \pm 8.39$ SD, $13.33\% \pm 3.33$ SD, $15.56\% \pm 5.09$ SD, and $14.44\% \pm 1.92$ SD, respectively. The lowest mortality was found in spat fed *P. lutheri* ($12.22\% \pm 3.85$ SD) and wheat flour ($12.22\% \pm 1.92$ SD) (Fig. 10A).

The highest mortality for juvenile pipi was found in animals fed on yeast ($33.33\% \pm 2.89$ SD), followed by *I. galbana* ($25.00\% \pm 10.00$ SD) and wheat flour ($25.00\% \pm 5.00$ SD) (Fig. 10B). Juveniles fed *T. suecica*, *C. muelleri*, *T. pseudonana* had the same percent mortality at 21.67%. Meanwhile, juveniles fed *C. muelleri* and corn flour had a similar percent mortality at $20.00\% \pm 5.00$ SD. The lowest mortality was found in juveniles fed on *P. lutheri* ($18.33\% \pm 2.89$ SD).

For adult pipi, the highest mortality was for pipi fed yeast ($33.33\% \pm 5.77$ SD), followed by adults fed wheat flour ($30.00\% \pm 10.00$ SD), and unfed ($30.00\% \pm 10.00$ SD). Adults fed *T. suecica* displayed a mortality of $26.67\% \pm 5.77$ SD. Juveniles fed *P. lutheri*, *C. muelleri*, and *T. pseudonana* showed the same mortality at $23.33\% \pm 5.77$ SD. The lowest mortality was found in adults fed corn flour ($16.67\% \pm 5.77$ SD) (Fig. 10C).

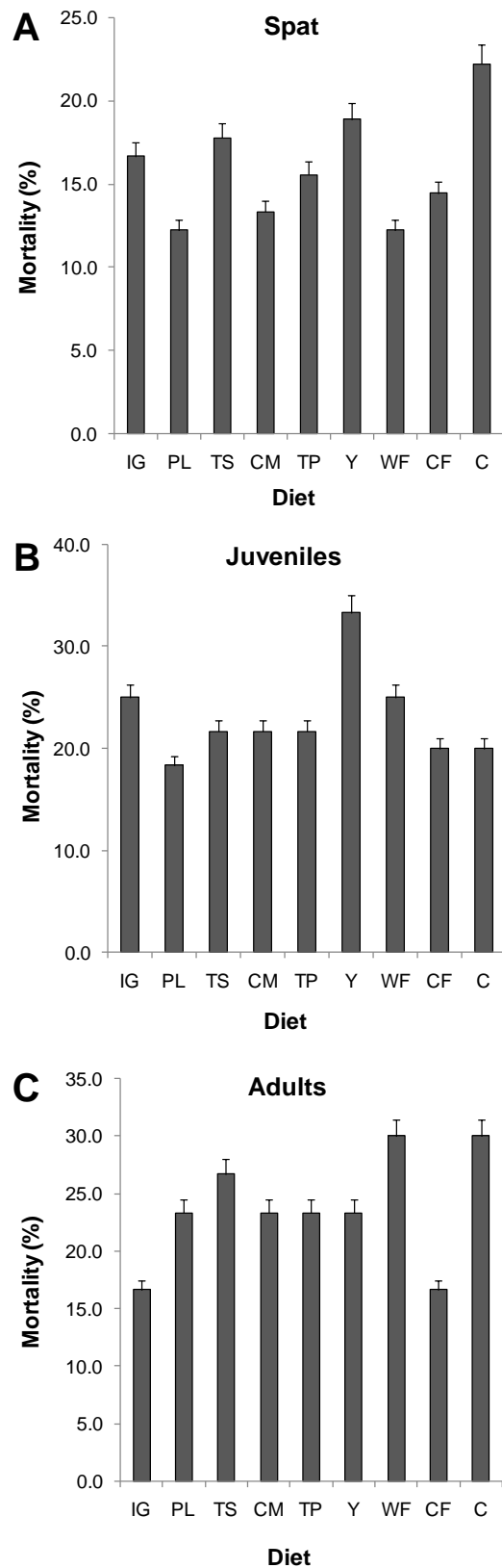


Fig. 10: Mortality of spat (A), juvenile (B), and adult (C) pipi during the 21-day feeding experiment. Clams were fed IG: *I. galbana*, PL: *P. lutheri*, TS: *T. suecica*, CM: *C. muelleri*, TP: *T. pseudonana*, Y: yeast, WF: wheat flour, and CF: corn flour. Control group (C) was the unfed clams. No statistical differences were observed among all treatments for spat, juvenile, and adult pipi. Error bars represent 95% confidence interval of the mean.

Biochemical composition of diets and adult tissues

Biochemical composition of the experimental diets (microalgal and processed diets) fed to spat, juvenile, and adult pipi are presented in Table 6. In general, the microalgal diets (*I. galbana*, *P. lutheri*, *T. suecica*, *C. muelleri*, and *T. pseudonana*) contained high proportion of protein and lipid, and low carbohydrate. In contrast, the processed diets (baker's yeast, corn flour, and wheat flour) were low in protein and lipid but high in carbohydrate. The highest protein content ($33.54\% \pm 1.54$ SD) among all diets was observed in *T. pseudonana* (3H). Microalga *P. lutheri* contained the highest proportion of lipids ($23.21\% \pm 2.93$ SD), while wheat flour had the highest amount of carbohydrates ($80.18\% \pm 2.81$ SD) (Table 6). Microalga *I. galbana* contained $21.30\% \pm 0.59$ SD protein, $19.06\% \pm 0.65$ SD lipid, and $19.59\% \pm 1.86$ SD carbohydrate.

Tissue quality data of adult pipi of the initial samples and individuals fed the experimental diets are shown in Table 7. Protein and lipid increased in the tissues for all treatments except for the controls (ANOVA, $F_{(9, 20)} = 338.82$, $p < 0.05$). The largest proportion of total protein content was found in adults fed *P. lutheri* ($33.75\% \pm 0.87$ SD). However, the protein content did not differ from that of those fed yeast (Tukey test, $p > 0.05$). Lipid content was highest in adult pipi fed *I. galbana* ($6.13\% \pm 0.53$ SD). However, the lipid content was not significantly different from those fed *P. lutheri* ($5.41\% \pm 0.45$ SD) and corn flour ($4.46\% \pm 0.36$ SD) (Tukey test, $p > 0.05$). The control group lost 11.8% of its lipid reserve and indeed, the value was similar with the initial value (Tukey test, $p = 0.961$).

Meanwhile, carbohydrate levels in the tissues decreased during the 21-day experimental period for all fed groups and the control. However, there were insignificant decreases for carbohydrate levels in adult pipi fed *T. suecica*, *C. muelleri*, wheat flour, and the control group (Tukey test, $p < 0.05$).

Table 6: Biochemical composition (%) of the microalgal and substitute diets used for rearing *P. australis* spat, juveniles, and adults in the laboratory (mean \pm SD).

Diets	Protein	Lipid	Carbohydrate
<i>Isochrysis galbana</i> (T-ISO)	21.30 \pm 0.59	19.06 \pm 0.65	19.59 \pm 1.86
<i>Pavlova lutheri</i>	27.23 \pm 1.69	23.21 \pm 2.93	9.44 \pm 3.29
<i>Tetraselmis suecica</i>	29.74 \pm 1.51	10.90 \pm 0.60	18.68 \pm 3.49
<i>Chaetoceros muelleri</i>	31.79 \pm 0.48	15.63 \pm 1.37	20.34 \pm 3.02
<i>Thalassiosira pseudonana</i> (3H)	33.54 \pm 1.54	18.86 \pm 1.55	13.56 \pm 5.31
Yeast	5.36 \pm 0.12	1.80 \pm 0.24	77.19 \pm 1.66
Wheat flour	8.34 \pm 0.16	1.57 \pm 0.38	80.18 \pm 2.81
Corn flour	8.39 \pm 1.01	5.56 \pm 0.68	76.79 \pm 0.47

Table 7: Biochemical composition (%) of adult *P. australis* at the beginning and at the end of the experimental period (mean \pm SD).

Diets	Protein	Lipid	Carbohydrate
Initial	6.99 \pm 0.43 ^a	1.02 \pm 0.15 ^a	17.20 \pm 3.47 ^c
<i>Isochrysis galbana</i> (T-ISO)	24.27 \pm 0.88 ^e	6.13 \pm 0.53 ^e	7.03 \pm 0.94 ^{a,b}
<i>Pavlova lutheri</i>	33.75 \pm 0.87 ^f	5.41 \pm 0.45 ^{d,e}	4.04 \pm 0.20 ^a
<i>Tetraselmis suecica</i>	10.77 \pm 0.44 ^b	2.67 \pm 0.81 ^b	16.32 \pm 2.38 ^c
<i>Chaetoceros muelleri</i>	14.06 \pm 0.39 ^c	2.92 \pm 0.27 ^{b,c}	10.28 \pm 2.37 ^{b,c}
<i>Thalassiosira pseudonana</i> (3H)	18.19 \pm 1.60 ^d	3.33 \pm 0.54 ^{b,c}	7.53 \pm 1.52 ^{a,b}
Yeast	23.47 \pm 1.43 ^e	4.28 \pm 0.28 ^{c,d}	7.98 \pm 1.58 ^{a,b}
Wheat flour	18.86 \pm 0.28 ^d	2.08 \pm 0.70 ^b	13.31 \pm 4.08 ^{b,c}
Corn flour	18.92 \pm 0.39 ^d	4.46 \pm 0.36 ^{c,d,e}	7.90 \pm 1.49 ^{a,b}
Control	7.53 \pm 0.28 ^b	0.90 \pm 0.14 ^a	12.18 \pm 3.97 ^{b,c}

3.5.3. Growth and dietary content of pipi

Biochemical composition of the experimental diets affected the growth of pipi of all size classes. In spat, dietary lipid contents were found to be a factor for growth determination. High lipid levels increased the shell length and weight gain (Figs. 11B & 11E). Dietary protein also increased shell length in spat (Fig. 12A) but had no effect on spat weight gain (Fig. 11D) (Pearson correlation = 0.006, $p > 0.05$). Carbohydrate level displayed a negative relationship with both spat shell length and weight gain (Figs. 11C & 11F).

For juvenile pipi, dietary protein and lipid contents positively affected shell length and weight gain (Figs. 12A-12E). However, the relationship between protein level and shell growth was weak ($R^2 = 0.007$) (Fig. 12A). Weight gain and protein content displayed a moderate positive relationship ($R^2 = 0.2778$), while carbohydrates did not have effect on shell length or weight gain of juvenile pipi (Figs. 12C & 12F).

Weight gain in adult pipi was significantly correlated with dietary protein and lipid contents. However, the correlations were weak, $R^2 = 0.0057$ and $R^2 = 0.0328$ for protein and lipid, respectively (Figs. 13D & 13E). Protein and lipids had no effects on the shell length of adult pipi. (Figs. 13A & 13B). Meanwhile, levels of carbohydrates in the diets had a significant effect on shell length of adult pipi (Pearson correlation = 0.624, $R^2 = 0.3894$) (Fig. 13C).

SPAT PIPI

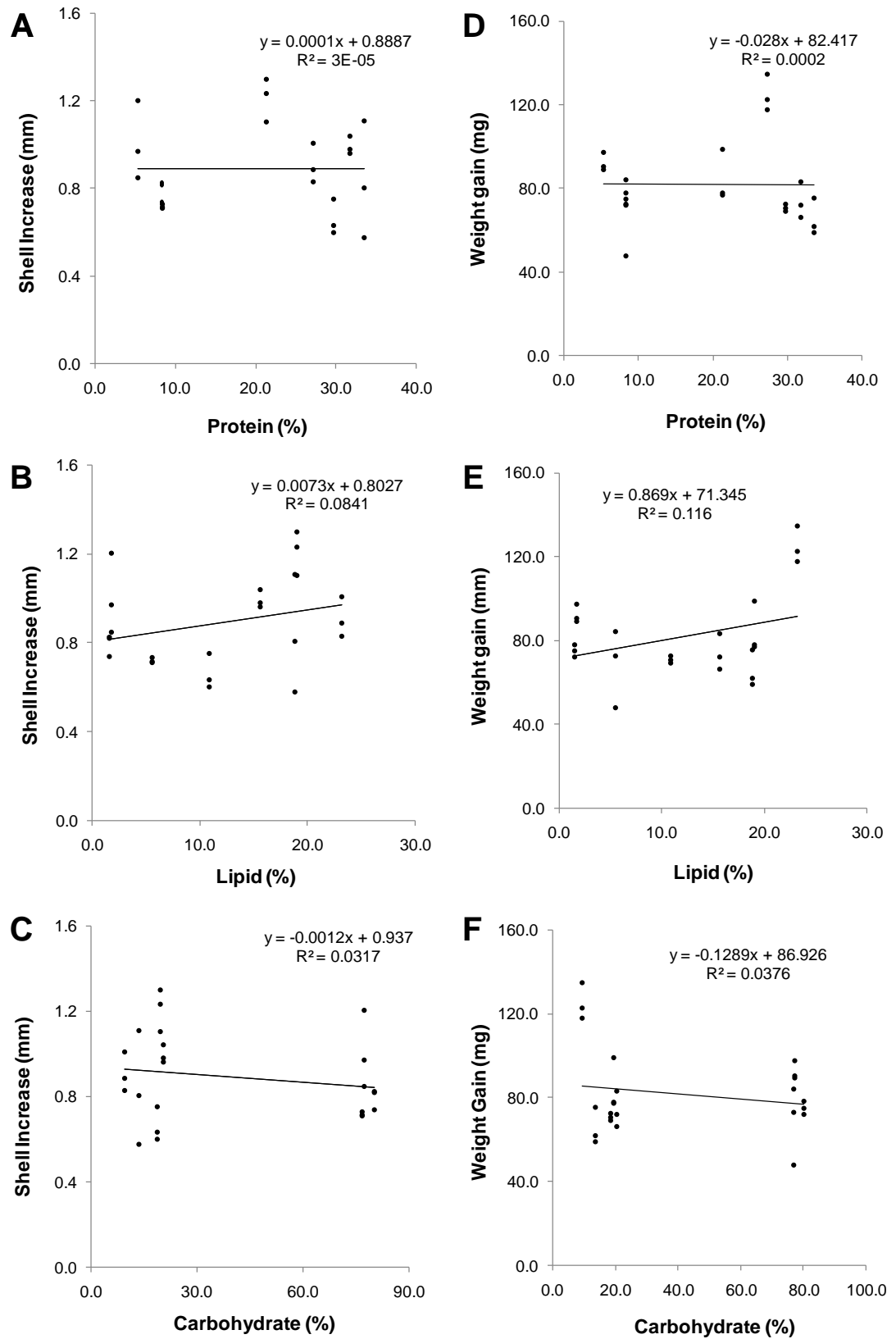


Fig. 11: Relationships between growth (shell increase and weight gain) and biochemical composition of the diets fed to the spat of *P. australis*. The linear regression equations are shown on the graphs

JUVENILE PIPI

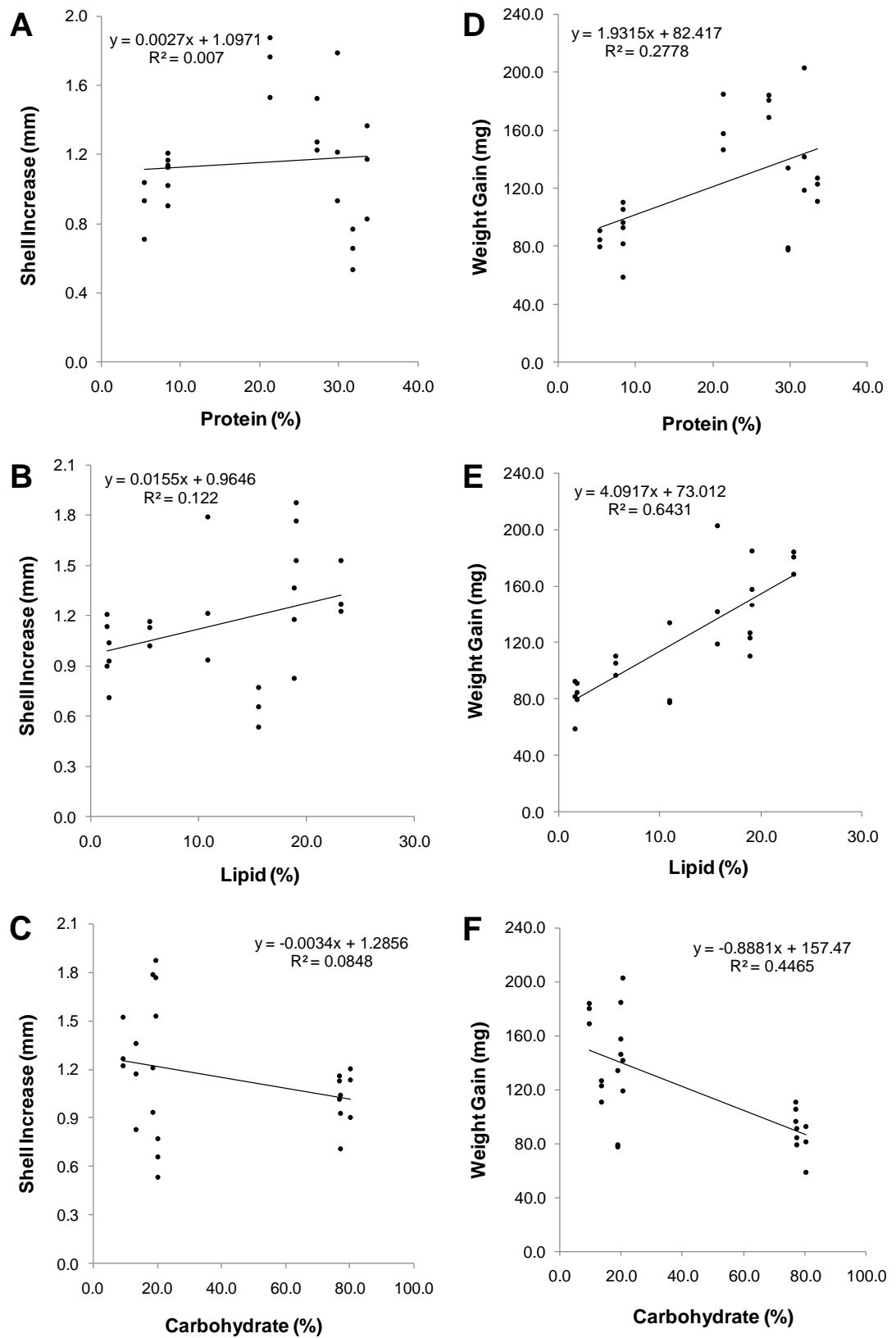


Fig. 12: Relationships between shell increase (shell increase and weight gain) and biochemical composition of the diets fed to the juveniles of *P. australis*. The linear regression equations are shown on the graphs.

ADULT PIPI

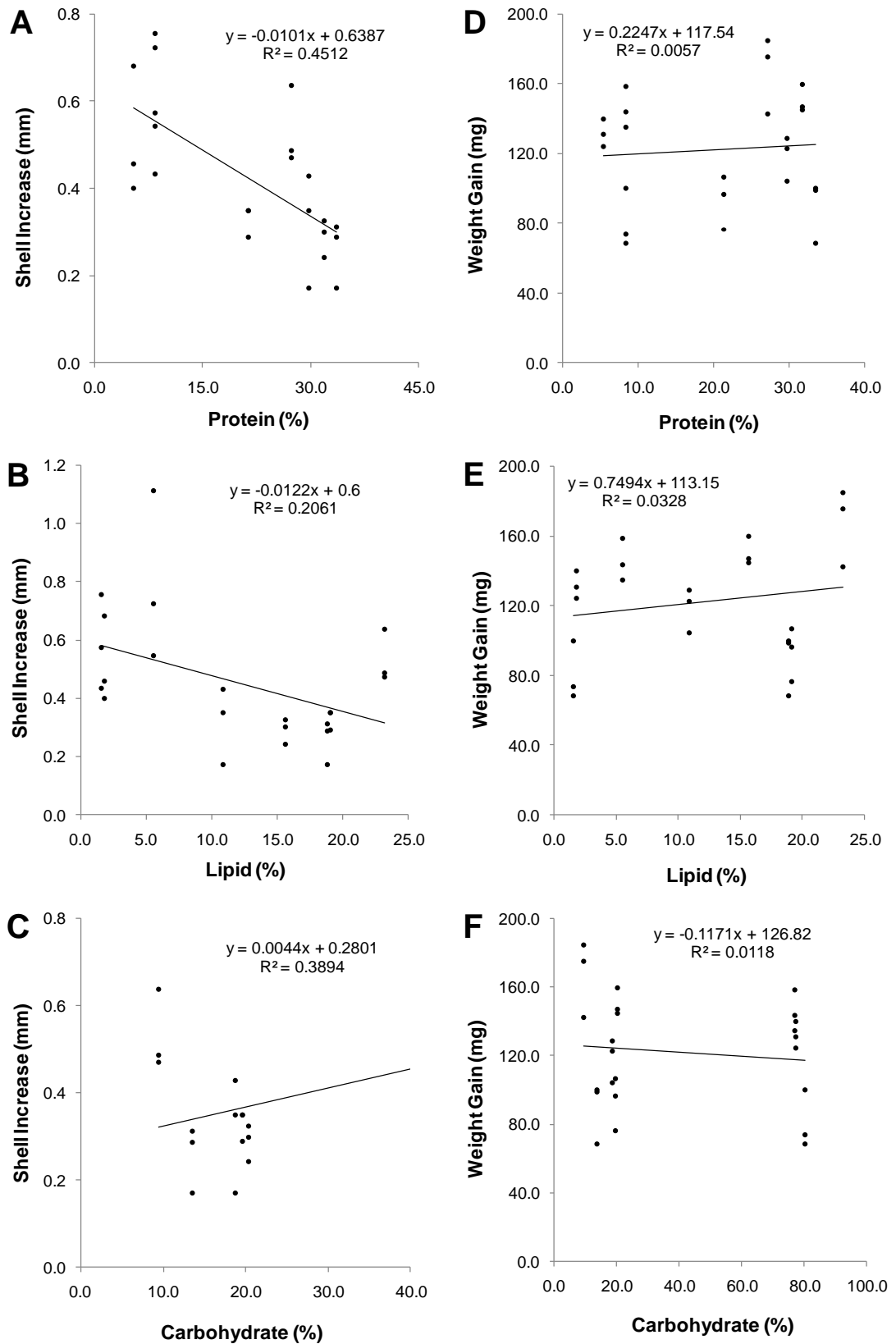


Fig. 13: Relationships between shell increase (shell increase and weight gain) and biochemical composition of the diets fed to the adults of *P. australis*. The linear regression equations are shown on the graphs.

3.6. DISCUSSION

3.6.1. Effects of the different diets on growth and mortality

Results of these experiments demonstrate that the processed diets (yeast, wheat flour, and corn flour) are potential substitutes for pipi feeds grown under hatchery conditions. The use of yeast for spat pipi, for example, showed similar shell growth as *I. galbana* (t-test, $p > 0.05$). Spat fed wheat and corn flours showed no differences in shell growth when compared to those fed the microalgal diets, except for *I. galbana* (t-test, $p < 0.05$). For juveniles, the corn flour fed animals had similar weight gain with *I. galbana* ($p > 0.05$). Microalga *I. galbana* was the main source of energy for weight gain in juvenile pipi (Fig. 9D). All fed clams, regardless of size class, grew relatively better than the no food control. It was apparent that *P. lutheri* microalga mostly supported weight gains for all size classes.

In general, spat and juvenile pipi grew faster than adults. During the earlier life stages (larvae, spat, and juveniles), the body is actively metabolising, mainly to support growth. Therefore, nutrients derived from diets are catabolised to provide energy for growth. The same growth pattern has been observed in other bivalve species such as in the rock-boring clam *Penitella penita* (Evans, 1968), the fingernail clam *Sphaerium striatinum* (Hornbach *et al.*, 1983), the Asiatic clam, *Corbicula fluminea* (Welch & Joy, 1984), the hard clam *M. mercenaria* (Lorio & Malone, 1995), the Antarctic clam *Laternula elliptica* (Ahn & Shim, 1998), the black clam *Villorita cyprinoides* (Arun, 2009).

Unfed spat and adult pipi showed highest mortalities, while for juveniles, the highest mortality was found in yeast-fed animals. These results indicate the inability of pipi to survive a long period of starvation. Starvation may not have only caused reduction in wet weight mass of pipi, but also depletion of energy level needed to maintain vital functions.

The use of yeast, wheat flour, and corn flour have been reported as potential substitutes for microalgae in other bivalve species. For example, Perez-Camacho *et al.* (1998) suggested the use of corn-based diet to substitute 50% of the 2% phytoplankton daily ration in the little neck clam, *R. decussatus*. In another study, Albentosa *et al.* (2002) found no significant difference between spat fed 100% *I. galbana* and a mixed diet consisting of 50% *I. galbana* and 50% wheat germ flour in the Manila clam (*R. philippinarum*). In regard to substituting yeast for microalgal diets, many studies have shown positive results for growth in *R. philippinarum* (Coutteau *et al.*, 1992), the hard clam *M. mercenaria* (Epifanio, 1979; Coutteau *et al.*, 1994), the blue mussel *Mytilus edulis* (Epifanio, 1979), the bay scallop *Argopecten irradians* (Epifanio, 1979), the Pacific oyster *C. gigas* (Coutteau *et al.*, 1992), and the Sydney rock oyster *Saccostrea commercialis* (Brown *et al.*, 1996). However, growth in the soft tissue of *C. gigas* oysters decreased with the increasing amount of yeast in the diet (Epifanio, 1979).

3.6.2. Effect of dietary contents of the diets on growth performance

High protein contents in dietary microalgae were reported to enhance growth performance in the short-neck clam *Tapes japonica* (Gallager & Mann, 1981),

the Pacific oyster *C. virginica* (Webb & Chu, 1983), the European flat oyster *Ostrea edulis* (Enright *et al.*, 1986), and the Mediterranean mussel *Mytilus galloprovincialis* (Langdon & Onal, 1999) juveniles. On the other hand, Uriarte and Farias (1999), working with the scallop *Argopecten purpuratus*, and Ceron-Ortiz *et al.* (2006) with *Lyropecten (Nodipecten) subnodosus*, found that spat of both species grew better with higher lipid levels in the diets. Results of the present study are in agreement with Uriarte and Farias (1999) and Ceron-Ortiz *et al.* (2006). Smaller pipi (spat and juveniles) demonstrated positive relationships between length and weight increments and dietary lipid contents (Figs. 11B, 11E, 12B, & 12E). On the basis of biochemical composition of the experimental diets used in this study (Table 5), it is inferred that lipid plays a major role in determining weight gain in spat and juveniles. In support to this, growth of *O. edulis* spat also was influenced by the amount of lipids in larval tissues during metamorphosis and in the diets (Laing & Millican, 1986). Similarly, a significant correlation between growth and dietary lipid content was observed in *C. virginica* spat (Wikfors *et al.*, 1984). However, Waldock and Nascimento (1979) and Langdon and Waldock (1981) found that level of lipids in diets was negatively correlated with growth rates of *C. gigas* larvae and spat.

Conversely, adult pipi showed preference for diet with high carbohydrate level, particularly corn flour, which contained $76.79\% \pm 0.47$ SD carbohydrates (Table 7). The highest percentage of shell increase in the adults was obtained with corn flour, while the greatest weight gain was found in *P. lutheri*-fed animals. It is noteworthy that adults fed with 100% corn flour had low mortality over 21 days (16.67%), and the value was same with *I. galbana*-fed adults (Fig. 13C). In addition, adult pipi showed a positive relationship between the total

carbohydrate content and shell increase (Table 6). Hence, corn flour could be a potential diet to adult pipi. Carbohydrates are important to balance the utilisation of proteins and lipids in catabolic pathways for energy production (Whyte *et al.*, 1989) and are also regarded as the principal energy reserve for adult marine bivalves; both for gametogenesis and during food deficiency especially in winter time (Reid, 1969).

Growth of bivalves is not merely elucidated by the biochemical composition in diets, but is also dependent on other factors, more likely to be cell attributes and interactive effects of nutrients in diets (Knaeur & Southgate, 1999). For example, *P. lutheri* and *I. galbana* cells are small in size and more round in shape, making them easier to be ingested and further digested in the guts, particularly during the earlier life stages. Additionally, *I. galbana* cells have a balanced proportion of proteins, lipids, and carbohydrates (Table 6). Diatom species (e.g., *C. muelleri* and *T. pseudonana*) are less digestible due to their siliceous cell walls and presence of spines around the margin of the cells. Feeding a number of microalgal species is more beneficial for rapidly growing bivalves than a single species (Brown, 1991).

3.6.3. Effect of the diets on body composition of adult pipi

Proximate analyses of adult pipi revealed that proteins and lipids were accumulated in the tissues for all fed groups, while carbohydrate levels depleted in all treatments including the control group. The concomitant increase in the levels of proteins and lipids and carbohydrate depletions, were due to accumulation of mature oocytes. These results suggest that the gonadal

development have initiated during the breeding season. This conforms with other studies where accumulation of proteins in bivalve tissues are simultaneously an indicator to maturation of gonads (Devi *et al.*, 1985; Kreeger, 1993; Rivonker & Parulekar, 1995), and as a reserve prior to spawning activity (Wafer *et al.*, 1976; Nagabhushanam & Mane, 1978; Gabbott & Peek, 1991). Proteins and lipids were continuously metabolised to support energy demand whenever needed (food scarcity or insufficient carbohydrates).

Meanwhile, carbohydrates in adult tissues were rapidly catabolised to meet the energy requirements for reproduction (Table 7). Similar observations were made by Rodriguez *et al.* (1993) who regarded carbohydrates as the main energy source for gametogenesis in clams *T. decussatus* and *R. philippinarum*. For adult oysters, cornstarch feeds have been used to complement natural microalgae to improve condition index of the animals (Haven, 1965; Ingle, 1967; Dunathan *et al.*, 1969). These findings emphasised the importance of carbohydrates accumulation as energy reserves in the form of glycogen in gametogenesis (Gabbott, 1976). Starvation in adult pipi resulted to expenditures of lipid and carbohydrate resources. This is explained by the functions of lipids and carbohydrates during food scarcity (Gabbott & Bayne, 1973; Beninger & Lucas, 1984; Whyte *et al.*, 1990).

3.6.4. Microalgal and substitute diets

In recent years, there have been numerous studies on microalgal replacements for bivalve cultures. Among these, partial substitutions with alternative diets have been studied in other clam species such as *R. philippinarum* (Albentosa *et*

al., 1989; Coutteau *et al.*, 1993; Caers *et al.*, 1999; Albentosa *et al.*, 2002), *R. decussatus* (Albentosa *et al.*, 1997; Albentosa *et al.*, 1999), *C. fluminea* (Foe & Knight, 1986), and *M. mercenaria* (Coutteau, 1991). The use of low-cost processed diets used in the present study would allow an increase in pipi production in hatcheries by reducing the operational costs.

Microalgae are still the main nutrient sources for pipi, particularly for spat and juveniles. This is true for larval and juvenile cultures as observed in other bivalve species (Knauer & Southgate, 1999; Pales Espinosa & Allam, 2006; Martinez-Fernandez & Southgate, 2007). However, baker's yeast would be a good substitute for feeding spat and juvenile pipi. The experimental microalgae contained higher protein and lipid levels, while wheat and corn flours were rich in carbohydrates. Growth in smaller pipi was promoted by higher lipid contents, so, high carbohydrate diets (especially wheat and corn flours) were less suitable for their growth. In contrast, adult pipi required carbohydrates to support growth and reproduction. Thus, it is suggested that wheat and corn flours would be good substitutes for adult feeds. The outcomes of this study are useful in providing better understandings on nutrients required by pipi and proposing the use of processed diets in their feeding.

CHAPTER FOUR
EFFECT OF DIETS ON MATURITY OF *Paphies australis*

4.1. INTRODUCTION

The breeding period in pipi occurs from late winter to late summer with increased spawning activity in spring (Hooker, 1995). Other New Zealand mesodesmatids have also been reported to have similar breeding periods (Dickie, 1986; Grant, 1994). The maturity of bivalve gonads is affected by endogenous and exogenous factors. Exogenous factors, such as food availability and water temperature play a major role in controlling gametogenesis and spawning in bivalves (Bayne *et al.*, 1978; Rodhouse *et al.*, 1984; Pipe, 1985). In spring, summer, and early autumn, phytoplankton are more abundant, when water temperatures are higher compared to the rest of the year (Rao, 1955).

Reproduction requires large amounts of energy to sustain the energy required for developing gonads. Reproductive success depends on the energy from diets or where food is limited, energy reserves will be required to support gamete development and spawning activities. In general, the diet of bivalve broodstock diets plays a major role on their reproduction and the quality of eggs (Robinson, 1992; Samain *et al.*, 1992). During the conditioning period, nutrition and temperature are normally manipulated to promote gametogenesis and gonad development (Heasman *et al.*, 1996).

Hooker (1995) conditioned pipi broodstock for 23 days under hatchery conditions. The pipi were kept at 22°C and fed daily with two microalgal species, *Isochrysis galbana* and *Thalassiosira pseudonana* throughout the conditioning period. At day 23, pipi were induced to spawn using a combination

of temperature shock (27°C) and a dilute sperm solution. These results show that pipi could be conditioned and spawned under hatchery conditions.

4.1.1. Effect of diets on broodstock conditioning

The quality of food offered to bivalve broodstock during the conditioning period has a major influence on their reproductive performance and on the quality of eggs and larvae produced (Robinson, 1992; Farias & Uriarte, 2001; Uriarte *et al.*, 2001). In broodstock conditioning of the bay scallop *Aequipecten irradians* Lamarck, the broodstock failed to spawn in response to increasing seawater temperature (Sastry, 1966). However, it was apparent in the following trials that phytoplankton was necessary to bring the adults to spawning condition (Sastry, 1968). These studies have indicated food items are important for bivalves to reach gonadal maturation and conditioning success.

Fecundity and larval survival also have been found to be related to dietary content (Uriarte *et al.*, 2004; Utting & Millican, 1998). In the Chilean scallop, *Argopecten purpuratus* broodstock, higher protein content in the microalgal diet has significantly reduced the time to reach maturity, increased the fecundity in the females, and improved the energy balance of the broodstock (Farias *et al.*, 1997). The fecundity of broodstock and larval survival of the Pacific oyster *Ostrea edulis* also improved when broodstock were fed a diet containing microalga *I. galbana*. Uriarte *et al.* (2004), in their study on the Chilean scallop (*A. purpuratus*) and the pacific oyster (*Crassostrea gigas*), found that a conditioning diet containing microalgae rich in protein (high protein *I. galbana*

and *Chaetoceros neogracile*) improved the larval growth and survival of both species.

With regard to conditioning diets, bivalve species respond differently to different types of diets. For example, Millican and Helm (1994) reported that the fecundity and larval survival of *O. edulis* oysters were higher when offered diet of *I. galbana* (clone T-ISO). Similarly, Soudant and co-workers (1996a; 1996b) found that the maturity of the scallop, *Pecten maximus* broodstock and the number of D-larvae were improved with T-ISO cells. In contrast, in the scallop *Minachlamys asperima*, the highest fecundity was obtained by the broodstock fed on *Chaetoceros gracilis*. Therefore, it is necessary to identify the optimum dietary items that promote better performance of bivalve broodstock, particularly for hatchery production of the target species.

4.1.2. Temperature

Water temperature also plays a profound role in promoting maturity in the gonads of bivalves (Heasman *et al.*, 1996; Uriarte *et al.*, 2004; Liu *et al.*, 2008). Temperature has been shown to possibly induce maturity of bivalves outside of their natural reproductive cycle (Loosanoff & Davis, 1952; Heasman *et al.*, 1996; Chavez-Villalba *et al.*, 2002; Ojea *et al.*, 2008). Chavez-Villalba *et al.* (2002), in their study with *C. gigas*, concluded that the hatchery production of oyster could be improved by shortening the period of broodstock conditioning. This can be achieved by exposing them to a higher temperature of 22°C instead of 19°C. The maturity of the European clam, *Ruditapes decussatus* was

improved when the broodstock were conditioned at $20 \pm 1^{\circ}\text{C}$ and $22 \pm 1^{\circ}\text{C}$ compared to those kept at $18 \pm 1^{\circ}\text{C}$ (Matias et al., 2008).

Considerable attention has been devoted to understanding the role of diets and physical parameters (e.g., temperature) on reproduction of bivalves (Sastry, 1968; Loosanoff & Davis, 1952; Heasman *et al.*, 1996; Chavez-Villalba *et al.*, 2002; Ojea *et al.*, 2008). The outcomes of these studies have been applied in hatcheries by keeping bivalve broodstock at elevated temperatures with adequate nutrition supply to promote gamete maturation.

4.1.3. Objective of the study

The objective of this study was to evaluate the effect of different conditioning diets on reproductive maturity of pipi broodstock. The broodstock were conditioned for 28 days at the AUT Laboratory. Diets were chosen based on the results of the growth performances and biochemical composition of adult pipi in Chapter Three of this thesis. The tested conditioning diets were *Pavlova lutheri* and corn flour. Broodstock were subjected to spawning induction procedure at the end of the conditioning period.

4.5. MATERIALS AND METHODS

4.5.1. Broodstock collection and conditioning

Pipi broodstock from the Onehunga Bay were collected in November 2009 with a shell length of $59.30 \text{ mm} \pm 2.39 \text{ SD}$ and a wet weight of $27.80 \text{ g} \pm 4.07 \text{ SD}$. A group of 100 animals were collected during low tide and transported back to the AUT Aquaculture Laboratory. They were acclimatised in a 50 L tank at 14°C for 3 days. During the acclimatisation, they were not fed to purge the contents of their stomach.

Pipi were divided randomly into four conditioning groups, consisting of fifteen animals each. Pipi were placed in 30 L tanks with $1 \mu\text{m}$, UV-treated seawater recirculating system. The water temperature was gradually increased from 14°C to 18°C over two days. The pipi were conditioned with three different diets: diet A) 100% *Pavlova lutheri*, diet B) 100% corn flour, and diet C) a mixed diet of *Pavlova*/corn flour at 1:1 ration. They were fed with 6% of their body weight for all diets. Unfed animals were the control group. Microalgae were cultured in 15 L carboys with the addition of f/2 medium (Guillard, 1975) under 24-h light conditions, at a temperature of $21 \pm 1^{\circ}\text{C}$.

Histological observations were performed at the start of the experiment. Thirty animals were subjected to the initial histological study to determine the state of gonad development.

4.5.2. Histology

Gonad samples were processed using the standard wax histological technique (Humason, 1967). Tissue samples were sealed in paraffin and 5 µm slices were taken (Leica RM2235, Germany). From each clam, 3 slices were obtained corresponding to different depths in the body. Sectioned tissue cuts were then placed on glass slides and stained with haematoxylin and eosin-Y. Slides were mounted in DPX adhesive and cover-slipped. Sex and gametogenic development were completed under a compound light microscope (Leica, Germany) at 100x and 400x magnifications. The identification of the phases was done using a scale from that proposed for this species by Hooker (1995) (Table 8).

4.5.3. Spawning induction experiments

At the beginning of the induction, the shells were gently rinsed with seawater to remove any attached debris and fouling organisms. This step is to eliminate any contaminants that may affect the larval quality (Spencer, 2002). The broodstock were placed in 50 L spawning tanks and subjected to two water bath temperatures, $10 \pm 1^\circ\text{C}$ and $26 \pm 1^\circ\text{C}$, at 30-minute intervals. To initiate the pumping activity, *P. lutheri* and *Tetraselmis suecica* cells were added to the bath tanks. Generally, adults will respond after 3-4 hours during the early reproductive season and within an hour in the later stage of reproduction (Spencer, 2002; Michael & Neil, 2004). At day 21, the first spawning trial was carried out. However, the broodstock failed to spawn and were returned to the conditioning tanks. A week later, spawning trial was repeated.

Table 8: Phases of gonadal development for female and male of *P. australis* (modified from Hooker, 1995).

Phase	Characteristics	
	Female	Male
Early active	<p>A lot of ovogenic activity.</p> <p>Abundant mature oocytes are present in the lumen and attached to the follicle walls by a stalk or broad cytoplasmic base.</p> <p>The nucleus occupies most of the cell volume, gonad volume is small.</p>	<p>Spermatogonia, spermatocytes, spermatids and spermatozoa co-exist in follicles.</p> <p>However, in less developed samples, there is no dominant cell type.</p> <p>Proliferation of spermatocytes and spermatides; lumen central often with small numbers of spermatozoa.</p>
Late active	<p>There are still many more mature oocytes.</p> <p>Follicle walls are thin and the gonad occupied larger surface in the lumen with a polygonal configuration.</p>	<p>Approximately one third to one half of the lumina are occupied by spermatocytes and spermatids.</p> <p>The centre of the lumina is filled with dense radiating bands of spermatozoa.</p>
Mature	<p>The lumen is filled with mature ova.</p> <p>Follicle walls are thin and there is still little ovogenic activity.</p> <p>Gonad volume is large.</p>	<p>A thin layer of spermatogonia attaches to the follicle walls.</p> <p>Spermatocytes and spermatid layers are less thick than in the late active phase.</p> <p>Spermatozoa formed concentric bands and filled the majority of the lumina and often pointing to the centre of the lumen.</p>
Spawned/partially spawned	<p>Disappearance of follicle walls.</p> <p>The walls of follicles have not contracted; therefore, there are large spaces in the lumen and few ova or oocytes.</p> <p>Presence of overlapping stages (with mature or early active phase) occurs as gametogenesis is a cyclic process.</p>	<p>Spermatozoa occupy the lumina with numerous gaps.</p> <p>Few spermatocytes or spermatids.</p> <p>Spermatogonia as in mature phase.</p>

4.6. RESULTS

Following the three weeks conditioning, pipi were induced to spawn by means of temperature shock (10°C and 26°C). However, after 12 hours of induction, the broodstock did not show any sign of spawning. They were returned to the conditioning tanks for a further week. The second spawning induction trial was repeated on day 28. The second trial also failed to bring the broodstock to spawn. Therefore, histological analyses were carried out for all individuals to determine the gonadal maturity. The evaluation of the dietary impact on the reproductive maturity of pipi was based on the histological observations.

The experiment began with early active phase pipi. Broodstock conditioned with *P. lutheri* displayed 40% maturity while the remaining pipi were in the late active stage. When fed with corn flour, 42.86% reached maturity and 57.14% were in the late active stage. Most of the pipi fed the mixed diet (*P. lutheri* and corn flour) were sexually matured (76.92%) and only 23.08% were in the late active stage. Inadequate food led to very low maturity. In the unfed group, only 6.67% of the individuals reached maturity, 73.33% were in the late active stage, and 20.0% were in the early active stage (Figs. 14 & 15).

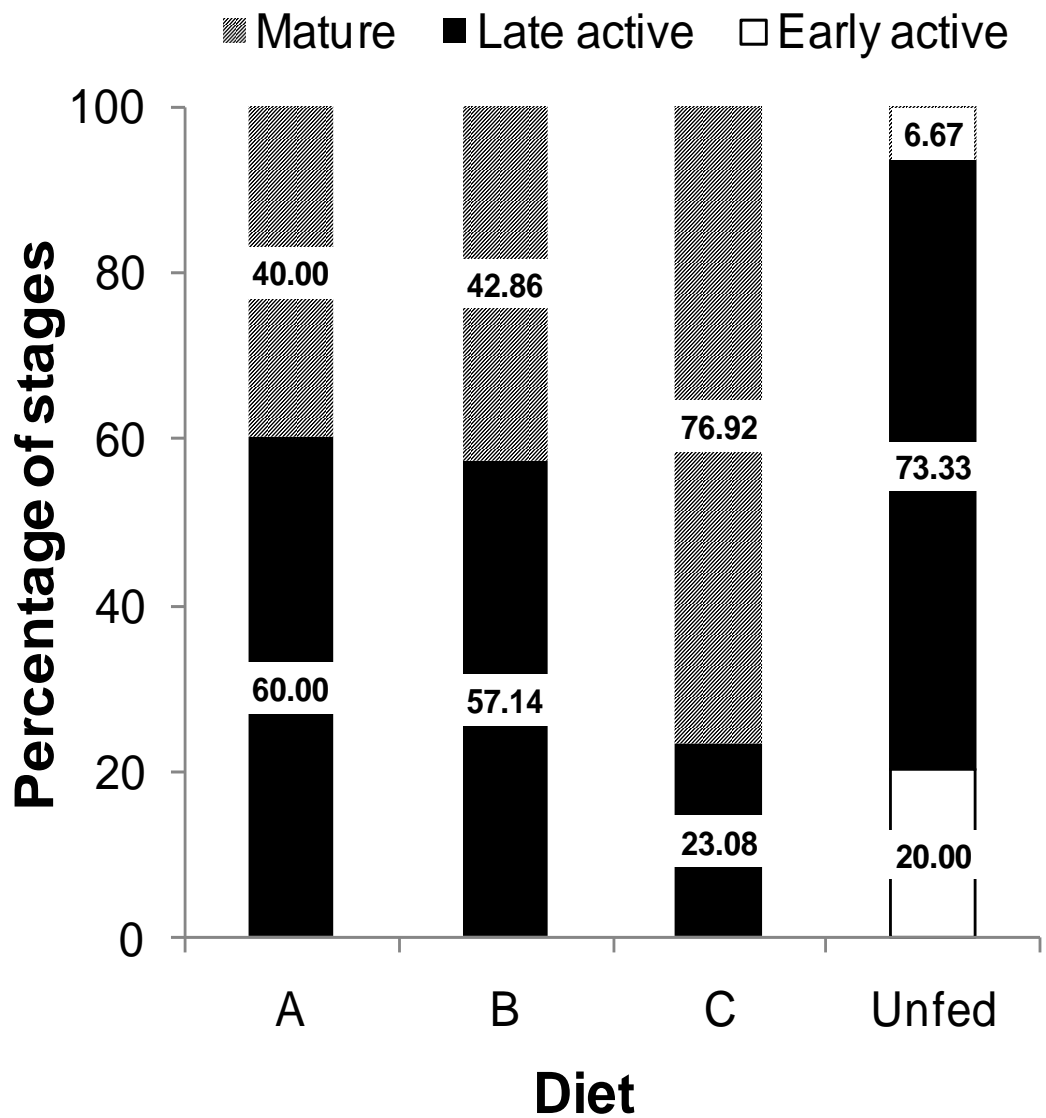


Fig. 14: Percentage of pipi broodstock at different phases of gonadal development. Pipi were conditioned for 28 days with different diets. A) 100% *Pavlova lutheri*, B) 100% corn flour, C) *P. lutheri*:corn flour (1:1), and unfed pipi.

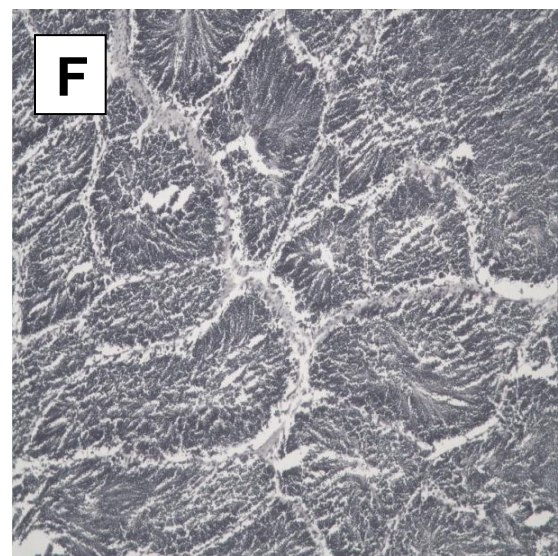
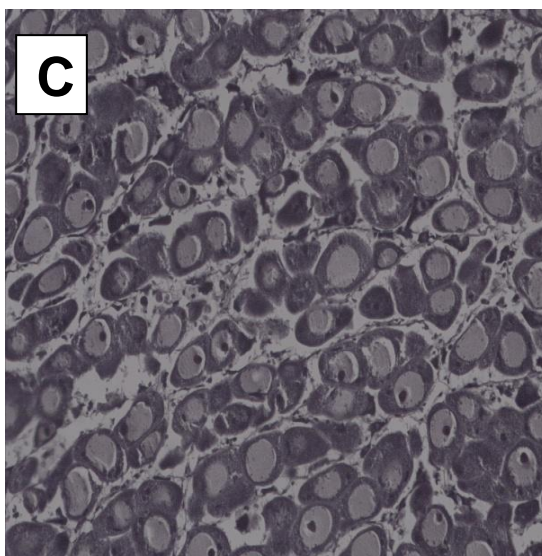
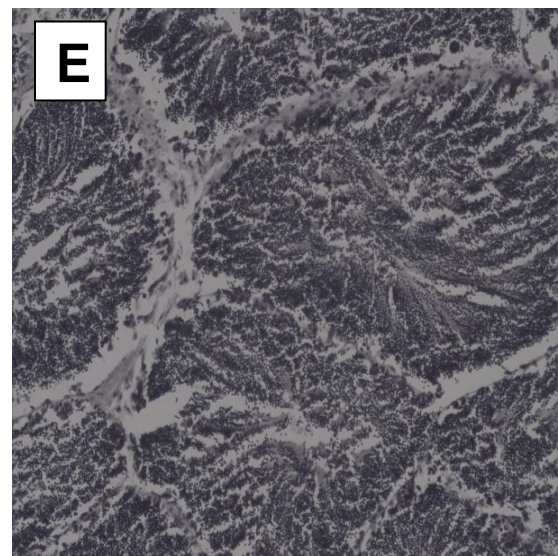
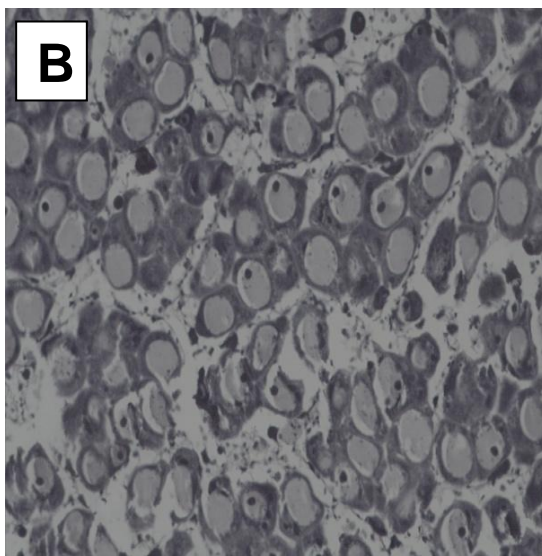
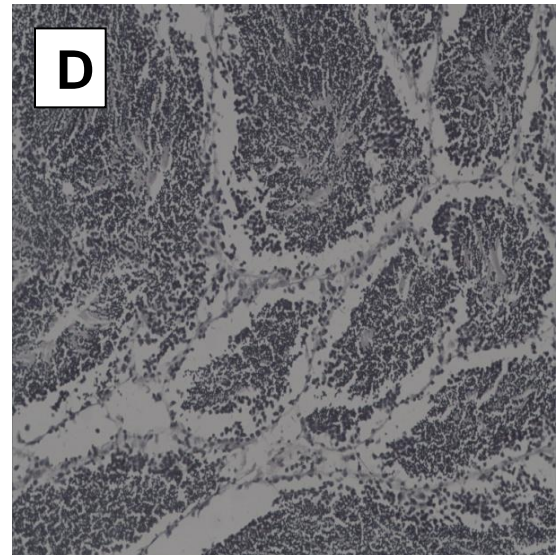
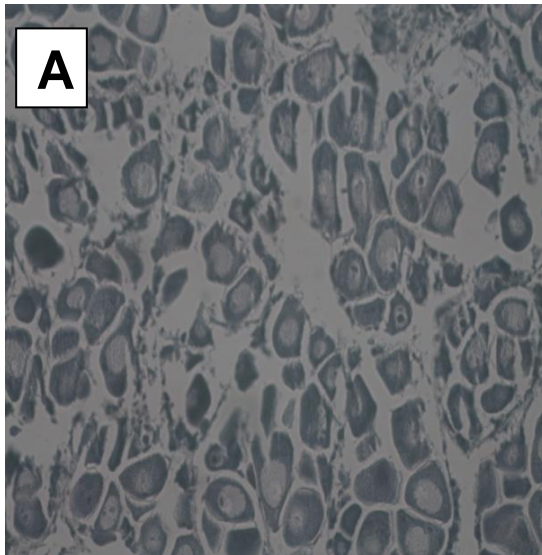


Fig. 15: Developmental stages of pipi maturity. Females: A) Early active, B) Late active, C) Mature. Males: D) Early active, E) Late active, F) Mature.

4.7. DISCUSSION

In general, the chances in spawning success vary with biological factors (e.g., insufficiently ripe gonads due to stress) (Morse, 1984; Hooker, 1995) and human error (incorrect techniques or lack of practical skills) (Hahn, 1989). During the period of conditioning, broodstock bivalves are held at elevated temperatures and provided with adequate food quality and quantity (Utting & Spencer, 1991; Utting & Millican, 1997). However, in the present study, the application of the same standard technique failed to bring broodstock pipi to spawn. Hooker (1995) succeeded in the conditioning and spawning induction of broodstock pipi within the 23-day experiment. The broodstock were fed a mixed diet consisted of *I. galbana* and *T. pseudonana* during the conditioning period. Hooker's study showed that pipi can be conditioned and spawned in hatcheries.

Because previously Hooker (1995) succeeded to induce spawning in broodstock pipi after only 23 days of conditioning, spawning failures in this study are unlikely to be the result of poor conditioning period or insufficient nutrition. All the fed groups (*P. lutheri*, corn flour, and the mixed diet) had at least reached 40% of maturity, with the highest maturity observed in clams fed diet C (76.92% maturity). The feeds, particularly diet B, were sufficient to fuel development of gonads from the early active phase (Figs. 15A & 15D) to mature phase (Figs. 15C & 15F). The findings, therefore, demonstrated that the experimental diets were nutritionally adequate for pipi broodstock during the conditioning period.

Observations during the spawning experiments indicated that the broodstock had completely closed their valves when they were exposed to the water bath temperatures. This behavior may indicate that the pipi were stressed due to the temperature shock method. In the first spawning trial, pipi broodstock were exposed to the temperature stimulant for 8 hours before they were reconditioned for another week. The 8 hour exposure may have exacerbated or caused stress in the pipi, especially with the repeated cycles of cold and hot water temperatures. Similarly, the same behavior was observed in the second spawning induction trial.

4.7.1. Environmental factors during broodstock conditioning

In aquaculture practices, the main aim is to maximise the yield of production. To this end, environmental cues (e.g., temperature, photoperiod) can be manipulated so that the biological processes of the organisms can be controlled (e.g., reproduction, metabolism) (Saout *et al.*, 1999; Chavez-Villalba *et al.*, 2002; Martinez & Perez, 2003; Fabioux *et al.*, 2005). In the present study, food supply had been the only factor considered during the pipi conditioning period. Although the pipi maturity was obtained with the food supply, it may have been succeeded to induce spawning if other environmental factors are taken into consideration (e.g., temperature and/or photoperiod) during the conditioning period.

Previous studies on bivalves have shown that environmental factors do not act alone on the gonad growth. Instead, combinations of several factors often are involved in the development and maturity of the gonads (Janzel & Villalaz,

1994; Sastry, 1968). Sastry (1968) proved that temperature alone had no impact on the scallop (*Aequipecten irradians*) spawning maturity. Alternatively, both temperature and food supply/phytoplankton factors have an influence on the oocytes sizes. Similarly, Janzel and Villalaz (1994), in their study on *Argopecten ventricosus*, showed that the interaction between temperature, food ration, and conditioning period promoted changes in the *A. ventricosus* gonads.

The interaction between water temperature and photoperiod may exert the regulatory role of sexual cycles in marine bivalves which has been shown by Saout *et al.* (1999) with *P. maximus* scallop. They found that changes in temperature and day-length affected the partitioning between previtellogenic (before yolk formation) and vitellogenic (yolk formation) oocytes. In their study, they manipulated the light regimes and temperature to approximate 'spring' and 'winter' conditions. Animals maintained within the 'spring' condition had a significantly higher gonadal index than those conditioned under the 'winter' environment. In addition, these factors also had favoured changes in the accumulation of total proteins, carbohydrates as well as lipids content in the female part of the gonad. In another study, Fabioux *et al.* (2005) analysed the role of temperature and photoperiod in the reproduction of the oyster, *Crassostrea gigas* by manipulating both temperature and photoperiod conditions to natural, accelerating and winter conditions. In the natural conditions, the temperature and photoperiod were manipulated according to the mean natural cycles recorded in Marennes-Oleron Bay, France. The temperature and photoperiod were accelerated twice to that of the natural cycle and was referred as the 'accelerating cycle', while for the third condition, the animals were maintained under winter conditions, 8°C and 8-hour light

exposure. The results obtained were consistent with Saout *et al.* (1999), where, the oysters cultured under accelerated conditions were found to have the highest percentage of the gonad occupation rate in contrast to 'natural' and 'wintering' conditions. They also demonstrated that under an optimal food level, temperature together with and/or photoperiod drove the reproductive internal clock of *C. gigas*, particularly for the regulation of gonial proliferation and germ cell maturation.

To summarise, it appears that a combination of suitable conditioning diet, water temperature, and photoperiod are expected to enhance the chances of successful spawning induction in pipi broodstock. However, a combination effect of these factors needs further examination to test the hypothesis. Nevertheless, a combination of skills and practical experience also is vital for spawning induction success.

CHAPTER FIVE
GENERAL DISCUSSION

5.1. INTRODUCTION

This thesis discusses the feeding and broodstock conditioning aspects of the New Zealand pipi, *Paphies australis* cultured under hatchery conditions. Chapter two of this thesis investigated the cell clearance rates of pipi fed different microalgal species (*Isochrysis galbana*, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros muelleri*, and *Thalassiosira pseudonana*). The clearance mechanism is used to remove particles from water column and dilute the suspension (Dame, 1996). Pipi (31.0-44.0 shell length) filtered *T. pseudonana* cells at the fastest rate ($0.81 \pm 0.05 \text{ L hour}^{-1}$), while the slowest rate was showed in pipi filtered *P. lutheri* ($0.19 \pm 0.04 \text{ L hour}^{-1}$). While food selectivity was observed, the relative pattern of food ingestion for all microalgae was the same throughout the experiment. The clearance rates in descending order were *T. pseudonana* > *I. galbana* > *C. muelleri* > *T. suecica* > *P. lutheri*. This study has shown that pipi are capable of filtering a variety of different microalgae while at the same time being a selective filter feeder. The shape of microalgal cells may have affected selectivity of the experimental microalgae. In addition, it also may have been affected by the chemo-selection based on the signalling mechanism from microalgal exudates (Shumway *et al.*, 1985; Baldwin, 1995). However, the hypothesis warrants further investigations to elucidate feeding selectivity on the chemo-selection basis.

Generally, in bivalve cultures the larval and spat rearings are fully dependent on live microalgae as the main food source, either fed mono-specifically or offered as a mixed diet. A more balanced composition in mixed diets generally produces better growth and survival rates (Lora-Vilchis *et al.*, 2004; Martinez-

Fernandez *et al.*, 2006; Martinez-Fernandez & Southgate, 2007). Once they reach the juvenile stage, they are shifted to the open sea to feed on phytoplankton naturally contained in seawater. In bivalve hatcheries, microalgae are usually cultured under fully controlled conditions in indoor laboratories. The maintenance costs are relatively high and therefore, the spat production of bivalves would likely be restricted by the costs.

The application of alternative diets in pipi feeds was considered a success and showed promising results (Chapter 3). The same microalgal species used in Chapter 2, along with the three processed diets (baker's yeast, wheat flour, and corn flour) were fed to pipi of different size classes for 21 days. Spat, juvenile, and adult pipi showed varying preferences to the experimental diets. The greatest shell length increase for spat and juvenile pipi were obtained with *I. galbana*, while the weight gains were greatly supported by *P. lutheri*. However, spat fed yeast displayed a similar shell growth as *I. galbana*. For the juveniles, animals fed yeast showed a similar weight gain with those that fed *P. lutheri* and corn flour. For adult pipi, the corn flour diet promoted the highest shell increase whereas the weight gain was mostly supported by *P. lutheri*. Lipids were found to affect growth in smaller pipi (spat and juveniles). Conversely, carbohydrates have been found to affect growth of the adults. The role of carbohydrates in adult pipi was explained by the needs of glucogen reserves to fuel development of gonads during reproduction. At the end of the feeding experiment, adult pipi were subjected to proximate analyses of the soft tissues. The accumulations of lipids and proteins, and depletion of carbohydrates indicated that the adults may have initiated their gonadal activities. The great expenditures of carbohydrates by the adults were due to energy requirements needed during the development

of gonads. Results from Chapter 3 suggested the use of baker's yeast as a potential in feeding spat and juvenile pipi, while wheat and corn flours would be promising substitutes for adult pipi feeds. However, the use of processed diets for pipi cultures needs further investigation on a commercial scale.

The use of alternative processed diets for growing bivalves in hatcheries would greatly reduce the cost of production (Coutteau & Sorgeloos, 1992). Bivalves generally depend on microalgae as their main nutrition throughout their life cycle. The production of microalgae in hatcheries is expensive and the quality depends on the main molecular components such as proteins, carbohydrates and lipids (especially fatty acids), qualifying them as good or bad quality food (Napolitano *et al.* 1990). At the same time, the biochemical content of the microalgae depends on several factors, such as the strain, type and amount of nutrients used in the culture medium, temperature and light conditions, stage of the growth curve at harvest and water quality (Abalde *et al.*, 1994; Duerr *et al.*, 1998). Studies with other bivalve species on the use of alternative diets also show positive results. Development of formulated diet for pipi clams in the future would increase potential of pipi clams to become an important aquaculture species in New Zealand.

Hooker (1995) and results from histological studies in this thesis (Chapter 4) also suggest that broodstock pipi can be conditioned, induced to spawn, and produced larvae within hatcheries. This indicates the great potential of pipi to be cultured in hatcheries and further released into the wild. Other clam species have been successfully produced and cultured in hatcheries, such as the hard clam *Mercenaria mercenaria* (Castagna & Kraeuter, 1981; Malouf, 1989; Lorio

& Malone, 1995; Cepeda-Perez, 2004), the grooved carpet-shell clam *Ruditapes decussatus* (Albentosa *et al.*, 1996; 1999), the Manila clam *Ruditapes philippinarum* (Albentosa *et al.*, 2002; Zhang & Yan, 2006), the soft-shell clam *Mya arenaria* (Hidu & Newell, 1989), and the surf clam *Spisula solidissima* (Goldberg, 1989).

Based on the results in the adult feeding trial, diets that provided the best growth and the highest survival were chosen for the conditioning experiment in Chapter Four. The broodstock were conditioned to bring them to spawn in the hatchery. At the end of the conditioning period, they were induced to spawn by means of the temperature shock method. Spawning induction trials were carried out at days 21 and 28 following the conditioning period. Unfortunately, the spawning trials failed to induce the broodstock. This could be due to stress factor arisen from the temperature shock stimulant. The mixed diet consisted of microalga *P. lutheri* and corn flour greatly fuelled the energy requirements needed for gonadal development. In the group of broodstock fed the mixed diet, 76.92% reached mature stage. The remaining broodstock were in the late active phase. The results suggested the use of the mixed diet during the conditioning period of broodstock pipi prior to spawning induction. The potential of the mixed diet to induce spawning of pipi can be re-examined, incorporating a combination of other environmental factors (e.g., temperature and/or photoperiod).

Since food is a large portion of the total expenditure of bivalve hatcheries, studies associated with feeding are very important to optimise the costs. In addition, high quality diets are required for commercial aquaculture to be

successful (Howerton, 2001). Therefore, the findings within this thesis will provide valuable information on the feeding requirements during the growing and conditioning periods of pipi. This information is necessary for a successful aquaculture of pipi in the future.

REFERENCES

- Abalde, J., Cid, A., Reiriz, S., Torres, E., & Herrero, C. (1994). Response of the marine microalga *Dunaliella tertiolecta* (Chlorophyceae) to copper toxicity in short time experiences. *Bulletin of Environmental Contamination and Toxicology* (in press).
- Ahn, I-Y. & Shim, J. H. (1998). Summer metabolism of the Antarctic clam, *Laternula elliptica* (King and Broderip) in Maxwell Bay, King George Island and its implications. *Journal of Experimental Marine Biology and Ecology*, 224(2), 253-264, doi: 10.1016/S0022-0981(97)00201-3.
- Albentosa, M., Fernandez-Reiriz, M. J., Perez-Camacho, A., & Labarta, U. (1999). Growth performance and biochemical composition of *Ruditapes decussatus* spat fed on microalgal and wheatgerm flour diets. *Journal of Experimental Marine Biology and Ecology*, 232, 23-37. doi: 10.1016/S0022-0981(98)00086-0
- Albentosa, M., Naessens, E., Leger, P., Coutteau, P., Lavens, P., & Sorgeloos, P. (1989). Promising results in the seed culturing of the Manila clam *Tapes semidecussata* with a manipulated yeast product as a partial substitute for algae. *European Aquaculture Society: special publication*, 10, 7-8. Retrieved from <http://www.vliz.be/imis/imis.php?module=ref&refid=2801>
- Albentosa, M., Perez-Camacho, A., Fernandez-Reiriz, M. J., & Labarta, U. (2002). Wheatgerm flour in diets for Manila clam, *Ruditapes philippinarum*, spat. *Aquaculture*, 212(1-4), 335-345. doi:10.1016/S0044-8486(02)00121-7
- Albentosa, M., Perez-Camacho, A., Labarta, A., & Fernandez-Reiriz, M. J. (1996). Evaluation of live microalgal diets for the seed culture of *Ruditapes decussatus* using physiological and biochemical parameters. *Aquaculture*, 148(1), 11-23. doi: 10.1016/S0044-8486(96)01405-6
- Andrew, S. (2007). *Paphies australis*. Retrieved May 20, 2008, from <http://www.mollusca.co.nz/speciesdetail.php?speciesid=2453&species=Paphies%20australis>
- AOAC. (2005). *Official Methods of Analysis of AOAC INTERNATIONAL* (18th Edition). (W. Horwitz, Ed.) Gaithersburg, Maryland, USA: AOAC International.
- Arun, A. (2009). An assessment on the influence of salinity in the growth of black clam (*Villorita cyprinoides*) in cage in Cochin Estuary with a special emphasis on the impact of Thanneermukkom salinity barrier. *Aquaculture, Aquarium, Conservation and Legislation International Journal of the Bioflux Society*, 2 (3), 319-330. Retrieved from <http://library.enaca.org/AquacultureAsia/Articles/july-sept-2009/7-assessment-influence-salinity-black-clam.pd>

- Babinchak, J. & Ukeles, R. (1979). Epifluorescence microscopy, a technique for the study of feeding in *Crassostrea virginica* veliger larvae. *Marine Biology*, 51(1), 69-76. doi: 10.1007/BF00389032
- Bacon, G. S., MacDonald, B. A., & Ward, J. E. (1998). Physiological responses of infaunal (*Mya arenaria*) and epifaunal (*Placopecten magellanicus*) bivalves to variations in the concentration and quality of suspended particles: I. Feeding activity and selection. *Journal of Experimental Marine Biology and Ecology*, 219, 105-125. doi:10.1016/S0022-0981(97)00177-9
- Baldwin, B. S. (1995). Selective particle ingestion by oyster larvae (*Crassostrea virginica*) feeding on natural seston and cultured algae. *Marine Biology*, 123(1), 95-107. doi: 10.1007/BF00350328
- Baptist, G., Meritt, D., & Webster, D. (1993). Growing microalgae to feed bivalve larvae. *NRAC Fact Sheet* (160), p. 8.
- Baron, J. (1992). Reproductive cycles of the bivalve molluscs *Atactodea striata*, *Garfarium tumidum* (Roding) and *Anadara scapha* in New Caledonia. *Australian Journal of Marine and Freshwater Research*, 43, 393-402. Retrieved from http://www.publish.csiro.au/?act=view_file&file_id=MF9920393.pdf
- Barber, B. J. & Blake, N. J. (1981). Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). *Journal of Experimental Marine Biology and Ecology*, 52, 121-134. doi:10.1016/0022-0981(81)90031-9
- Bass, D., Howe, A., Brown, N., Barton, H., Demidova, M., Michelle, H., Li, L., Sanders, H., Watkinson, S. C., Willcock, S., Richards, T. A. (2007). Yeast forms dominate fungal diversity in the deep oceans. *Proceedings of the Royal Society B*, 274(1629), 3069-3077. doi: 10.1098/rspb.2007.1067
- Bayne, B. L., Bayne, C. J., Carefoot, T. C., & Thompson, R. J. (1976). The physiological ecology of *Mytilus californianus* Conrad. *Oecologia*, 22(3), 211-228. doi: 10.1007/BF00344793
- Bayne, B. L., Holland, D. L., Moore, M. N., Lowe, D. M., & Widdows, J. (1978). Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom*, 58, 825-841. doi: 10.1017/S0025315400056794
- Bayne, B. L., Widdows, J., Newell, R. I. E. (1977). Physiological measurements on estuarine bivalve molluscs in the field. In B. F. Keegan, P. O. Ceidigh, & P. J. S. Boaden (Eds.), *Biology of Benthic Organisms: 11th European Symposium on Marine Biology, Galway, October 1976. European Marine Biology Symposia*, 11 (pp. 57-68). Oxford, UK: Pergamon Press.

- Becker, W. E. (1986). Nutritional properties of microalgae: Potential and constraints. In: A. Richmond (Ed.), *Handbook of Microalgal Mass Culture* (pp. 339-420). CRC Press, Boca Raton: Florida.
- Beninger, P. G. & Lucas, A. (1984). Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adams & Reeve). *Journal of Experimental Marine Biology and Ecology*, 79(1), 19-37. doi:10.1016/0022-0981(84)90028-5
- Berntsson, K. M., Jonsson, P. R., Wangberg, S. A., & Carlsson, A. S. (1997). Effects of broodstock diets on fatty acid composition, survival and growth rates in larvae of the European flat oyster, *Ostrea edulis*. *Aquaculture*, 154(2), 139-153. doi: 10.1016/S0044-8486(97)00041-0
- Berry, P. F. & Schleyer, M. H. (1983). The brown mussel *Perna perna* on the Natal coast, South Africa: utilization of available food and energy budget. *Marine Ecology Progress Series*, 13, 201-210. Retrieved from <http://www.int-res.com/articles/meps/13/m013p201.pdf>
- Beukema, J. J. & de Bruin, W. (1979). Calorific values of the soft parts of the tellinid bivalve *Macoma balthica* (L.) as determined by two methods. *Journal of Experimental Marine Biology and Ecology*, 37(1), 19-30. doi:10.1016/0022-0981(79)90023-6
- Binnie, N. (2008). Statistics with SPSS. Auckland, NZ: Lyceum Press.
- Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 39, 911-917. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/13671378>
- Bradstock, M. (1985). *Between the tides. New Zealand shore and estuary life*. Reed Methuen Publishers, Auckland. 158 pp.
- Brillant, M. G. S. & MacDonald, B. A. (2000). Postingestive selection in the sea scallop, *Placopecten magellanicus* (Gmelin): the role of particle size and density. *Journal of Experimental Marine Biology and Ecology*, 253, 211-277. doi: 10.1016/S0022-0981(00)00258-6
- Brown, M. R. (1991). The amino acid and sugar composition of 16 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, 145, 79-99. doi: 10.1016/0022-0981(91)90007-J
- Brown, M. R. (2002). Nutritional value of microalgae for aquaculture. In: L. E. Cruz-Suárez, D. Ricque-Marie, M. Tapia-Salazar, M. G. Gaxiola-Cortés, N. Simoes (Eds.). *Avances en Nutrición Acuicola VI. Memorias del VI Simposium Internacional de Nutrición Acuicola*. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

- Brown, M. R., Barrett, S. M., Volkma, J. K., Nearhos, S. P., Nell, J. A. & Allan, G. L. (1996). Biochemical composition of new yeasts and bacteria as food for bivalve aquaculture. *Aquaculture*, 143, 341-360. doi: 10.1016/0044-8486(96)01286-0
- Brown, M. R., Dunstan, G. A., Jeffrey, S. W., Volkman, J. K., Barrett, S. M., & Leroi, J. M. (1993b). The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis* sp. (clone T-ISO). *Journal of Phycology*, 29, pp. 601–612. doi: 10.1111/j.0022-3646.1993.00601.x
- Brown, M. R., Garland, C. D., Jeffrey, S. W., Jameson, I. D., Leroi, J. M. (1993a). The gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis* sp. (clone T-ISO), *Pavlova lutheri* and *Nannochloropsis oculata*. *Journal of Applied Phycology*, 5(3), 285-296. doi: 10.1007/BF02186231
- Brown, M.R., Jeffrey, S.W., & Garland, C.D. (1989). Nutritional aspects of microalgae used in mariculture; a literature review. *CSIRO Marine Laboratory Reort*. No. 205, p. 44.
- Brown, M. R., Jeffrey, S. W., Volkman, J., & Dunstan, G. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, 151, 315-331. doi:10.1016/S0044-8486(96)01501-3
- Brown, M. R. & Robert, R. (2002). Preparation and assessment of microalgal concentrates as feeds for larval and juvenile Pacific oyster (*Crassostrea gigas*). *Aquaculture*, 207, 289-309. doi: 10.1016/S0044-8486(01)007426
- Caers, M., Coutteau, P., & Sorgeloos, P. (1999). Dietary impact of algal and artificial diets, fed at different feeding rations, on the growth and fatty acid composition of *Tapes philippinarum* (L). spat. *Aquaculture*, 170, 307-322. doi: 10.1016/S0044-8486(98)00410-4
- Camacho, A. P., Albentosa, M., Fernandez-Reiriz, M. J., & Labarta, U. (1998). Effect of microalgal and inert (cornmeal and cornstarch) diets on growth performance and biochemical composition of *Ruditapes decussatus* seed. *Aquaculture*, 160(1-2), 89-102. doi:10.1016/S0044-8486(97)00232-9
- Campos, J. & Veer, H. W. V. D. (2008). Autecology of *Crangon crangon* (L.) with an emphasis on latitudinal trends. *Oceanography and Marine Biology: An Annual Review*, 46. 65-104. Retrieved from <http://www.crcnetbase.com/doi/pdf/10.1201/9781420065756.ch3>
- Castagna, M. & Kraeuter, J. N. (1981). Manual for growing the hard clam *Mercenaria*. Special report in Applied Marine Science and Ocean Engineering No. 249. Virginia Institute of Marine Science, Gloucester Point, VA 23062, USA. 110 pp.

- Cepeda-Perez, E. (2004). Induced spawning behavior and larval development of the hard clam, *Mercenaria mercenaria* (Linné, 1758) in Puerto Rico. Unpublished PhD thesis. Universidad de Puerto Rico.
- Ceron-Ortiz, A. N., Cordero, B., Arredondo-Vega, B. O., & Voltolina, D. (2006). Effect of algal diet and temperature on survival, growth and biochemical composition of spat of the lion's paw scallop *Nodipecten subnodosus*. *Aquaculture*, 298(1-2), 64-69. doi: 10.1016/j.aquaculture.2009.10.006
- Chavez-Villalba, J., Pommier, J., Andriamiseza, J., Pouvreau, S., Barret, J., Cochard, J. -C., & Pennec, M. L. (2002). *Aquaculture*, 214(1-4), 115-130. doi: 10.1016/S0044-8486(01)00898-5
- Chotipuntu, P. (2005). Marine diatom (*Chaetoceros calcitrans*) as a monospecies diet for conditioning oyster (*Crassostrea belcheri* Sowerby) broodstock. *Walailak Journal of Science and Technology*, 2(2), 201-207. Retrieved from <http://masterorg.wu.ac.th/file/ird-20090614-ZEd8X.pdf>
- Chu, F. E. & Webb, K. L., (1984). Polyunsaturated fatty acids and neutral lipids in developing larvae of the oyster, *Crassostrea virginica*. *Lipids*, 19, 815-820
- Clausen, I. & Riisgard, H. U. (1996). Growth, filtration and respiration in the mussel *Mytilus edulis*: no evidence for physiological regulation of the filter-pump to nutritional needs. *Marine Ecology Progress Series*, 141, 37-45. Retrieved from <http://www.int-res.com/articles/meps/141/m141p037.pdf>
- Coughlan, J. & Ansell, A. D. (1964). A direct method for determining the pumping rate of siphonate bivalves. *Journal du Conseil International pour l'Exploration de la Mer*, 29(2), 205-213. doi: 10.1093/icesjms/29.2.205
- Coutteau, P., Dravers, M., Dravers, P., Leger, P., & Sorgeloos, P. (1993). Manipulated yeast diets and dried algae as a partial substitute for live algae in the juvenile rearing of the Manila clam *Tapes philippinarum* and the Pacific oyster *Crassostrea gigas*. In G. Barnabe, & P. Kestemont (Eds.) *Production, Environment and Quality. Bordeaux Aquaculture '92* (pp. 523-531).
- Coutteau, P., Hadley, N. H., Manzi, J. J., & Sorgeloos, P. (1991). Manipulated yeast diets as a partial algal substitute for the nursery culture of the hard clam *Mercenaria mercenaria*: 77-78. In: *Aquaculture and the environment. Aquaculture Europe '91*, Dublin, Ireland, June 10-12, 1991. European Aquaculture Society Special Publication 14.
- Coutteau, P., Hadley, N. H., Manzi, J. J., & Sorgeloos, P. (1994). Effect of algal ration and substitution of algae by manipulated yeast diets on the growth of juvenile *Mercenaria mercenaria*. *Aquaculture*, 120, 135-150. doi:10.1016/0044-8486(94)90229-1

- Coutteau, P., Lavens, P., & Sorgeloos, P. (1990). Baker's yeast as a potential substitute for live algae in aquaculture diets: *Artemia* as a case study. *Journal of the World Aquaculture Society*, 21 (1), 1-9. Retrieved from <http://www.vliz.be/imisdocs/publications/3064.pdf>
- Coutteau, P., & Sorgeloos, P. (1992). The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. *Journal of Shellfish Research*, 11, 467-476. Retrieved from <http://www.vliz.be/imisdocs/publications/113466.pdf>
- Cranford, P. J. & Hill, P. S. (1999). Seasonal variation in food utilization by the suspension-feeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. *Marine Ecology Progress Series*, 190, 223-239. doi: 10.3354/meps190223
- Crimp, D. (2007). Out of the blue – aquaculture. *New Zealand Geographic*, 84, 98.
- Chavez-Villalba, J., Pommier, J., Andriamiseza, J., Pouvreau, S., Barret, J., Cochard, J. –C., & Pennec, M. L. (2002). *Aquaculture*, 214(1-4), 115-130. doi: 10.1016/S0044-8486(01)00898-5
- Couturier, C. & Aiken, D.E. (1989). Possible role of photoperiod in sea scallop reproduction. *Proceedings of the annual meeting 1989, Aquaculture Association of Canada Symposium*, 89(3), 65-67.
- Dame, R. F. (1996). *Ecology of Marine Bivalves: An Ecosystem Approach*. Florida: CRC Press LLC.
- Davenport, J. & Woolmington, A. D. (1982). A new method of monitoring ventilatory activity in mussels and its use in a study of the ventilatory patterns of *Mytilus edulis* L. *Journal of Experimental Marine Biology and Ecology*, 62(1), 55-67. doi:10.1016/0022-0981(82)90216-7
- Dawber, C. (2004). Lines in the water: A history of Greenshell mussel farming in New Zealand. Picton, NZ: River Press.
- Defosse, J. –M. & Hawkins, A. J. S. (1997). Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus*. *Marine Biology*, 129(1), 139-147. doi: 10.1007/s002270050154
- Delgado, M. & Perez-Camacho, A. (2007). Influence of temperature on gonadal development of *Ruditapes philippinarum* (Adams and Reeve, 1850) with special reference to ingested food and energy balance. *Aquaculture*, 264(1-4), 398-407. doi: 10.1016/j.aquaculture.2006.11.009
- Devauchelle, N. & Mingant, C. (1991). Review of the reproductive physiology of the scallop, *Pecten maximus*, applicable to intensive aquaculture. *Aquatic Living Resources*, 4(1), 41-51. doi: 10.1051/alr:1991004

- Devi, V. U., Rao, Y. P., & Rao, D. G. V. P. (1985). Seasonal changes in the biochemical composition of a tropical intertidal prosobranch *Morula granulata* (Duclos). *Journal of Molluscan Studies*, 51, 248-256. Retrieved from <http://mollus.oxfordjournals.org/cgi/content/abstract/51/3/248>
- de Zwaan, A. & Zandee, D. I. (1972). The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis* L. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 43(1), 47-54. doi: 10.1016/0305-0491(72)90200-3
- Dickie, B. N. (1986). The reproductive cycle of the *Paphies australis* at four locations in the lower Whangarei Harbour. Whangarei Harbour Water Quality Management Plan. Unpublished Report to the Northland Catchment Commission and Regional Water Board.
- Duerr, E. O., Molnar, A., & Sato, V. (1998). Cultured microalgae as aquaculture feeds. *Journal of Marine Biotechnology*, 6(2), 65-70. Retrieved from <http://www.springerlink.com/content/ce31urywb0t3mnp/>
- Dunathan, J. P., Ingle, R. M., & Havens Jr., W. K. (1969). Effects of artificial foods upon oyster fattening with potential commercial applications. *Florida Board of Conservation. Marine Research Laboratory*, 58, pp 1-38.
- Dupuy, C., Vaquer, A., Lam-Hoai, T., Rougier, C., Mazouni, N., Lautier, J., Collos, Y., & Le Gall, S. (2000). Feeding rate of the oyster *Crassostrea gigas* in a natural planktonic community of the Mediterranean Thau Lagoon. *Marine Ecology Progress Series*, 205, 171-184. Retrieved from <http://archimer.ifremer.fr/doc/2000/publication-812.pdf>
- Enright, C. T., Newkirk, G. F., Craigier, J. S., & Castell, J. D. (1986). Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros gracilis* Schütt of varied chemical composition. *Journal of Experimental Marine Biology and Ecology*, 96(1), 15-26. doi: 10.1016/0022-0981(86)90010-9
- Epifanio, C. (1979). Comparison of yeast and algal diets for bivalve molluscs. *Aquaculture*, 16, 187-192. doi: 10.1016/0044-8486(79)90106-6
- Espinosa, E. P. & Allam, B. (2006). Comparative growth and survival of juvenile hard clams, *Mercenaria mercenaria*, fed commercially available diets. *Zoo Biology*, 25(6), 513-525. doi: 10.1002/zoo.20113
- Evans, J. W. (1968). The effect of rock hardness and other factors on the shape of the burrow of the rock-boring clam, *Penitella penita*. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 4(4), 271-278. doi:10.1016/0031-0182(68)90094-1

- Fabioux, C., Huvet, A., Le Soucho, P., Le Pennec, M., & Pouvreau, S. (2005). Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. *Aquaculture*, 250(1-2), 458-470. doi: 10.1016/j.aquaculture.2005.02.038
- Famme, P., Riisgard, H. U., & Jorgensen, C. B. (1986). On direct measurements of pumping rates in the mussel *Mytilus edulis*. *Marine Biology*, 92, 323-327. doi: 10.1007/BF00392672
- Farias, A. & Uriarte, I. (2001). Effect of microalgae protein on the gonad development and physiological parameters for the scallop *Argopecten purpuratus* (Lamarck, 1819). *Journal of Shellfish Research*, 20(1), 97-106. Retrieved from <http://direct.bl.uk/bld/PlaceOrder.do?UIN=101231550&ETOC=RN&from=searchengine>
- Farias, A., Uriarte, I., & Varas, P. (1997). Nutritional study on the broodstock conditioning of the Chilean scallop, *Aequipecten purpuratus* (Lamarck, 1819). *Revista de biología marina y oceanografía*, 32(2), 127-136.
- FAO (1993). *Fishery statistics. Catches and landings. 1991*. Retrieved from <http://www.fao.org/fishery/statistics/programme/publications/yearbooks/en>
- FAO (2009). *FAO yearbook: Fishery and aquaculture statistics. 2007*. Retrieved February 10, 2010, from Food and Agriculture Organization of the United Nations website: <ftp://ftp.fao.org/docrep/fao/012/i1013t/i1013t.pdf>
- FAO (2010). *National Aquaculture Sector Overview: New Zealand*. Retrieved April 25, 2010, from http://www.fao.org/fishery/countrysector/naso_newzealand/en
- Fistarol, G. O., Legrand, C., & Graneli, E. (2005). Allelopathic effect on a nutrient-limited phytoplankton species. *Aquatic Microbial Ecology*, 41, 153-161. Retrieved from http://www.bom.hik.se/ngred/Artiklar/110_Fistarol_etal_AME.pdf
- Fjell, K. M. (1996). Method for ash determination by conductivity. *Cereal Chemistry*, 73(4), 510-511. Retrieved from <http://www.scopus.com/record/display.url?eid=2-s2.0-0029913550&origin=inward&txGid=Z0ZnkTnPCPcAES0AquVWJjd%3a10>
- Foe, C. & Knight, A. (1986). Growth of *Corbicula fluminea* (Bivalvia) fed artificial and algal diets. *Hydrobiologia*, 133, 155-164.
- Foster-Smith, R. L. (1975). The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). *Journal of Experimental Marine Biology and Ecology*, 17(1), 1-22. doi:10.1016/0022-0981(75)90075-1

- Foster-Smith, R. L. (1976). Some mechanisms for the control of pumping activity in bivalves. *Marine and Freshwater Behaviour and Physiology*, 4(1), 41-59. doi: 10.1080/10236247609386941
- Gabbott, P. A. (1975). Storage cycles in marine bivalve molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: H. Barnes (Ed.), *Ninth European Marine Biology Symposium* (pp. 191-211). Aberdeen University Press, Aberdeen.
- Gabbott, P.A. (1983). Developmental and seasonal metabolic activities in marine molluscs. In: P. Hochachka (Ed.). *Mollusca Vol. 2* (pp. 165-219). Academic Press, New York.
- Gabbott, P. A. & Bayne, B. L. (1973). Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom*, 53, 269-286. doi: 10.1017/S0025315400022268
- Gabbott, P. A. & Peek, K. (1991). Cellular biochemistry of the mantle tissue of the mussel *Mytilus edulis* L. *Aquaculture*, 94(2-3), 165-176. doi:10.1016/0044-8486(91)90116-O
- Gallager, S. M. (1988). Visual Observations of particle manipulation during feeding in larvae of a bivalve mollusc. *Bulletin of Marine Science*, 43(3), 344-365. Retrieved from <http://docserver.ingentaconnect.com/deliver/connect/umrsmas/0007497/v43n3/s3.pdf?expires=1271461364&id=56237840&titleid=10983&acnname=Auckland+University+of+Technology&checksum=4F08FF94A9650C20E3D0D9EE658C38E8>
- Gallager, S. M. & Mann, R. (1981). The effect of varying carbon-nitrogen ratio in the phytoplankter *Thalassiosira pseudonana* (3H) on its food value to the bivalve *Tapes japonica*. *Aquaculture*, 26(1), 95-105.
- Galtsoff, P. S. (1928). The effect of temperature on the mechanical activity of the gills of the oyster (*Ostrea virginica* Gm.). *Journal of General Physiology*, 11, 415-431. Retrieved from <http://jgp.rupress.org.ezproxy.aut.ac.nz/content/11/4/415.full.pdf+html>
- Gillespie, L., Ingle, R. M., & Havens Jr., W. K. (1966). Nutritional studies with adult oysters, *Crassostrea virginica* (Gmelin). *Florida Board of Conservation. Marine Research Laboratory*, 51, pp 1-26.
- Grant, C. M. (1994). Demographics and reproduction of the tuatua *Paphies subtriangulata*. Unpublished MSc thesis, University of Auckland, New Zealand.
- Greenway, J. P, C. (1981, June). Dargaville tuatua. *Catch*, 14-15.
- Griffiths, R. (1980). Filtration, respiration and assimilation in the black mussel *Choromytilus meridionalis*. *Marine Ecology Progress Series*, 3, 63-70. Retrieved from <http://www.int-res.com/articles/meps/3/m003p063.pdf>

- Grzimek, B., Kleiman, D. G., Geist, V., & McDade, M. C. (2004). *Grzimek's Animal Life Encyclopedia*. Detroit: Thomson-Gale.
- Guillard, R. (1975). Culture methods. In G. Hallegraeff, D. Anderson, & A. Cembella (Eds.), *Manual on Harmful Marine Microalgae*. IOC Manual and Guides No. 33 (pp. 45-62). Paris: UNESCO.
- Goldberg, R. (1989). Biology and culture of the surf clam. In J. J. Manzi & M. Castagna (Eds.), *Clam Mariculture in North America (Development in Aquaculture and Fisheries Science)* (pp. 263-276). Amsterdam: Elsevier.
- Hahn, K. O. (1989). Induction of settlement in competent abalone larvae. In K. O. Hahn (Ed.), *Hand Book of Culture of Abalone and Other Marine Gastropods* (pp. 101-112). Boca Raton: CRC Press.
- Harrison, P. J., Waters, R. E. & Taylor, F. J. R. (1980). A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *Journal of Phycology*, 16, 28-35. doi: 10.1111/j.0022-3646.1980.00028.x
- Haven, D. S. (1965). Supplemental feeding of oysters with starch. *Chesapeake Science*, 6, 43-51. doi: 10.2307/1350622
- Hawkins, A. J. S., Smith, R. F. M., Bayne, B. L., & Heral, M. (1996). Novel observations underlying the fast growth of suspension-feeding shellfish in turbid environments: *Mytilus edulis*. *Marine Ecology Progress Series*, 131, 179-190. doi: 10.3354/meps131179
- Hay, M. E. (1996). Marine chemical ecology: what's known and what's next? *Journal of Experimental Marine Biology and Ecology*, 200(1-2), 103-134. doi:10.1016/S0022-0981(96)02659-7
- Healy, J. M. (1998). The mollusca. In D. T. Anderson (Ed.), *Invertebrate zoology* (pp. 123-173). Melbourne, SM: Oxford.
- Heasman, M. P., Diemar, J., O'Connor, W. A., Sushames, T., & Foulkes, L. (2000). Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs – a summary. *Aquaculture Research*, 31, 637–659. doi: 10.1046/j.1365-2109.2000.318492.x
- Heasman, M. P., O'Connor, W. A., & Frazer, A. W. (1996). Temperature and nutrition as factors in conditioning broodstock of the commercial scallop *Pecten fumatus* Reeve. *Aquaculture*, 143(1), 75-90. doi: 10.1016/00448486(95)01231-1
- Helm, M. (1990). Modern progettazione e gestione di schiuditoi per molluschi bivalve e nuovi sviluppi. In: *Tapes philippinarum, Ente Sviluppo Agricolo Veneto*. Dosson di Casier: Treviso, p. 63.

- Helm, M., & Bourne, N. (2004). *Hatchery culture of bivalves: A practical manual*. Rome: Food and Agriculture Organization. 177 p.
- Hewitt, J., Pridmore, R., Thrush, S., & Cummings, V. (1997). Assessing the short-term stability of spatial patterns of macrobenthos in a dynamic estuarine system. *Limnology and Oceanography*, 42 (2), 282-288. Retrieved from http://72.48.224.78/lo/toc/vol_42/issue_2/0282.pdf
- Hidu, H. & Newell, C. R. (1989). Culture and ecology of the soft-shelled clam, *Mya arenaria*. In J. J. Manzi & M. Castagna (Eds.), *Clam Mariculture in North America (Development in Aquaculture and Fisheries Science)* (pp. 277-292). Amsterdam: Elsevier.
- Hildreth, D. I. & Crisp, D. J. (1976). A corrected formula for calculation of filtration rate of bivalve molluscs in an experimental flowing system. *Journal of Marine Biology Association U. K.*, 56(111-120). doi:10.1017/S0025315400020476
- Hodgson, P. A., Henderson, R. J., & Sargent, J. R. (1991). Patterns of variation in the lipid class and fatty acid composition of *Nannochloropsis oculata* (Eustigmatophyceae) during batch culture. *Journal of Applied Phycology*, 3(2), 169-181. doi: 10.1007/BF00003699
- Holland, D. A. & Chew, K. K., (1974). Reproductive cycle of the Manila clam (*Venerupis japonica*) from Hood Canal, Washington. In: *Proceedings of the National Shellfisheries Association*, 64, pp. 53–58.
- Hooker, S. H. (1995). Life history and demography of the pipi *Paphies australi* (Gmelin, 1790) (Bivalvia: Mesodesmatidae). Unpublished PhD thesis. University of Auckland, New Zealand.
- Hooker, S. H. (1997). Larval and postlarval development of the New Zealand pipi, *Paphies Australis* (Bivalvia: Mesodesmatidae). *Bulletin of Marine Science*, 61(2), 225-240. Retrieved from <http://www.ingentaconnect.com/content/umrsmas/bullmar/1997/0000001/00000002/art00003>
- Hornbach, D. J., Wissing, T. E., & Burky, A. J. (1983). Seasonal variation in the metabolic rates and Q₁₀-values of the fingernail clam, *Sphaerium striatinum* Lamarck. *Comparative Biochemistry and Physiology Part A: Physiology*, 76(4), 783-790. doi:10.1016/0300-9629(83)90141-X
- Hosie, A. M. (n.d.). *Descriptions of major taxonomic groups*. Retrieved April 15, 2010 from <http://www.marlin.ac.uk/taxonomydescriptions.php>
- Howerton, R. (2001). Best management practices for Hawaiian aquaculture. *Center for Tropical and Subtropical Aquaculture Publication No. 148*. Retrieved December 20, 2009 from http://www.ctsa.org/upload/publication/CTSA_148631672853284080260.pdf

- Hull, P. J., Cole, R. G., Creese, R. G., & Healy, T. R. (1998). An experimental investigation of the burrowing behaviour of *Paphies australis* (bivalvia: Mesodesmatidae). *Marine and Freshwater Behaviour and Physiology*, 31(3), 167-183. doi: 10.1080/10236249809387071
- Ingle, R. M. (1967). Artificial food for oysters. *Sea Frontiers*, 13, 296–303.
- Janzel, R. & Villalaz, G. (1994). Laboratory study of food concentration and temperature effect on the reproductive cycle of *Argopecten ventricosus*. *Journal of Shellfish Research*, 13(2), 513-519.
- Jeffs, A. G., Holland, R. C., Hooker, S. H., & Hayden, B. J. (1999). Overview and bibliography on the greenshell mussel, *Perna canaliculus* from New Zealand waters. *Journal of Shellfish Research*, 18, 347-360.
- Jones, H. D., Richards, O. G., & Southern, T. A. (1992). Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis* L. *Journal of Experimental Marine Biology and Ecology*, 155(2), 213-237. doi:10.1016/0022-0981(92)90064-H
- Jones, M. (1883). Animals of the estuary shore. Illustrated guide and ecology. *University of Canterbury Publication No. 32*. 162 p.
- Jorgensen, C. B. (1949). The rate of feeding by *Mytilus* in different kinds of suspension. *Journal of Marine Biology Association U. K.*, 28, 333-344. doi: 10.1017/S0025315400023249
- Jorgensen, C. B. (1975a). On gill function in the mussel *Mytilus edulis* L. *Ophelia*, 13, 187-232.
- Jorgensen, C. B. (1975b). Comparative physiology of suspension feeding. *Annual Review Physiology*, 37, 57-79. doi:10.1146/annurev.ph.37.030175.000421
- Jorgensen, C. B. (1990). *Bivalve filter feeding: hydrodynamics, bioenergetics, physiology and ecology*. Denmark, F: Olsen & Olsen.
- Jorgensen, C. B. & Goldberg, E. D. (1953). Particle filtration in some ascidians and lamellibranchs. *The Biological Bulletin*, 105(3), 477-489. Retrieved from <http://www.biolbull.org/cgi/reprint/105/3/477>
- Jorgensen, C. B., Larsen, P. S., Mohlenberg, F., & Riisgard, H. U. (1988). The bivalve pump: properties and modeling, *Marine Ecology Progress Series*, 45, 205–216.
- Jorgensen, C. B., Larsen, P. S., & Riisgard, H. U. (1990). Effects of temperature on the mussel pump. *Marine Ecology Progress Series*, 64, 89-97.
- Jorgensen, C. B. & Riisgard, H. U. (1988). Gill pump characteristics of the soft clam *Mya arenaria*. *Marine Biology*, 99(1), 107-109. doi: 10.1007/BF00644983

- Judge, M. L., Coen, L. D., & Heck, Jr. K. L. (1992). The effect of long term alteration of in situ currents on the growth of *Mercenaria mercenaria* in the northern Gulf Mexico. *Limnology and Oceanography*, 37, 1550-1559. Retrieved from <http://www.jstor.org/stable/2837969>
- Khoi, C. M., Guong, V. T., & Merckx, R. (2006). Growth of the diatom *Chaetoceros calcitrans* in sediment extracts from *Artemia franciscana* ponds at different concentrations of nitrogen and phosphorus. *Aquaculture*, 259(1-4), 354-364. doi: 10.1016/j.aquaculture.2006.05.050
- Kiorboe, T., & Mohlenberg, F. (1981). Particle selection in suspension feeding bivalves. *Marine Ecology Progress Series*, 5, 291-296. Retrieved from <http://www.int-res.com/articles/meps/5/m005p291.pdf>
- Knauer, J. & Southgate, P. (1997). Growth and fatty acid composition of Pacific oyster (*Crassostrea gigas*) spat fed a spray-dried freshwater microalga (*Spongiococcum excentricum*) and microencapsulated lipids. *Aquaculture*, 154, 293-303. doi: 10.1016/S0044-8486(97)00056-2
- Knauer, J., & Southgate, P. (1999). A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture. *Reviews in Fisheries Science*, 7, 241-280. doi: 10.1080/10641269908951362
- Knuckey, R. M., Brown, M. R., Robert, R., & Frampton, D. M. F. (2006). Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. *Aquaculture Engineering*, 35, 300-313.
- Kreeger, D. A. (1993). Seasonal patterns in utilization of dietary protein by the mussel *Mytilus trossulus*. *Marine Ecology Progress Series*, 95, 215-232. Retrieved from <http://www.int-res.com/articles/meps/95/m095p215.pdf>
- Kreeger, D. A. & Langdon, C. J. (1994). Digestion and assimilation of protein by *Mytilus trossulus* (Bivalvia: Mollusca) fed mixed carbohydrate/protein microcapsules. *Marine Biology*, 118, 479-488. doi: 10.1007/BF00350305
- Kreeger, D. A., Langdon, C. J., & Newell, R. (1988). Utilization of refractory cellulosic carbon derived from *Spartina alterniflora* by the ribbed mussel *Geukensia demissa*. *Marine Ecology Progress Series*, 42, 171-179.
- LaBarbera, M. & Vogel, S. (1976). An inexpensive thermistor flow meter for aquatic biology. *Limnology and Oceanography*, 21, 750-756. Retrieved from <http://www.scopus.com/record/display.url?eid=2-s2.0-0016889240&origin=inward&txGid=BIU5eHzly844CSPKsUstDoa%3a10>
- Laing, I. (1987). The use of artificial diets in rearing bivalve spat. *Aquaculture*, 65(3-4), 24-249. doi: 10.1016/0044-8486(87)90237-7

- Laing, I. & Millican, P. F. (1986). Relative growth and growth efficiency of *Ostrea edulis* L. spat fed various algal diets. *Aquaculture*, 54(4), 245-262. doi: 10.1016/0044-8486(86)90270-X
- Langdon, C. J., & Newell, R. (1990). Comparative utilization of detritus and bacteria as food sources by two bivalve suspension feeders, the oyster *Crassostrea virginica* and the mussel, *Geukensia demissa*. *Marine Ecology Progress Series*, 58, 299-310.
- Langdon, C. J. & Onal, E. (1999). Replacement of living microalgae with spray-dried diets for the marine mussel *Mytilus galloprovincialis*. *Aquaculture*, 180(3-4), 283-294. doi: 10.1016/S0044-8486(99)00197-0
- Langdon, C. J. & Waldock, M. J. (1981). The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. *Journal of the Marine Biological Association of the United Kingdom*, 61, 431-448. doi: 10.1017/S0025315400047056
- Larcombe, M. (1968). Distribution and recognition of intertidal organisms in the Whangateau Harbour, and a classification for sheltered soft shores. Unpublished BSc (Hons) thesis. University of Auckland, New Zealand.
- Leach, B. F., Davidson, J. M., Robertshawe, M., & Leach, P. C. (2001). Identification, nutritional yield, and economic role of Tuatua shellfish, *Paphies* spp., in New Zealand archaeological sites. *People and Culture in Oceania*, 17, (1-26). Retrieved from <http://www.soc.nii.ac.jp/jsos/mcoabs17.html>
- Leger, P., Bieber, G. F., & Sorgeloos, P. (1985). International study on *Artemia* XXXIII. Promising results in larval rearing of *Penaeus stylirostris* using a prepared diet as algal substitute and for *Artemia* enrichment. *Journal of World Mariculture Society*, 16, 354-367.
- Leising, A. W., Horner, R., Pierson, J. J., Postel, J., & Halsband-Lenk, C. (2005). The balance between microzooplankton grazing and phytoplankton growth in a highly productive estuarine fjord. *Progress in Oceanography*, 67, 366-383. doi: 10.1016/j.pocean.2005.09.007
- Liu, W., Alabi, A. O., & Pearce, C. M. (2008). Fertilization and embryonic development in the basket cockle, *Clinocardium nuttallii*. *Journal of Shellfish Research*, 27(2), 393-397. doi: 10.2983/0730-8000(2008)27[393:FAEDIT]2.0.CO;2
- Loosanoff, V. L. & Davis, H. C. (1952). Temperature requirements for maturation of gonads of northern oysters. *The Biological Bulletin*, 103, 80-96. Retrieved from <http://www.biolbull.org/cgi/reprint/103/1/80>
- Lora-Vilchis, M., Ruiz-Velazco-Cruz, E., Reynoso-Granados, T., & Voltolina, D. (2004). Evaluation of five microalgae diets for juvenile pen shells *Atrina maura*. *Journal of the World Aquaculture Society*, 35(2), 232-236. doi: 10.1111/j.1749-7345.2004.tb01079.x

- Lorio, W. J. & Malone, S. (1995). Biology and culture of the northern quahog clam (*Mercenaria mercenaria*), *Southern Regional Aquaculture Center, SRAC Publication Number 433*. Retrieved from http://aquanac.org/publicat/usda_rac/efs/srac/433fs.pdf
- Malouf, R. E. (1989). Clam culture as a resource management tool. In J. J. Manzi & M. Castagna (Eds.), *Clam Mariculture in North America (Development in Aquaculture and Fisheries Science)* (pp. 427-447). Amsterdam: Elsevier.
- Mann, R. (1979a). The effect of temperature on growth, physiology, and gametogenesis in the Manila clam *Tapes philippinarum* (Adams & Reeve, 1850). *Aquaculture*, 38(2), 121-133. doi: 10.1016/0022-0981(79)90016-9
- Mann, R. (1979b). Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. *Journal of the Marine Biological Association of the United Kingdom*, 59, 95-110. doi: 10.1017/S0025315400046208
- Marsden, I. D. (1999). Respiration and feeding of the surf clam *Paphies donacina* from New Zealand. *Hydrobiologia*, 405, 179-188. doi: 10.1023/A:1003823527514
- Martinez-Fernandez, E., Acosta-Salmon, H., & Southgate, P. (2006). The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae. *Aquaculture*, 257, 491-503. doi: 10.1111/j.1749-7345.2004.tb01079.x
- Martinez-Fernandez, E., & Southgate, P. (2007). Use of tropical microalgae as food for larvae of the black-lip pearl oyster *Pinctada margaritifera*. *Aquaculture*, 263, 220-226. doi:10.1016/j.aquaculture.2006.09.040
- Martinez, G. & Perez, H. (2003). Effect of different temperature regimes on reproductive conditioning in the scallop *Argopecten purpuratus*. *Aquaculture*, 228(1-4), 153-167. doi: 10.1016/S0044-8486(03)00321-1
- Matias, D., Joaquim, S., Leitao, A., & Massapina, C. (2008). Effect of geographic origin, temperature and timing of broodstock collection on conditioning, spawning success and larval viability of *Ruditapes decussatus* (Linné, 1758). *Aquaculture International*, 17(3), 257-271. doi: 10.1007/s10499-008-9197-3
- Mazon-Suastegui, J. M., Ruiz-Ruiz, K. M., Parres-Haro, A. P., & Saucedo, P. E. (2008). Combined effects of diet and stocking density on growth and biochemical composition of spat of the Cortez oyster *Crassostrea corteziensis* at the hatchery. *Aquaculture*, 284(1-4), 98-105. doi:10.1016/j.aquaculture.2008.07.022

- McLeod, R. & Wing, S. (2008). Influence of an altered salinity regime on the population structure of two infaunal bivalve species. *Estuarine, Coastal and Shelf Science*, 78 (3), 529-540. doi:10.1016/j.ecss.2008.01.019
- Millican, P. F. & Helm, M. (1994). Effects of nutrition on larvae production in the European flat oyster, *Ostrea edulis*. *Aquaculture*, 123, 83-94. doi: 10.1016/0044-8486(94)90121-X
- Minaur, J. (1969). Experiments on the artificial rearing of the larvae of *Pinctada maxima* (Jameson) (Lamellibranchia). *Australian Journal of Marine and Freshwater Research*, 20(2), 175-188. doi: 10.1071/MF9690175
- Ministry of Fisheries. (2004). *Paphies australis*. Retrieved July 7, 2008, from http://fpcs.fish.govt.nz/science/documents/plenary/PPI%201A_FINAL%2008.pdf
- Ministry of Fisheries (2008). *Aquaculture*. Retrieved April 19, 2010, from <http://fs.fish.govt.nz/Page.aspx?pk=24&tk=62>
- Mohlenberg, F. & Riisgard, H. U. (1978). Efficiency of particle retention in 13 species of suspension-feeding bivalves, *Ophelia*, 17, 239-246.
- Mohlenberg, F. & Riisgard, H. U. (1979). Filtration rate, using a new indirect technique, in thirteen species of suspension-feeding bivalves. *Marine Biology*, 54(2), 143-147. doi: 10.1007/BF00386593
- Morse, D. E. (1984). Biochemical and genetic engineering for improved production of abalones and other valuable molluscs. *Aquaculture*, 39(1-4), 263-282. doi:10.1016/0044-8486(84)90271-0
- Morton, J. E. & Miller, M. C. (1968). The New Zealand seashore. Collins, London. 653 p.
- Muranaka, M. S. & Lannan, J. E. (1984). Broodstock management of *Crassostrea gigas*: Environmental influences on broodstock conditioning. In Morse, D. E. and Mann, R. (Eds.), *Recent Innovations in Cultivation of Pacific Molluscs. Developments in Aquaculture and Fisheries Science* (pp. 217-228). Elsevier, Amsterdam.
- NABIS (2004). *Pipi*. Retrieved July 19, 2008, from National Aquatic Biodiversity Information System website: <http://www2.nabis.govt.nz/Map.aspx>
- Nagabhushnam, R. & Mane, U. H. (1978). Seasonal variation in the biochemical composition of *Mytilus viridis* at Ratnagiri on the West Coast of India. *Hydrobiologia*, 57(1), 69-72. doi: 10.1007/BF00018630
- Nakamura, Y. (2001). Filtration rates of the Manila clam, *Ruditapes philippinarum*: dependence on prey items including bacteria and picocyanobacteria. *Journal of Experimental Marine Biology and Ecology*, 266(2), 181-192. doi:10.1016/S0022-0981(01)00354-9

- Napolitano, G. E., Ackman, R. G., & Ratnayake, W. M. N. (1990). Fatty acid composition of three cultured algal species (*Isochrysis galbana*, *Chaetoceros gracilis* and *Chaetoceros calcitrans*) used as food for bivalve larvae. *Journal of the World Aquaculture Society*, 21(2), 122-130. doi: 10.1111/j.1749-7345.1990.tb00532.x
- Nell, J. A. (1985). Comparison of some single cell proteins in the diet of the Sydney rock oyster (*Saccostrea commercialis*). *Progressive Fish Culturist*, 47, 110-113.
- Nell, J., Diemar, J., & Heasman, M. (1996). Food value of live yeasts and dry yeast-based diets fed to Sydney rock oyster *Saccostrea commercialis* spat. *Aquaculture*, 145(1-4), 235-243. doi: 10.1016/S0044-8486(96)01337-3
- Nell, J. & O'Connor, W. (1991). The evaluation of fresh algae and storage algae concentrates as a food source for Sydney rock oyster *Saccostrea commercialis* (Iredale & Roughley) larvae. *Aquaculture*, 99, 277-284. doi: 10.1016/0044-8486(91)90248-6
- Nevejan, N., Davis, J., Little, K., & Kilonia, A. (2007). Use of formulated diet for mussel spat (*Mytilus galloprovincialis*) in a commercial hatchery. *Journal of Shellfish Research*, 26, 357-363.
- Nevejan, N. M., Pronker, A. E., & Peene, F. (2008). Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus, 1758). Part II. New formulated feeds offer new perspectives to commercial hatcheries. *Aquaculture International*, 16(6), 483-495. doi: 10.1007/s10499-007-9160-8
- Nielsen, N. F., Larsen, P. S., Riisgard, H. U., & Jorgensen, C. B. (1993). Fluid motion and particle retention in the gill of *Mytilus edulis*: video recordings and numerical modeling. *Marine Biology*, 116(1), 61-71. doi: 10.1007/BF00350732
- Numaguchi, K. & Nell, J. A. (1991). Effects of gelatin-acacia microcapsule and algal meal supplementation of algal diets on growth rates of Sydney rock oyster, *Saccostrea commercialis* (Iredale & Roughley), larvae. *Aquaculture*, 94(1), 65-78. doi: 10.1016/0044-8486(91)90129-U
- O'Connor, W. A., Nell, J. A., & Diemar, J. A. (1992). The evaluation of twelve algal species as food for juvenile Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley). *Aquaculture*, 108(3-4), 277-283. doi:10.1016/0044-8486(92)90112-X
- Ohba, S. (1959). Ecological studies in the natural population of a clam, *Tapes japonica*, with special reference to seasonal variations in the size and structure of the population and to individual growth. *Biological Journal of Okayama University*, 5, 13-42.

- Ojea, J., Pazos, A. J., Martinez, D., Novoa, S., Garcia-Martinez, P., Sanchez-Lopez, J. L., & Abad, M. (2008). Effects of temperature regime on broodstock conditioning of *Ruditapes decussatus*. *Journal of Shellfish Research*, 27(5), 1093-1100. doi: 10.2983/0730-8000-27.5.1093
- Pales Espinosa, E., & Allam, B. (2006). Comparative growth and survival of juvenile had clams, *Mercenaria mercenaria*, fed commercially available diets. *Zoo Biology*, 25, 513-525. Retrieved from: <http://somas.stonybrook.edu/~MADL/pubspdf/Emma-clamgrowth.pdf>
- Pales Espinosa, E., Barille, E., & Allam, B. (2007). Use of encapsulated live microalgae to investigate pre-ingestive selection in the oyster *Crassostrea gigas*. *Journal of Experimental Marine Biology and Ecology*, 343(1-3), 118-126. doi:10.1016/j.jembe.2006.12.002
- Patrick, S. Faury, N., & Goulletquer, P. (2006). Seasonal changes in carbohydrate metabolism and its relationship with summer mortality of Pacific oyster *Crassostrea gigas* (Thunberg) in Marennes–Oléron bay (France). *Aquaculture*, 252(2-4), 328-338. Retrieved from <http://archimer.ifremer.fr/doc/2006/publication-1116.pdf>
- Perez-Camacho, A., Albentosa, M., Fernandez-Reiriz, M. J., & Labarta, U. (1998). Effect of microalgal and inert (cornmeal and cornstarch) diets on growth performance and biochemical composition of *Ruditapes decussatus* seed. *Aquaculture*, 160(1-2), 89-102. doi:10.1016/S0044-8486(97)00232-9
- Pipe R. K. (1985). Seasonal cycles and effects of starvation on egg development in *Mytilus edulis*. *Marine Ecology Progress Series (Halstenbek)*, 24, 121-128. Retrieved from <http://scholar.google.com/scholar?q=Seasonal+cycles+and+effects+of+starvation+on+egg+mytilus>
- Pirini, M., Manuzzi, M. P., Pagliarani, A., Trombetti, F., Borgatti, A. R., & Ventrella, V. (2007). Changes in fatty acid composition of *Mytilus galloprovincialis* (Lmk) fed on microalgal and wheat germ diets, *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 147, 616-626. doi:10.1016/j.cbpb.2007.04.003
- Ponis, E., Parisi, G., Chini-Zittelli, G., Lavista, F., Robert, R., & Tredici, M. R. (2008). *Pavlova lutheri*: Production, preservation and use as food for *Crassostrea gigas* larvae. *Aquaculture*, 282(1-4), 97-103. doi:10.1016/j.aquaculture.2008.06.010
- Ponis, E., Probert, I., Veron, B., Mathieu, M., & Robert, R. (2006). New microalgae for the Pacific oyster *Crassostrea gigas* larvae. *Aquaculture*, 253, 618-627. doi:10.1016/j.aquaculture.2005.09.011
- Porter, D. & Earl, O. (1990). Nutrition labeling issues and directions for the 1990s. National Academy Press, Washington , DC .

- Powell, A. W. (1979). *New Zealand Mollusca*. Marine, land and freshwater snails. Collins, London.
- Provasoli, L. (1968). Media and prospects for the cultivation of marine algae. In H. Watanabe, & A. Hattori (Ed.), *Proceedings, U. S.-Japan Conference. Japanese Society of Plant Physiology*, pp. 63-75. Hakone, Japan.
- Raby, D., Mingelbier, M., Dodson, J. J., Klein, B., Lagadeuc, Y., Legendre, L. (1997). Food-particle size and selection by bivalve larvae in a temperate embayment. *Marine Biology*, 124, 665-672. doi: 10.1007/s002270050057
- Rao, C. B. (1955). On the distribution of algae in a group of six small ponds: II. algal periodicity. *Journal of Ecology*, 43(1), 291-308. Retrieved from <http://www.jstor.org/stable/pdfplus/2257136.pdf>
- Redpath, K. J. & Davenport, J. (1988). The effect of copper, zinc and cadmium on the pumping rate of *Mytilus edulis* L. *Aquatic Toxicology*, 13(3), 217-225. doi:10.1016/0166-445X(88)90054-9
- Reid, R. G. B. (1969). Seasonal observations on diet, and stored glycogen and lipids in the horse clam, *Tresus capax* (Gould, 1850). *Veliger*, 11, 378-381.
- Riisgard, H. U. (1977). On measurements of the filtration rates of suspension feeding bivalves in a flow system, *Ophelia*, 17, 167–173.
- Riisgard, H. U. (1981). Cell-volume response in the naked marine flagellated *Dunaliella marina* transformed from darkness to light of different intensities. *Botanica Marina*, 24, 657-659.
- Riisgard, H. U. (1988). Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. *Marine Ecology Progress Series (Halstenbek)*, 45, 217-223. Retrieved from <http://www.int-res.com/articles/meps/45/m045p217.pdf>
- Riisgard, H. U. (2001). On measurement of filtration rates in bivalves—the stony road to reliable data: review and interpretation. *Marine Ecology Progress Series*, 211, 275-291. Retrieved from <http://www.int-res.com/articles/meps/211/m211p275.pdf>
- Riisgard, H. U. & Larsen, P. S. (2000). Comparative ecophysiology of active zoobenthic filter feeding, essence of current knowledge. *Journal of Sea Research*, 44(3-4), 169-193. doi: 10.1016/S1385-1101(00)00054-X
- Riisgard, H. U. & Mohlenberg, F. (1979). An improved automatic recording apparatus for determining the filtration rate of *Mytilus edulis* as a function of size and algal concentration. *Marine Biology*, 52 (1), 61-67. doi: 10.1007/BF00386858

- Riisgard, H. U., Poulsen, L., & Larsen, P. S. (1996). Phytoplankton reduction in near-bottom water caused by filter-feeding *Nereis diversicolor*—implications for worm growth and population grazing impact. *Marine Ecology Progress Series* (in press).
- Riisgard, H. U. & Randlov, A. A. (1981). Energy budget, growth and filtration rates in *Mytilus edulis* at different algal concentrations. *Marine Biology*, 61(2-3), 227-234. doi: 10.1007/BF00386664
- Riisgard, H. U., Randlov, A. A., & Kristensen, P. S. (1980). Rates of processing, oxygen consumption and efficiency of particle retention in veligers and post-metamorphic *Mytilus edulis*. *Ophelia*, 19, 37-47.
- Rivonker, C. U. & Parulekar, A. H. (1995). Proximate biochemical composition and caloric potential in the raft-grown green mussel *Perna viridis*. *Journal of Marine Biological Association of India*, 37, 231-236. Retrieved from <http://drs.nio.org/drs/handle/2264/2304>
- Robert. R., Parisi, G., Rodolfi, L., Poli, B. M., & Tredici, M. R. (2001). Use of fresh and preserved *Tetraselmis suecica* for feeding *Crassostrea gigas* larvae. *Aquaculture*, 192(2-4), 333-346. doi:10.1016/S0044-8486(00)00456-7
- Robinson, A. (1992). Dietary supplements for reproductive conditioning of *Crassostrea gigas kumamoto* (Thunberg): I. Effects on gonadal development, quality of ova and larvae through metamorphosis. *Journal of Shellfish Research*, 11, 437-441.
- Rodhouse, P. G., Roden, C. M., Burnell, G. M., Hensey, M. P., McMahon, T., Ottway, B., & Ryan, H. (1984). *Journal of the Marine Biological Association of the United Kingdom*, 64, 513-529. doi:10.1017/S0025315400030204
- Rodriguez, M. E., Arnaiz, R., Coa, A., Martinez, D., Silva, A., Varela, J. A., (1993). Proceso de maduración y acondicionamiento de *Tapes decussatus* (Linné, 1787), fuera de época natural: I. Histoquímica, histología y composición bioquímica. In: Cerviño, A., Landín, A., de Coa, A., Guerra, A., Torre, M. (Eds.), Proc. IV Nat. Congress Aquaculture, 21-24 September 1993. Consellería de Pesca, Marisqueo e Acuicultura, Xunta de Galicia, España, pp. 335–340.
- Round, F. E., Crawford, R. M., & Mann, D. G. (1990). *The diatoms: Biology and morphology of the genera*. New York, NY: Cambridge University Press.
- Ruiz, C., Abad, M., Sedano, F., Garcia-Martin, L. O., & Sanchez-Lopez, J. L. (1992). Influence of seasonal environmental changes on the gamete production and biochemical composition of *Crassostrea gigas* (Thunberg) in suspended culture in El Grove, Galicia, Spain. *Aquaculture*, 155(2), 249-262. doi: 10.1016/0022-0981(92)90066-J
- Samain, J. –F., Segueineau, C., Cochard, J. –C., Delaunay, F., Nicolas, J. –L., Marty, Y., Galois, R., Mathieu, M., & Moal, J. (1992). What about growth variability for *Pecten maximus* production? *Oceanis*, 18, 49-66.

- Saout, C., Quere, C., Donval, A., Paulet, Y. –M., & Samain, J. –F. (1999). An experimental study of the combined effects of temperature and photoperiod on reproductive physiology of *Pecten maximus* from the Bay of Brest (France). *Aquaculture*, 172(3-4), 301-314. doi: 10.1016/S0044-8486(98)00406-2
- Sastry, A. N. (1968). The relationships among food, temperature, and gonad development of the bay scallop, *Aequipecten irradians* Lamarck. *Physiological Zoology*, 41, 44-53.
- Sastry, A. N. (1969). Temperature effects in reproduction of the big scallop, *Aequipecten irradians* Lamarck. *The Biological Bulletin*, 130, 118-134. Retrieved from <http://www.biolbull.org/cgi/reprint/130/1/118>
- Sastry, A. N. (1979). Pelecypoda (excluding Ostreidae). Reproduction in Marine Invertebrates. In Giese, A. C. and Pearse, J. S. (Eds.), *Volume V; Molluscs: Pelecypods and Lesser Classes* (pp. 113-292). Academic Press, New York.
- Schmidt, L. E. & Hansen, P. J. (2001). Allelopathy in the prymnesiophyte *Chrysochromulina polylepis*: effect of cell concentration, growth phase and pH. *Marine Ecology Progress Series*, 216, 67-81. Retrieved from <http://www.int-res.com/articles/meps/216/m216p067.pdf>
- Seguineau, C., Laschi-Loquerie, A., Moal, J., & Samain, J. F. (1996). Vitamin requirements in great scallop larvae. *Aquaculture International*, 4(4), 315-324. doi: 10.1007/BF00120948
- Shaw, W. (1962). Seasonal gonadal changes in female soft-shelled clams, *Mya arenaria* in the Tred Avon river, Maryland. In: *Proceedings of the National Shellfisheries Association*, 53, 121-132.
- Shumway, S. E., Cucci, T. L., Newell, R. C., & Yentsch, C. M. (1985). Particle selection, ingestion, and absorption in filter-feeding bivalves. *Journal of Experimental Marine Biology and Ecology*, 54(2), 143-147. doi:10.1016/0022-0981(85)90222-9
- Smith, P. J., MacArthur, G. J., & Michael, K. P. (1989). Regional variation in electromorph frequencies in the tuatua, *Paphies subtriangulata*, around New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 23, 27-33.
- Sobral, P. & Widdows, J. Effects of increasing current velocity, turbidity and particle-size selection on the feeding activity and scope for growth of *Ruditapes decussatus* from Ria Formosa, southern Portugal. *Journal of Experimental Marine Biology and Ecology*, 245(1), 111-125. doi: 10.1016/S0022-0981(99)00154-9

- Soudant, P., Marty, Y., Moal, J., Robert, R., Quere, C., Le Coz, J. R., & Samain, J. F. (1996a). Effect of food fatty acid and sterol quality on *Pecten maximus* gonad composition and reproduction process. *Journal of Experimental Marine Biology and Ecology*, 143, 361-378. doi: 10.1016/0044-8486(96)01276-8
- Soudant, P., Moal, J., Marty, Y., & Samain, J. F. (1996b). Impact of the quality of dietary fatty acids on metabolism and the composition of polar lipid classes in female gonads of *Pecten maximus* (L.). *Journal of Experimental Marine Biology and Ecology*, 205(1-2), 149-163. doi:10.1016/S0022-0981(96)02608-1
- Spencer, B. E. (2002). Molluscan shellfish farming. United Kingdom, UK: Blackwell Science.
- Taylor, J. J., Rose, R. A., Southgate, P. C., & Taylor, C. E. (1997). Effects of stocking density on growth and survival of early juvenile silver-lip pearl oysters, *Pinctada maxima* (Jameson), held in suspended nursery culture. *Aquaculture*, 153, 41-49. doi:10.1016/S0044-8486(97)00015-X
- Thompson, P. A. & Harrison, P. J. (1992). Effects of monospecific algal diets of varying biochemical composition on the growth and survival of Pacific oyster (*Crassostrea gigas*) larvae. *Marine Biology*, 113(4), 645-654. Retrieved from <http://www.springerlink.com/content/g333137322r38124/>
- Tillmann, U. & John, U. (2002). Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. *Marine Ecology Progress Series*, 230, 47-58. Retrieved from <http://www.int-res.com/articles/meps2002/230/m230p047.pdf>
- Urban, Jr. E. R. & Langdon, C. J. (1984). Reduction in costs of diets for the American oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements. *Aquaculture*, 38(4), 277-291. doi:10.1016/00448486(84)90333-8
- Uriarte, I. & Farias, A. (1999). The effect of dietary protein content on growth and biochemical composition of Chilean scallop *Argopecten purpuratus* (L.) postlarvae and spat. *Aquaculture*, 180(1-2), 119-127. doi: 10.1016/S0044-8486(99)00145-3
- Uriarte, I., Farias, A., & Castilla, J. C. (2001). Effect of antibiotic treatment during larval development of the Chilean scallop *Argopecten purpuratus*. *Aquacultural Engineering*, 25(3), 139-147. doi:10.1016/S01448609(01)00078-4
- Uriarte, I., Farias, A., Hernandez, J., Schafer, C., & Sorgeloos, P. (2004). Reproductive conditioning of Chilean scallop (*Argopecten purpuratus*) and the Pacific oyster (*Crassostrea gigas*): effects of enriched diets. *Aquaculture*, 230(1-4), 349-357. doi:10.1016/S0044-8486(03)00419-8

- Uronen, P., Lehtinen, S., Legrand, C., Kuuppo, P., & Tamminen, T. (2005). Haemolytic activity and allelopathy of the haptophyte *Prymnesium parvum* in nutrient-limited and balanced growth conditions. *Marine Ecology Progress Series*, 299, 137-148. Retrieved from <http://www.int-res.com/articles/meps2005/299/m299p137.pdf>
- Utting, S. D. & Millican, P. F. (1997). Techniques for the hatchery conditioning of bivalve broodstocks and the subsequent effect on egg quality and larval viability. *Aquaculture*, 155(1-4), 45-54. doi: 10.1016/S0044-8486(97)00108-7
- Utting, S. D. & Millican, P. F. (1998). The role of diet in hatchery conditioning of *Pecten maximus* L.: a review. *Aquaculture*, 165, 167-178. doi: 10.1016/S0044-8486(98)00268-3
- Utting, S. D. & Spencer, B. E. (1991). *The hatchery culture of bivalve mollusc larvae and juveniles*. Laboratory Leaflet Number 68, Ministry of Agriculture, Fisheries and Food Directorate of Fisheries Research Lowestoft. 31p. Retrieved from http://www.lmm.ufsc.br/data/files/larvicultura_Utting_and_Spencer.pdf
- Venus, G. (1984). *Paphies australis* (pipis) in the Whangarei Harbour. *Whangarei Harbour Study Technical Report No. 6. Unpublished Report to the Northland Harbour Board, Whangarei, New Zealand*.
- Vogel, S. *Life in moving fluids*. 2nd ed. Chichester, WS. Princeton University Press.
- Wafer, M. V. M., Vijayaraghawan, S., & Krishnakumari, L., (1976). Seasonal changes in the nutritive value of the Green mussel *Mytilus viridis* L. *Indian Journal of Marine Sciences*, 5, 252-254.
- Waite, A. M., Olson, R. J., Dam, H. G., & Passow, U. (1995). Sugar-containing compounds on the cell surfaces of marine diatoms measured using concanavalin A and flow cytometry. *Journal of Phycology*, 31(6), 925-933. doi: 10.1111/j.1529-8817.1995.tb03847.x
- Waldock, M. J. & Nascimento, I. A. (1979). The triacylglycerol composition of *Crassostrea gigas* larvae fed on different algal diets. *Marine Biological Letters*, 1, 77-86.
- Walne, P. (1970). Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria*, and *Mytilus*. *Fish Investigations*, 26, 1-62.
- Walrond, C. (2009, March 2). *Coastal fish - New Zealand's coastal fish*. Retrieved February 11, 2010, from Te Ara - the Encyclopedia of New Zealand website: <http://www.TeAra.govt.nz/en/coastal-fish/1>

- Ward, J. E. & MacDonald, B. A. (1996). Pre-ingestive feeding behaviors of two sub-tropical bivalves (*Pinctada imbricata* and *Arca zebra*): responses to an acute increase in suspended sediment concentration. *Bulletin of Marine Science*, 59(2), 417-432. Retrieved from <http://www.ingentaconnect.com/content/umrsmas/bullmar/1996/00000059/00000002/art00010>
- Ward, J. E. & Targett, N. M. (1989). Influence of marine microalgal metabolites on the feeding behaviour of the blue mussel *Mytilus edulis*. *Marine Biology*, 101, 313-321. doi: 10.1007/BF00428127
- Webb, K. L. & Chu, F. E. (1983). Phytoplankton as a food source for bivalve larvae. In Pruder, G. D., Langdon, C. J., & Conklin, D. (Eds.), *Biochemical and Physiological Approaches to Shellfish Nutrition. Proceedings of the Second International Conference on Aquaculture Nutrition* (pp. 272-291). Louisiana State University: Baton Rouge.
- Welch, K. J. & Joy, J. E. (1984). Growth Rates of the Asiatic Clam, *Corbicula fluminea* (Muller), in the Kanawha River, West Virginia. *Freshwater Invertebrate Biology*, 3(3), 139-142. Retrieved from <http://www.jstor.org/stable/1467187>
- Whyte, J. N. C., Bourne, N., & Hodgson, C. A. (1989). Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. *Aquaculture*, 78(3-4), 333-347. doi:10.1016/0044-8486(89)90110-5
- Whyte, J. N. C., Bourne, N., & Hodgson, C. A. (1990). Nutritional condition of rock scallop, *Crassadoma gigantea* (Gray), larvae fed mixed algal diets. *Aquaculture*, 86, 25-40. doi: 10.1016/0044-8486(90)90219-D
- Whyte, J. N. C., Englar, J. R., Carswell, B. L. (1990). Biochemical composition and energy reserves in *Crassostrea gigas* exposed to different levels of nutrition. *Aquaculture*, 90(2), 157-172. doi:10.1016/0044-8486(90)90338-N
- Wikfors, G. H., Ferris, G. E., & Smith, B. C. (1992). The relationship between gross biochemical composition of cultured algal foods and growth of the hard clam, *Mercenaria mercenaria* (L.). *Aquaculture*, 108, 135-154. doi: 10.1016/0044-8486(92)90324-E
- Wikfors, G. H., Twarog, Jr. J. W., & Ukeles, R. (1984). Influence of chemical composition of algal food sources on growth of juvenile oysters, *Crassostrea virginica*. *The Biological Bulletin*, 167, 251-263. Retrieved from <http://www.biolbull.org/cgi/reprint/167/1/251>
- Williams, J. & Babcock, R. (2004). Comparison of multiple techniques to evaluate reproductive variability in a marine bivalve: application to the scallop *Pecten novaezelandiae*. *Marine and Freshwater Research*, 55, 457-468.

- Winter, J. E. (1973). The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by a continuous automatic recording apparatus. *Marine Biology*, 22(4), 317-328. doi: 10.1007/BF00391388
- Woodcock, S. & Benkendorff, K. (2008). The impact of diet on the growth and proximate composition of juvenile whelks, *Dicathais orbita* (Gastropoda: Mollusca). *Aquaculture*, 276, 162170. doi:10.1016/j.aquaculture.2008.01.036
- Wright, J. E. (1973). The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by continuous automatic recording apparatus. *Marine Biology*, 22, 317-328. doi: 10.1007/BF00391388
- Wright, R. T., Coffin, R. B., Ersing, C. P., & Pearson, D. (1982). Field and laboratory measurements of bivalve filtration of natural marine. *Limnology and Oceanography*, 27(1), 91-98. Retrieved from <http://www.jstor.org/stable/2836088>
- Xie, Q. & Burnell, G. M. (1994). A comparative study of the gametogenic cycles of the *Tapes philippinarum* (Adams and Reeve, 1850) and *Tapes decussatus* (Linnaeus) on the south coast of Ireland. *Journal of Shellfish Research*, 13(2), 467-472.
- Zhang, G. & Yan, X. (2006). A new three-phase culture method for Manila clam, *Ruditapes philippinarum*, farming in northern China. *Aquaculture* (in press).

APPENDIX I

Microalgal cell concentrations fed to pipi within 360 minutes ($\times 10^6$ cells/mL).

Microalgal species		Time (minutes)				
		0	30	60	180	360
<i>Isochrysis galbana</i>	Rep. 1	3.21	1.30	0.91	0.53	0.44
	Rep. 2	3.36	1.59	0.79	0.57	0.37
	Rep. 3	3.17	1.28	0.81	0.46	0.33
<i>Pavlova lutheri</i>	Rep. 1	4.01	3.09	2.13	1.55	1.17
	Rep. 2	3.84	3.20	2.20	1.53	1.11
	Rep. 3	3.90	2.99	2.17	1.41	1.21
<i>Tetraselmis suecica</i>	Rep. 1	2.70	1.39	0.90	0.61	0.44
	Rep. 2	2.56	1.26	1.06	0.57	0.46
	Rep. 3	2.61	1.25	0.91	0.69	0.49
<i>Chaetoceros muelleri</i>	Rep. 1	3.51	1.67	1.10	0.72	0.61
	Rep. 2	3.39	1.71	1.34	0.75	0.56
	Rep. 3	3.42	1.65	1.28	0.87	0.54
<i>Thalassiosira pseudonana</i>	Rep. 1	3.21	1.08	0.65	0.35	0.25
	Rep. 2	2.89	1.10	0.61	0.49	0.33
	Rep. 3	2.76	1.04	0.67	0.27	0.11

APPENDIX II

Shell length (mm), wet weight (mg), and mortality data for spat pipi.

Diet	mm ind. ⁻¹	mg ind. ⁻¹	mortality %
<i>Isochrysis galbana</i>	1.2124	84.5252	16.67
<i>Pavlova lutheri</i>	0.9083	125.1358	12.22
<i>Tetraselmis suecica</i>	0.6616	70.6945	17.78
<i>Chaetoceros muelleri</i>	0.9941	73.6429	13.33
<i>Thalassiosira pseudonana</i>	0.8295	65.3019	15.56
Yeast	1.0081	92.3304	18.89
Wheat flour	0.7945	74.9383	12.22
Corn flour	0.7189	68.1282	14.44
Control	0.0640	-24.0404	22.22

Shell length (mm), wet weight (mg), and mortality data for juvenile pipi.

Diet	mm ind. ⁻¹	mg ind. ⁻¹	mortality %
<i>Isochrysis galbana</i>	1.7213	163.2726	25.00
<i>Pavlova lutheri</i>	1.3391	177.9412	18.33
<i>Tetraselmis suecica</i>	1.3106	96.7190	21.67
<i>Chaetoceros muelleri</i>	0.6528	154.6250	21.67
<i>Thalassiosira pseudonana</i>	1.1203	120.2222	21.67
Yeast	0.8916	84.8663	33.33
Wheat flour	1.0807	77.4683	25.00
Corn flour	1.1023	104.2266	20.00
Control	0.0328	-37.0147	20.00

Shell length (mm), wet weight (mg), and mortality data for adult pipi.

Diets	mm ind. ⁻¹	mg ind. ⁻¹	mortality %
<i>Isochrysis galbana</i>	0.3296	93.0556	16.67
<i>Pavlova lutheri</i>	0.5321	167.7381	23.33
<i>Tetraselmis suecica</i>	0.3167	118.6310	26.67
<i>Chaetoceros muelleri</i>	0.2893	150.7143	23.33
<i>Thalassiosira pseudonana</i>	0.2571	89.1071	23.33
Yeast	0.5135	131.7460	23.33
Wheat flour	0.5885	80.6944	30.00
Corn flour	0.7940	145.8796	16.67
Control	0.0145	-87.8373	30.00

APPENDIX III

Phase 1: Early active

Phase 2: Late active

Phase 3: Mature

Number of pipi individuals at different maturity phases.

100% <i>Pavlova lutheri</i>					100% corn flour				
No.	Sex	L	W	Phase	No.	Sex	L	W	Phase
1	M	65.4	34.97	2	16	M	58.6	26.46	2
2	M	63.5	35.02	2	17	F	58.4	27.77	3
3	M	58.3	24.65	3	18	M	59.5	24.49	2
4	M	62.4	31.73	2	19	F	56.6	26.36	2
5	M	59.3	25.68	2	20	F	59.2	26.80	3
6	F	63.7	33.50	3	21	M	60.4	35.92	2
7	M	58.3	27.51	2	22	F	58.8	30.24	2
8	F	59.4	25.68	3	23	F	62.3	33.03	3
9	F	58.1	27.64	3	25	M	60.3	33.00	3
10	M	58.9	30.27	2	26	M	61.2	28.26	3
11	F	56.9	20.37	3	27	M	59.5	26.98	3
12	M	58.0	24.73	2	28	F	62.4	26.42	2
13	F	61.4	36.21	2	29	F	55.9	21.21	2
14	F	57.9	27.17	3	30	F	56.9	28.03	2
15	F	59.8	29.58	2					
<i>Pavlova</i>-corn flour					Deprived				
No.	Sex	L	W	Phase	No.	Sex	L	W	Phase
31	M	57.9	21.87	3	46	F	62.8	38.58	2
32	F	58.7	25.77	3	47	M	59.8	27.91	2
33	F	59.3	27.09	2	48	M	59.4	26.90	2
34	F	58.4	27.78	3	49	M	64.7	35.41	2
35	M	59.5	27.52	3	50	F	54.8	21.99	2
36	F	56.5	24.76	3	51	F	60.4	25.79	2
37	F	61.0	29.29	3	52	M	58.2	27.80	2
38	F	57.5	25.08	3	53	F	54.8	19.97	1
39	M	55.8	23.58	2	54	F	58.8	26.03	2
40	M	58.8	29.57	3	55	F	58.7	26.02	1
41	M	57.8	25.56	3	56	M	58.0	23.61	2
42	F	62.2	31.58	2	57	F	56.5	23.74	3
43	F	62.3	30.05	3	58	M	60.9	28.06	1
					59	M	54.8	25.24	2
					60	M	60.4	28.51	2