Optimising growth and survival in Irish aquaculture of Abalone and Sea Urchins

Colin Hannon

Submitted in fulfillment of the requirements of the degree of

Doctor of Philosophy

Supervised by Dr Rick A. Officer

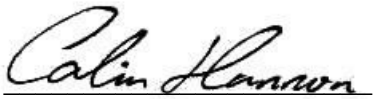
Submitted to the Galway-Mayo Institute of Technology

September 2015

GMIT
INSTITIÚID TEICNEOLAÍOCHTA NA GAILLIMHE-MAIGH EO
GALWAY-MAYO INSTITUTE OF TECHNOLOGY

Declaration:

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed:  Candidate

ID No. G00165703

Date: 24/09/15

Corrigendum:

This corrigendum excludes a co-authored manuscript that was not considered as part of the PhD examination but was previously presented as an appendix to the thesis:

> Domínguez-Godino, J.A., Slater, M.J., Hannon, C., González-Wangüermert, M., 2015. A new species for sea cucumber ranching and aquaculture: Breeding and rearing of *Holothuria arguinensis*. Aquaculture 438, 122–128.

This appendix, previously published without the authorisation of all co-authors, has been removed at the request of a co-author.

The title of the thesis has also been corrected to one consistent with the scope of the work examined. All references to the now omitted appendix and its context have also been removed from this version of the thesis.

Abstract

Optimising growth and survival in Irish abalone and sea urchin aquaculture

Increased demand for abalone and sea urchins globally has resulted in increased production of these commercially important invertebrates in aquaculture. Transfer of aquaculture technology from established producing countries to Europe has resulted in the application and implementation of new technologies by farm operators in Ireland. The industry-based research presented in this thesis addresses key industry barriers in invertebrate aquaculture, focusing on production and on-growing methods for the European abalone *Haliotis tuberculata* and the commercially important native sea urchin *Paracentrotus lividus*. To resolve knowledge gaps in the production of algivorous invertebrates in Ireland four main issues were investigated:

1- Industry, market and production constraints for the non-endemic species of abalone *H. tuberculata* and *H. discus hannai* were reviewed and evaluated. Findings indicated the current status and direction of the culture of abalone in Europe.

2- New culture methods were developed for the green encrusting macroalgae *Ulvella lens* as it is known to increase settlement rates of swimming abalone and sea urchin larvae. Both abalone and sea urchins have similar preferences to settlement cues and the technology for settlement is common to both industries. *Ulvella lens* has been used and implemented successfully into the abalone industry in producing countries such as Japan and Australia.

3- Culture methods developed for *U. lens* were applied to the *P. lividus* culture with the aim of implementing this developed technology into sea urchin aquaculture in Ireland. Due to the decline of the commercial *P. lividus* fishery in Ireland, increased interest has developed in the commercial culture of this species. The findings of this industry-based research indicate the need for increased commercial scale research addressing the production constraints that hamper the industry as a whole.

4- Novel mixed macroalgal-meal diets enriched with bioavailable phosphorus were investigated for increased growth in the diet of *H. tuberculata*, and compared against *Palmaria palmata*. Weaning diets for juvenile abalone normally incorporate some form of animal protein, which does not belong in the natural diet of juvenile abalone as they are algivorous by nature. Phosphorus is limiting in the diets of abalone, and may not be available as it is bound in an indigestible form such as phytate.

Table of Contents

List of Figures

List of Tables

Acknowledgments

This PhD was partly funded by County Galway Vocational Education Committee.

I would firstly like to thank my supervisor Dr Rick Officer whose encouragement, support and patience was never lost over the years of this study, and only for a broken beam trawl I wouldn't taken the path that has lead to this study.

I would like to thank Jean Le Dorven for the years of training and motivation to continue work in aquaculture, and to John Chamberlain whose willingness to try new culture techniques even if they didn't work the first time. To Dr Matt Slater for continuingly involving me in projects that has furthered my research I would also like to say thanks.

To my colleague and friend Dr James Keating and the rest of the Marine & Freshwater Research Centre students and staff, thanks for all the good times.

Most importantly I'd like to thank my parents Willie and Maureen for their support and encouragement over the years of study, and to Aisling for her never ending support and kindness.

Chapter I: General Introduction

Global demand for valuable invertebrates has driven their increased production through aquaculture. FAO figures show constantly increasing trends in the production of valuable invertebrates such as abalone (Cook, 2014; Cook & Gordon, 2010) and sea urchins (Lawrence and Lawrence, 2014).

The main production regions for abalone are China, Korea, South Africa, Chile and Australia, with China producing 90,694 metric tonnes (mt) between 2013-2014 (Cook, 2014). Sea urchin production is based mainly in Chile, USA, Japan, Canada and Russia, with Chile producing over half the world's supply of urchins (Watson *et al*., 2004).

However demand from outside European markets for both abalone and sea urchins exceeds the production through aquaculture (Cook, 2014; Cook *et al*., 2007). This demand has resulted in increased production through the adoption of culture methods developed in known producing countries, and the transfer of these technologies to developing industries in Europe (Hannon, *et al*., 2013).

Culture methods for both abalone and sea urchins in countries with commercial levels of production are well documented. Therefore industry led approaches may best facilitate the increased production of these commercially important invertebrates (Daume, 2007; Lawrence and Lawrence, 2014). The adoption of defined production methods by the industry in Ireland would replace the trial and error approach currently taken by farm operators.

Current invertebrate species of interest for increased cultivation in Ireland include two species of abalone; *Haliotis discus hannai* [Ino 1953] and *H. tuberculata* [Linnaeus, 1758], with the former been highly valued worldwide (Cook & Gordon 2010), and the native sea urchin *Paracentrotus lividus* [Lamarck, 1816] (Watson *et al*., 2004).

1.1 Abalone: *Haliotis tuberculata* & *H. discus hannai*

Abalone are large subtidal marine algivorous gastropods that are classified under the one genus *Haliotis* which totals about 100 species globally (Hahn, 1989). *H. tuberculata*, or Ormer in English, is common to the eastern Atlantic coasts of Brittany and France with its most northern geographical range being the Channel Islands of Jersey and Guernsey (Huchette and Clavier, 2004). *H. tuberculata*'s southern range extends from France to the coasts of Spain and Portugal including the Canary Islands and the Azores. This species of abalone is also distributed in some areas of the North African coasts and Mediterranean (Mgaya, 1995). Due to a short larval period dispersal is limited by the time available prior to settlement and a predisposition to specific habitats (Mgaya, 1995).

A commercial fishery exists for *H. tuberculata* in Brittany with minimal landings and a small total allowable catch (Huchette and Clavier, 2004). *H. tuberculata* is subject to over exploitation and fishing pressure like other valuable abalone species such as *H. discus hannai* (Cook and Gordon, 2010). Pressure from fisheries and declining harvest has also stimulated the culture of this species of abalone. Culture of *H. tuberculata* began in the 1980s in France and since then production has increased in France, Spain, Ireland, and Jersey through the establishment of commercial hatcheries and farms (Courtois de Viçose *et al*., 2012; Mai *et al*., 1995; Mgaya and Mercer, 1995).

H. discus hannai is a temperate-water species of abalone with a large geographical range. This species is endemic to the Pacific coasts of mainly China, Japan and Korea. *H. discus hannai* is one of the most studied species of abalone (Leighton, 2008; Hahn, 1989). This species is considered one of the most prized species in market places in Japan, demanding high value (Cook, 2014; Cook and Gordon, 2010). The culture of *H. discus hannai* was established in China in the early 1970s, with the development of defined production methods (Nie *et al*., 1996). Due to the increased production through aquaculture and the decline of wild stocks from fishing pressure and poaching (Cook and Gordon, 2010), this species is now cultured in several countries where *H. discus hannai* is not native (USA (Hawaii), Chile, Iceland, Spain, and Ireland).

H. tuberculata were first introduced to Ireland in the late 1970s and *H. discus hannai* in the mid-1980s (Leighton, 2008). *H. discus hannai* was introduced due to a tolerance of low seawater temperatures in winter and higher market value (Hahn, 1989). As the only country in Europe that has imported both these species of abalone Ireland has become an important hub for the expansion of the abalone aquaculture industry in Europe. As both species of abalone are cultured in Ireland it allows for greater feasibility of exporting to other countries within Europe. Once species are brought into Europe there are less stringent controls on the movement of these animals within the European Union. Farm operators in other European countries have previously been unsuccessful in importing directly from endemic regions such as Japan or Korea. However once abalone are certified disease free by state authorities and are on-grown in a disease free area they can be transported from region to region, as the animals exists with the E.U. The main diseases affecting abalone farming in Europe are *Vibrio* spp. in particular *Vibrio harveyi* and polychaete infestations of the genus *Polydora* (Nicolas *et al*., 2002)

Whilst commercial production in Europe has not yet exceeded 10mt per annum, the industry is still growing and Irish farm operators alone produce 3mt annually (Cook, 2014). Both *H. tuberculata* and *H. discus hannai* have similar nutritional requirements, culture conditions and settlement substrates (Courtois De Viçose *et al., 2010;* Mai *et al*., 1995; Mgaya and Mercer, 1995; Takahashi and Koganezawa, 1988) therefore farming of these species can be integrated as production methods and equipment are common to both species.

1.2 Sea Urchin: *Paracentrotus lividus*

The purple edible sea urchin *Paracentrotus lividus* is an echinoderm belonging to the family Echinodermata. These marine invertebrates inhabit sloping rocky seabeds and subtidal rock-pools and are endemic to the exposed rocky coast of Ireland (Heffernan, 1999). The species has a large distribution extending from Scotland, Ireland, and France and into the Mediterranean (Lawrence and Lawrence, 2014), following a similar southerly distribution as the European abalone *H. tuberculata* (Mgaya, 1995).

P. lividus is a large sea urchin with a test diameter of a maximum of 7.5-8.0cm (Lawrence, 2007; Watson *et al*., 2004). This species of sea urchin is predominately algivorous by nature, however it is opportunistic and can be omnivorous (Fernandez *et al*., 1998).

The *P. lividus* fishery in Ireland was exploited to near collapse by fishermen and divers between 1970 to the mid-1990s with a maximum export of 350mt per year (Andrew *et al*., 1995; Hur *et al*., 2002). The main demand for this species is from the French market where it is valued for its high quality roe (Cook *et al*., 2007; Fernández-Boán *et al*., 2012).

Recently this commercially important sea urchin has become the focus of increased interest for commercial aquaculture production around Europe as declining wild harvest are unable to supply market demand. The traditional market for *P. lividus* harvested in Ireland is mainly European, with the bulk of harvested urchins going to the French market (Watson *et al*., 2004).

The undersupply of an established market for a known species such as *P. lividus* presents an opportunity for producers of the valuable sea urchin to establish commercial scale production of *P. lividus* in Europe.

1.3 Review of the technical challenges facing aquaculture of the European abalone *Haliotis tuberculata* in Ireland

This chapter section examines the current status of abalone aquaculture in Europe focusing mainly on the European abalone *H. tuberculata*. The production constraints faced by farm operators and the industry are examined and identified as a whole, which culminates with industry advice and the direction needed to overcome barriers in production. A version of chapter section 1.3 can be found in the Journal Aquaculture International published as:

Colin Hannon, Rick A. Officer and Jean Le Dorven (2013). Review of the technical challenges facing aquaculture of the European abalone *Haliotis tuberculata* in Ireland. Aquaculture International Volume **21**, Issue **2**, Pages: 243–254

1.3.1 Abstract

Insufficient supply of available market size abalone from the wild stocks has resulted in increasing efforts since the early 1990s to culture this valuable marine shellfish. Despite significant financial investment and the establishment and expansion of farms around Europe, the production of a saleable end product has remained undefined. The technical barriers to producing viable juvenile abalone spat still impair growth of the industry in Ireland. Critical developments required in the industry are identified. Uptake of these developments from research remains slow due to the trial and error approach taken by operators. Using technologies and procedures from producing countries and implementing them into Irish culture conditions will aid the development and expansion of the industry.

1.3.2 Introduction

Global production of wild caught and cultured abalone reached 22,600 metric tonnes in 2002, over 8,600 metric tonnes was farmed and the total value of the production was estimated as approximately $0.8 Billion USD in 2002 (Gordon & Cook 2004). Six years later in 2008, the total available amount of abalone to market was 44,510 metric tonnes, almost double the figure in 2002 and a reported increase in farmed production of 350% in the same time period (Cook & Gordon 2010; FAO 2010).

A decline in yields from wild fisheries triggered a rapid development of abalone aquaculture in the early 1990s, and cultivation is widespread in many countries (Gordon & Cook 2001; Hahn 1989; Huchette *et al*. 2004).

In 2006, the FAO (FAO 2007) put a value on the worldwide production of abalone from aquaculture at $2.8 billion USD and the industry, at that point, was growing at a rate of almost 900 percent p/a. The lack of published production figures of the European industry means production results are only speculative with many farms not having a saleable or marketable product at the end of a production cycle of 4 to 5 years (Huchette 2010; Cook & Gordon 2010). As yet there is no domestic market in Ireland for an

abalone product and no consistent market in Europe. Sales remain sporadic to the restaurant industry clients (Legg 2010).

In Ireland there are two species of abalone farmed *Haliotis discus hannai* and *H. tuberculata* with the former been highly valued worldwide (Cook & Gordon 2010). The first abalone species imported into Ireland was *Haliotis tuberculata* in the mid 1970s and followed a decade later by the Japanese species *H. discus hannai* (Watson *et al*. 2004). The pool of genetic resources of *H. discus hannai* broodstock has been unsecure in terms of the amount of viable broodstock abalone in Ireland since the 1980s due to restrictive legislation on importing new animals for breeding. The current stock of viable *H. discus hannai* are derived entirely from the first individuals imported 3 decades ago (Leighton 2008; Watson *et al.* 2004).

Due to the lack of genetic diversity reflecting the small population size of the Japanese abalone *H. discus hannai* brood stock in Ireland, the commercial culture of this species remains unproductive because of the duration of the growing cycle and uncompetitive due to the associated costs of producing reasonably priced spat (Soler Vila 2008; Leighton 2008). The nearest endemic stock of wild European abalone *H. tuberculata* is Jersey in the English Channel, where selection of wild brood stock for aquaculture is possible (Mgaya & Mercer 1995; Courtois De Viçose *et al*. 2010).

The European abalone *H. tuberculata* is the only species of the family Haliotidae, which is harvested commercially in Europe (Mgaya & Mercer 1995). *H. tuberculata* (ormer in English) is endemic to eastern Atlantic coasts of mainland Europe and the Mediterranean. The most northerly extension of their geographical range is North-western Brittany and the Channel Islands of Jersey and Guernsey. There are no endemic wild stocks of *H. tuberculata* present on the coasts of Ireland and England (Huchette & Clavier 2004; Mai *et al*. 1995).

The culture of the abalone in Europe is under-developed due to bureaucratic delays in licensing and establishment of new farms, technological problems, research advice slow to be implemented by industry and as yet, a lack of reasonably priced juveniles (Soler Vila 2008; Huchette 2010). The main producing countries in Europe (Ireland and the

Channel Islands) have an output of just 2- 3 tonnes of *H. tuberculata* per year (Huchette & Clavier 2004).

The aim of this review is to identify the technological problems experienced by the abalone aquaculture industry in Ireland and the failure of the Irish industry to successfully transfer technology and protocols from established producing countries such as China and Korea (Cook & Gordon 2010).

The cultivation of abalone in Ireland has attracted significant financial investment and industry development since the early 1990s resulting in the establishment of commercial hatcheries and on-growing facilities (Hahn 1989; Gordon & Cook 2004; Huchette & Clavier 2004). Ireland currently has three commercial scale farms with an undefined production and output, as figures are unavailable or unreliable.

These farms are all situated on the Atlantic coast where conditions provide suitable water quality, abundance of preferred macroalgae (Leighton 2008; Watson *et al*. 2004; Browne *et al*. 2007). *Laminaria* spp. and in particular the red macroalgae *Palmaria palmata* are the main feed sources (Kelly & Owen 2002; Mai *et al*. 1995).

In 2003 the FAO forecast that Ireland would achieve an overall production of 25 tonnes of market size animals by 2008, however the total production in Ireland prior to 2008 was 122kg which represented a value of less than €4000 per tonne.

Forty thousand juveniles were also sold at a value of €66,000; 50% of these were transported to Spain for on-growing and the remainder to Irish abalone farmers (FAO 2004). In total in 2006, €1.4 million of grant aid from the state was invested into the abalone industry in Ireland however the production envisaged has not yet been met by industry (Browne *et al*. 2008).

The relatively poor return on significant financial investment from the Irish state into the industry demands that production processes be improved to remove the bottlenecks that still impede production on economically viable scale. Bottlenecks to production in Ireland are:

- Energy and labour costs;
- Price of juvenile abalone;

- Production and development of feeds (live or artificial);
- Failure to implement technology transfer from established producing countries.

Internationally the commercial culture of abalone has faced similar industry development problems; which have impeded production and industry growth (Fleming & Hone 1996; Cook 2008). A focus of attention on improved juvenile abalone production has aimed to provide a continuous and high quality food source from initial settlement through nursery and on-growing stage, to final market size (Daume 2003; Daume 2007). The juvenile abalone spat market for on-growing, has been used to develop the abalone aquaculture industry in Ireland and in other European countries. The added cost and technical difficulty of producing spat may not make it economically feasible for farmers to produce their own spat.

This same need has led to research in other growing regions on production processes of juvenile abalone under commercial conditions, particularly with respect to settlement cues and preferred settlement substrate (Daume *et al*. 2000; Daume *et al*. 2003; Takami 2003; Pang *et al*. 2006; Roberts *et al*. 2007).

These studies have indicated bottlenecks in production processes that remain on-going impediments to industry development worldwide:

- Poor identification of behaviour and feeding transitions, as the animals behaviour changes so will the feeding strategy when weaning from microalgae to macroalgae (Takami & Kawamura 2003);
- Inconsistent animal husbandry increases stress, slows growth and increases mortality: taken in context with technical barriers, this makes abalone aquaculture unproductive (Huchette *et al*. 2003; Dyck *et al*. 2011);
- Duration and cost of a full production cycle, the longer the animal stays at each development stage, post larvae, weaning, on-growing the longer it will take to see a return. Therefore farmed animals need to make it to market in a economically viable time scale (Naidoo *et al*. 2006);
- Daume *et al*. (2000) stated that incorrect and inadequate settlement substrate as an initial barrier linked to the abalone's dependency on their initial diet as a settlement substrate and food source.

The Irish industry has been slow to adopt and transfer technologies from one producing region to another due to lack of available information to farm operators. This is explained by the slow adoption of *U. lens* in Irish hatcheries, and the development of the macroalgae as an industry standard as in Japan (Takahashi & Koganezawa 1988) and Australia (Daume 2003; Daume 2007). The existence and use of *U. lens* in aquaculture as a settlement substrate for swimming veliger larvae was established in the 1980s (Takahashi & Koganezawa 1988; Ohshiro *et al*. 1999). *U. lens* has been used successfully in the enhanced induction of settlement in both urchins and abalone (Daume & Ryan 2004; Takahashi *et al*. 2002).
It has taken over two decades for this known promoter of settlement growth and survival of urchins and abalone to see its way into a trial in Ireland (Leighton 2008).

This settlement substrate is a known inducer of settlement for swimming larvae; this shows reluctance in the Irish aquaculture industry for technology transfer from one region to another. The potential of *U. lens* has been cited in both scientific (Daume & Ryan 2004; Courtois De Viçose *et al*. 2010) and industry based research (Daume 2003; 2007). Two factors remain to slow the adoption of *U. lens* in Ireland:

- Lack of stock cultures of *U. lens* to produces adult sporophytes;
- Limited literature and guidelines on the correct cultivation of the macroalgae.

1.3.3 Settlement and Settlement Cues

Settlement is a most critical life stage for abalone. At settlement, veliger larvae metamorphose from a swimming lecithotrophic (non-feeding) larvae to a particle feeding, post larvae (Kawamura *et al*. 1998). Settlement must be completed within a short interval to avoid negative effects on the animal's survival and growth. The critical periods of mortality occur just after settlement, coinciding with the transition from lecithotrophy to particle feeding. Abalone nursery operations predominantly rely on natural or cultured benthic diatoms as a settlement cue and initial food source, for abalone to attach and metamorphose they need a benthic diatom food source (Daume *et al*. 2000; Pang *et al*. 2006).

Post larvae will feed on a range of different settlement substrata, principally, benthic diatoms. Diatoms and bacteria may influence the settlement response of abalone larvae but they are not the main driving force (Daume 2006). Settlement may be induced or influenced through the inoculation of substrates with particular monospecific benthic diatoms, particularly *Navicula* and *Cocconeis* spp. The neurotransmitter molecule gamma-aminobutyric acid (GABA) has also been identified as one of the catalysts that can induce settlement and metamorphosis in abalone larvae, as the veliger larvae are sensitive to chemical signals emitted by the presence of settlement substrates (Takahashi & Koganezawa 1988).

The optimisation of settlement substrates and biofilms has been identified by industry-based research as critical for industry development (Nie *et al*. 1996; Gordon *et al*. 2006).

Abalone nurseries still experience inconsistent juvenile abalone production coupled with high mortality rate and slow growth rates (Daume 2003). Survival and growth rates of abalone are considered to be affected by food type and the ability of these animals to use the available food sources (Takami 2003). These have direct implication for Irish aquaculture for the production of spat, therefore production of identified live alga diets for the juvenile abalone need to be implemented by farm operators.

Ulvella lens is a macroalgae that grows two dimensionally over surfaces by increasing the diameter of its disc shaped thallus (Nielsen 1977). Both *U. lens* and the red coralline crustose algae have been proven to increase settlement (Morse, 1985). Whilst these encrusting algae will not support initial growth or survival (Takahashi & Koganezawa 1988; Daume *et al*. 2000) the presence of either on settlement plates has been proven to induce increased settlement and metamorphosis of veliger larvae (Daume and Ryan, 2004). In the case of red coralline crustose algae Gama-amino Buteryic Acid (GABA) and for *U. lens* bromoperoxidase are the two-biochemical signals associated with recruitment substrata (Ohshiro 1999; Morse, 1985).

The culture of *U. lens* for use in abalone aquaculture initially began in Japan during the 1980s (Takahashi and Koganezawa 1988), even prior to this *U. lens* was used for the cultivation of sea urchins (Ohshiro *et al.* 1999). An earlier study explains a basic culture method based on a laboratory scale of collection of zoospores (Nielsen 1977).

Takahashi and Koganezawa (1988) developed a reliable method of mass production of *U. lens* for use in aquaculture. Since the development of these techniques by the previous authors, there have not been any in-depth studies on the cultivation of *U. lens* for aquaculture, and there has been no research to transfer culture technologies to Irish conditions. Leighton (2008) is the only mention of *U. lens* in Ireland, however trials were only on a minor scale on one abalone farm, which in the end resulted in the loss of the available adult sporophytes to the Irish industry.

U. lens can be used as an efficient settlement substrate for abalone larvae, and as a live algal diet for juveniles greater than 3mm in shell length (Takahashi & Koganezawa 1988; Daume *et al*. 2004; Roberts *et al*. 2007; Courtois De Viçose *et al*. 2010).
Feeding trials have shown that *U. lens* can support adequate growth and survival, but as yet this has only been tested on a limited scale in Ireland (Leighton 2008).

Biofilms of *U. lens* itself can support moderate growth. However when mixed with easily digestible diatoms *Navicula* or *Cocconies* spp. it can support higher growth and survival (Daume *et al*. 2004). Therefore incorporating monospecific diatoms in conjunction with *U. lens* has been proven to promote continued sustainable growth and survival of juvenile abalone (Daume 2003; 2007). Therefore by using mixed biofilms with selected species is not only providing a settlement cue, but also providing an easily digestible diatom as an initial food source.

For production of juvenile abalone in Ireland to increase the application of *U. lens* needs to be investigated and transferred to the Irish aquaculture in conjunction with monospecific diatoms. This technology can also be applied to the cultivation of the purple urchin *Paracentrotus lividus* due to their shared preference to specific settlement cues (Ohshiro *et al*. 1999; Daume & Ryan 2004).

1.3.4 Feeding Transitions

As abalone grow in size, they progress through different life stages that are controlled by their predisposition to a food source (Kawamura *et al*. 2001; Takami 2003). Larvae, post larvae and juvenile abalone then in turn adapt their feeding behaviour and strategy, to efficiently feed on different food sources from diatoms to macroalgae. This may be dependent on the species of diatom, as this is an important factor for growth and survival (Viana *et al*. 2007).

Benthic diatoms are the principal food for postlarvae after metamorphosis; animals smaller than 0.8mm in shell length (SL) will grow at similar rates regardless of the diatom strain (Onitsuka *et al*. 2007). Abalone bigger than 0.8mm can digest these diatom strains due to their ability to overcome the adhesive strength and frustule strength of the diatom (Kawamura *et al*. 2001).

Adhesive strength, frustule strength and size are different for each strain of diatom (Gallardo & Buen 2003), early feeding can be promoted with the selection of an easily digestible diatom for the initial diet of the postlarvae (Takami 2003).

As abalone progress through these feeding transitions, they also change physiologically and morphologically (Johnston *et al*. 2005). These developments alter their feeding behaviour, this happens for two reasons:

- The animal can no longer extract sufficient nutrients to maintain the energy expended on longer foraging trips due to increased feeding efficiency (Kawamura *et al*. 2001);
- Feeding efficiency has out grazed or removed all diatoms thus forcing the animal to switch to a higher energy food source such as macroalgae (Takami *et al.* 2003).

The abalone feed using a rasping tongue called a radula, as the abalone grows it will alter shape and structure to allow for more efficient feeding. In the early stages after metamorphosis, the radula will begin to change with the addition of different lateral teeth shaped to dislodge diatoms (Kawamura *et al*. 2001). The efficiency of the radula depends on the adhesive strength and frustule strength of the specific benthic diatom (Johnston *et al*. 2005).

As yet, there is limited published work on what effect specific benthic diatom cultures and induction cues have on the preference of larval and post larval of the European abalone compared to commercially important species worldwide (Courtois de Viçose *et al*. 2010; 2012).

High growth rates are important to ensuring that cultured animals reach a marketable size and condition within a time that is economically viable (Naidoo *et al*. 2006). Daume & Ryan (2004) suggested that use of commercial settlement plates seeded with the macroalgae *U. lens*, and inoculated with the benthic diatom *Navicula* spp., would promote sufficient food to support rapid growth of post larvae. However high growth rates can only be maintained until the animal can no longer extract sufficient energy to support growth, due to the animals' increased feeding efficiency.
Complete grazing of the biofilm by abalone can result in starvation situation. If biofilms cannot be maintained the abalone need to be transferred to a macroalgal or an artificial diet (Johnston *et al*. 2005).

It is this ability to out graze the re-growth of the biofilm that initiates these feeding transitions, it may be possible to introduce new plates of *U. lens* however this can only sustain continued growth until 5-7mm in shell length until algal biomass becomes inadequate to maintain continued growth (Daume *et al.* 2007).

Maintaining a constant biofilm of *U. lens* is only useful until the abalone reach 5-7mm in shell length after this their growth will slow and force the animal to change its feeding strategy and find a new food source, such as *Laminaria* spp. or *P. palmata* which is predominately the preferred feed for *H. discus hannai* and *H. tuberculata* (Kelly & Owen 2002; Mai *et al.* 1995). *Palmaria palmata* or *Ulva* spp. are used as an interim feed before larger macroalgae like *Laminaria* spp. or an artificial diet. Operators are then faced with the decision to use hand-harvested macroalgae with its own associated costs (labor, licensing, and access) or revert to using a formulated artificial feed.

1.3.5 Artificial Diet

As juvenile abalone increases in size the live algal diets become limiting (Takami 2003) and can no longer provide adequate nutrition to sustain a high growth rate. Aquaculturists have attempted to improve growth and reduce diet dependent mortality by providing artificial foods to supplement diminishing natural biofilms (Pang *et al* 2006; Dyck *et al* 2011).

Artificial diets have been developed with this in mind and it is highly recommended that growers switch to an artificial diet, once the juvenile abalone has reached a weaning stage (Johnston *et al*. 2005). Artificial diets are formulated to contain the specific requirements that produce sustainable growth in the animals, feeds are generally comprised of, proteins, carbohydrates, lipids, fiber, vitamins and minerals (Mai *et al.* 1995; Fleming & Hone 1996).

Protein is the most expensive ingredient of the feed as it is typically derived from animal sources, which do not belong in the natural diet of abalone (Johnston *et al.* 2005). Johnston *et al.* (2005) found that trypsin activity increased to hydrolyse the animal protein content of the formulated feeds. As the majority of feeds on the market contain animal protein there is a niche for a formulated macroalgal diet to replace the unpalatable components with macroalgae.

Well-balanced artificial diets support satisfactory growth of spat (Fleming & Hone 1996). The weaning on to a formulated feed is diet-specific and may depend on the initial food source of the abalone, and the particle size of the artificial diet (Stott *et al*. 2002).

The establishment of a weaning protocol; switching from a live algal culture to a powdered artificial diet, is common practice on many farms. However this manipulation may lead to more than 50% mortality (Pang *et al*. 2006). The timing of any diet regime switch needs to be implemented correctly and at the correct size class of abalone, to mitigate excessive mortality (Huchette *et al*. 2003).

However, it is difficult to implement a regime switch in diet at this stage as the juvenile abalone also develop a cryptic behaviour and they actively seek out shelter or hides to evade predation pressures inherent in the wild environment (Cenni *et al*. 2010). At this crucial stage the animal husbandry, farmers must accommodate this behaviour, and provide appropriately formulated diet with regards to particle size (Stott et al. 2002). Palatability of the feed will also affect consumption due to the binders that maintain the feed stability in the water, as they tend to be manufactured form components unpalatable to the animals (O'Mahoney *et al*. 2011).

In Europe there are only two companies that supply artificial diets for abalone Skretting (Norway) and Le Gouessant (France), outside of Europe the other main companies are Adam & Amos (Australia) and the company Abfeed (South Africa). The artificial feeds produced by Le Gouessant are formulated to suit *H. tuberculata* (Walsh & Watson 2011). As there is no indigenous company producing feed for abalone in Ireland, there is a possible market for feed here in Ireland if production is to increase to production forecasted by FAO (2010).

The cost of producing the feed would need to be below the cost of harvesting macroalgae for it to be taken onboard by farm operators. Feeds are readily available but their import into Ireland is constrained due to the animal protein content of the feed, and particular concerns over:

- From where the protein is sourced; and,
- From what species it is derived.

Extensive and unnecessary handling of juvenile abalone will have a negative effect on the growth and survival, as stress combined with the lack of a blood clotting mechanism in their physiology will cause high mortality (Malham *et al*. 2003). Therefore mechanical mortalities will occur during a transfer from settlement plates to weaning tanks.

Huchette *et al*. (2003) suggested that proper husbandry should take behaviour into account. Tailoring rearing techniques to natural behaviours may help to maximize the health and growth of the animals, therefore providing hides as they begin to show cryptic or sheltering behaviour at 3 to 5mm. The unavoidable use of artificial diets at a later stage, when the growth of spat is limited, is mainly due to the insufficient algal supply (Pang *et al*. 2006; Johnston *et al*. 2005; Fleming & Hone 1996).

Preferred macroalgae diets are harvested by hand, a practice creating substantial logistic difficulty during the winter months (Kelly & Owen 2002), and nutrient content of harvested algae will also change from season to season (O'Mahoney *et al.* 2011). There has been considerable comparative research on artificial feeds and macroalgal diets in Ireland (Mai *et al*. 1995; Mgaya & Mercer 1995) and worldwide (Daume *et al.* 2007; Naidoo *et al*. 2006; Fleming & Hone 1996; Stott *et al*. 2002; Johnston *et al*. 2005).

Benefits for the production of abalone may not be optimal for farm operators as labour and energy costs dictate the scale of production. The use of artificial feeds represents a strategy adopted by farms to reduce such costs (Naidoo *et al*. 2006).

Licensing of the harvesting of macroalgae from the foreshore may force some farms to culture their macroalgal diets in the initial stages, and increasingly adopt artificial feeds from weaning onwards to market size (O'Mahoney *et al*. 2011; Werner & Dring 2011). The nutritional models that the farm operators apply will affect the size and production of the farm. The quantity of *P. palmata* and *Laminaria* spp. required to meet Irelands need for abalone feed may not be achieved from macroalgae aquaculture (Edwards & Watson 2011; Werner & Dring 2011). Due to the cost differences between harvesting by hand and cultivation, farms will always employ the model that results in the least amount of cost. The equipment required to cultivate seaweed or harvest by hand incur their own respective and capital costs, however it does have the possibility to improve seaweed cultivation in Ireland with the integration into farm productions.

1.3.6 Discussion

Ireland continues to be highly placed on a list of ideal locations for abalone farming due to water quality and the abundance of preferred macroalgae (Hahn 1989; Mai *et al*. 1995). Ireland is also the only country in Europe that has naturalised the Japanese abalone *H. discus hannai* (Huchette & Clavier 2004). The uncompetitive nature of the industry must therefore be due to other limiting factors:

- High energy costs reduce the scale of production due to overheads incurred running farm plant equipment;
- Licensing constraints on the establishment of new onshore and off shore aquaculture enterprises;
- Legislation preventing the importation of artificial feeds and viable broodstock of both species;
- Research and industry based research for abalone farming are not linked in Ireland, thus leaving the operator with a trial and error approach to abalone farming due to lack of information on industry standards from producing countries globally.

If these problems are recognised, then the growth potential of the industry in Ireland should be on par with other producing nations provided that technical issues are overcome.

The economic down turn has had a knock on effect on the prices of marketable abalone both in Europe and worldwide, as they are sold to markets in a live or preserved ex-*situ* of the country of growth with no added value (Legg 2010; Cook & Gordon 2010). This is also true for Ireland production as it is possible to have an added value product for Irish abalone such as a canned abalone.

Farm operators in Ireland must adopt the most economically viable processes that secure their position as producers to spat markets, wholesale markets, and also as merchants of processed product. Bottlenecks and technical barriers add expenses, reduce productivity and constrain competitiveness (Malham *et al*. 2003; Huchette *et al*. 2003).

The commercial culture of abalone is inconsistent due to their complicated life history and the fact that it is interlinked with their food source from settlement to marketable product (Kawamura *et al*. 2001; Takami 2003). Insufficient food supply will decrease productivity.

Precise control of the settlement of veliger larvae and survival of postlarvae are major priorities for farm operators (Courtois De Viçose *et al*. 2010). Controlling postlarvae and juvenile abalone will increase production and supply of a marketable product. However trial and error approaches to abalone farming remain the norm as defined practices differ with respect to location, species, nutrition and time of year (Huchette 2010).

1.3.7 Summary of Technical Barriers to Industry Development and Potential Solutions

This review has identified barriers and critical areas of research and development needed in the Irish industry to attain an increased production (settlement, feeding transitions and artificial feeds). The rearing systems and facilities for abalone farming in

Ireland are currently in place and tailored to spat production. Therefore the use of available technology and procedures from external sources needs to be applied within the Irish context.

The concentration of technical barriers during the initial stages of the animals' life cycle, require research focus on the nature and physiology of the animal during its early life history (Soler Vila 2008; Takami 2003; Daume & Ryan 2004). The barriers that impede production have been identified by industry based research from established producing countries (Daume 2003; Daume 2007).

For the Irish industry to be competitive it needs to transfer technology from successful producing regions to Irish growers and adapt protocols that have shown success, such as the use of *U. lens* in conjunction with monospecific diatoms. Establishing settlement protocols with the use of specific benthic diatoms (*Navicula* spp.) with an encrusting macroalgae (*U. lens*) has been proven to increase settlement and survival (Daume *et al*. 2000; Dyck *et al*. 2011).

Uptake of industry advice in Ireland has been slow. *U. lens* and monospecific diatoms have been tested and shown to increase settlement growth and survival of *H. tuberculata coccinea* (Courtois de Viçose *et al.* 2010) therefore it is a clear solution to increasing settlement, survival and growth of abalone during a nursery stage (Courtois de Viçose *et al.* 2012).

These barriers have previously been seen world wide in the commercial production of farmed abalone, which then triggered industry and research to rethink and adapt production processes (Takahashi & Koganezawa 1988; Daume *et al*. 2004; Daume 2003; 2007).

Implementation of correct protocols at the earliest stage needs to be applied to settlement, identification of behaviour changes and matching the correct diet for the current life stage of the animal. This, along with consistent animal husbandry will reduce cost and production duration.

Inadequate bio-film and incorrect protocols for a mixed diet approach to abalone farming are two of the major factors that reduce production (Hahn 1989; Olin & McBride 2000; Stott *et al*. 2004). Lack of available stock alga cultures to the industry, combined with incorrect production methods, reduce productivity on a whole, as these are the most important parts of the production of juveniles.
Failure of the Irish industry to invest in overcoming these production obstacles and adopting improved production processes results in, commercial abalone enterprises in Ireland achieving production and economic performance that remains uncompetitive compared with best practices of commercial abalone enterprises worldwide (Daume 2003; Daume 2007; Takami *et al.* 2003; Courtois De Viçose *et al.* 2010).

The use of specific principal diets for abalone culture in Ireland will increase juvenile production rather than remaining reliant on biofilms of naturally occurring assemblages of diatoms that can have inconsistent results. Adapted culture techniques for production of juvenile abalone need to be established from advice from industry-based research carried out on specific barriers that impede production and that have been experienced by industry worldwide.

The specific nutritional requirement for both *H. tuberculata* and *H. discus hannai* (Mai *et al.* 1995) are known and feeds have been developed with this in mind. The use of *U. lens* or another easily digestible macroalgae such as *Ulva* Spp. are used to bridge the animal between diatoms, however the implementation of correct timing of a regime switch from a macroalgae to a artificial diet needs to be investigated to ascertain best practices for weaning of juvenile abalone in Ireland.

Apart from the specific live algal diets, the existing equipment for settlement of abalone larvae can be used for the cultivation of live algal diets previously mentioned. As the relevant procedures have been documented in literature (Takahashi & Koganezawa 1988; Daume & Ryan 2004) and the culture of *U. lens* has been documented in Ireland (Leighton 2008) it is imperative that the transfer of these proven techniques are applied to juvenile abalone production.

1.4 Introduction to the thesis structure

The research questions and objectives aimed to be addressed, for this thesis is outlined in this section to act as an introduction to the main research of this thesis as a whole.

1.4.1 Current Status of Abalone & Sea Urchin Aquaculture in Ireland

The culture of algivorous invertebrates in Ireland first began in the late 1970s with the importation of abalone and continued in the 1980s with the development of culture techniques for *P. lividus* at the Carna Shellfish Research Laboratory in Connemara, Co. Galway. The first farms were set up for both abalone and sea urchins in the early 1990s (Leighton, 2008; Watson *et al*., 2004).

There are currently two *P. lividus* hatcheries in Ireland, both based in Co. Cork. The developing industry in Ireland has commercialised the production of *P. lividus* in Europe. Unsatisfied demand for wild sea urchins from traditional markets has increased the demand for hatchery-produced sea urchins. The current demand for sea urchins exceeds supply from hatcheries.
One of the same constraints facing the expansion of aquaculture production of sea urchins and abalone is the supply and availability of reasonably priced juveniles.

Constraints on aquaculture production affecting both abalone and sea urchin in Ireland are exacerbated by a trial and error approach to farming and the lack of implementation of industry advice or recommendation (Huchette, 2010). To overcome these constraints implementation of commercial by relevant research must be a priority. Industry based research funded by agencies such as the Australian Fisheries Research and Development Corporation (FRDC) has focused on identified industry constraints, and has led to industry expansion after implementation of research outcomes (Daume, 2007, 2003). Daume 2000 identified the need for correct settlement cues and inducers for the Australian abalone industry to expand by overcoming inconsistent settlement rates experienced by growers.
Daume 2003 identified a means of overcoming low settlement rates with the utilisation of *Ulvella lens* for the wider Australian industry and proved its validity through industry-based research. The same application of industry advice to the developing invertebrate industry in Ireland may facilitate expansion once industry constraints can

be identified and overcome. Other constraints exacerbating the poor industry growth are the lack of availability of preferred settlement cues and expertise to provide industry with answers to knowledge gaps.

Industry has attempted to identify and resolve barriers to production through research. The 7th Framework (FP7) project SUDEVAB (Sustainable Development of European SMEs engaged in Abalone Aquaculture) investigated the issues facing abalone farmers through core research topics of nutrition, genetics, pathology and sustainable culture for the production of abalone in Europe. This research aided the expansion of a commercial farm in Brittany France Haliotis, which employed findings and recommendations from the SUDEVAB project.

A similar FP7 research project RESURCH (Research & technological development to improve economic profitability and environmental Sustainability of Sea Urchin farming) is investigating new culture technologies to optimise commercial land and sea based sea urchin farming around Europe. This FP7 project is researching the affect of cage and tank design with differing feed compositions aiming to reduce costs of the on-growing of juvenile sea urchins to market sized animals.

These two industry led projects are a prime example of the response needed by the industry in Europe to develop and expand through industry-based research in conjunction with research providers. However there is a need for the results from each respective project to be disseminated to the wider sea urchin and abalone industry as a whole.

1.4.2 Available Technology and Technology Transfer

Opportunities exist to accelerate industry development through the adoption of successful technologies and methods from established producing countries. The green encrusting macro algae *Ulvella lens* was identified as an important induction cue for swimming larvae of both abalone and sea urchins (Takahashi and Koganezawa, 1988). This settlement technology was transferred to the culture of *H. discus hannai* in Japan in the late 1980s with the development of mass culture techniques by Takahashi and Koganezawa (1988). Prior to use in abalone aquaculture *Ulvella lens* [P. L. Crouan and

H. M. Crouan, 1859] was used as a settlement cue for sea urchin culture in Japan (Ohshiro *et al*., 1999; Takahashi and Koganezawa, 1988; Takahashi *et al*., 2002).

The development of these culture techniques for this commercially important settlement cue enabled the Australian abalone industry to also implement this technology. It is now used as an industry standard for production of abalone in Australia (Daume, 2007; Daume *et al*., 2004, 2000).

1.4.3 Feeding Transitions, Weaning & On-growing

Settled abalone and sea urchins postlarvae go through feeding transitions from benthic diatoms to larger macroalgae (Daume *et al*., 1999; Kawamura *et al*., 2001; Ohshiro *et al*., 1999). These feeding transitions are necessary to sustain growth; longer foraging trips and insufficient nutrient content of food sources forces the animals to move on to food with a higher value as they get bigger in size (Johnston *et al*., 2005).

Some farm operators use formulated artificial feeds as part of their production of abalone and sea urchins. Other options available to growers include wild harvested macroalgae such as *Laminaria* spp. Both of these production methods have associated costs to the grower. Artificial formulated feeds usually contain proteins derived from animal sources (predominantly fishmeal) which in some cases is not a component of the species natural diet (O'Mahoney *et al*., 2014).

Currently an opportunity exists in European aquaculture for a feed manufacturer to produce a formulated diet for both abalone and sea urchin that does not include protein derived from fishmeal or other animal sources (Johnston *et al*., 2005; Lawrence, 2007). *P. lividus* are capable of utilising animal proteins in artificial feeds as they are omnivorous in nature, (Fernandez *et al.,* 1998), however *P. lividus* has a preference for macroalgae as it comprises a major component of their natural diet (Lawrence and Lawrence, 2014; Watson *et al*., 2004).

As fishmeal is a controlled substance within the E.U. (EC Regulation 999/2001 & EC Regulation 183/2005) it poses more ethical and sustainability problems for its inclusion in the diet of algivorous invertebrates. Feeding animal protein to animals that are

algivorous by nature is not ethical and can lead to the transfer of disease. Not only does animal protein carry a cost for its inclusion it may also reduce the market value of the animals grown through taste and colour.

Abalone can achieve a higher food conversation ratio (FCR) when receiving components naturally occurring in their diet rather than formulated feeds (Naidoo *et al*., 2006; O'Mahoney *et al*., 2014). Use of mixed macroalgal-meal in increased proportions of the formulated diet, may make it possible to exclude fishmeal, also eliminating the most expensive feed component (Cook and Kelly, 2007).

Phosphorus availability has been identified as a limiting component in the diet of abalone. Phosphorus has an important role in cellular function and production of several key enzymes, and is directly involved in energy producing cellular reactions (Sales *et al*., 2003). Phosphorus content and Phosphorus availability depends on the type of feed it is contained in and on the ability of the animal to utilise it. Sales *et al*. (2003) identified that components containing phosphorus bound in phytate (soybean) are unavailable to abalone. Abalone will achieve greater growth when given higher level of bioavailable phosphorus (Coote *et al*., 1996).

1.4.4 Objectives and Thesis Structure

This thesis aims to identify the production constraints of the invertebrate aquaculture industry in Ireland and, through the application of industry practices proven in producing countries, enable these constraints to be redressed.

Objectives

1 To identify opportunities to overcome production constraints of industry expansion for abalone aquaculture;
2 To develop new culture methods of the commercially important encrusting macroalgae *Ulvella lens* for the Irish and European sea urchin and abalone industry;
3 To investigate the efficacy of *U. lens* as a settlement cue for *P. lividus* with the aim of industry implementation and application;
4 To investigate the development of a novel macroalgal-meal feed for *H. tuberculata* and investigate the effect of bioavailable phosphorus enrichment.

1.4.5 Chapter Overview

Chapter 2: Culture methods of live algal feeds for European aquaculture: optimising culture conditions for *Ulvella lens*, published as:

Hannon, C., Officer, R.A., Le Dorven, J. & Chamberlain, J. 2014. Culture methods of live algal feeds for European Aquaculture: Optimising culture conditions for *Ulvella lens*. *Aquaculture International* **22 (6)**: 1813-1822.

This chapter examines the use of *U. lens* as a settlement inducer in producing countries and the methods employed to culture this settlement cue. The developments presented in this chapter outline a new method, which greatly reduces the culture time for *U. lens.* These findings result in a substantial time savings, diminishing down time for farm operators seeking to incorporate *U. lens* into their production. The chapter also identified *U. lens* as an ideal candidate for technology transfer to the invertebrate aquaculture in Europe. Culture methods developed in this chapter have been applied to commercial aquaculture enterprises in Ireland.

Chapter 3: Evaluation of the efficacy of algal conditioned substrates for inducing settlement of *Paracentrotus lividus* larvae, submitted to Aquaculture Research (Under review).
U. lens has been identified as a settlement cue for multiple species of invertebrates in commercial aquaculture. This chapter investigated the affect and the efficacy of *U. lens* as a settlement inducer for the commercially important sea urchin *P. lividus.* Methods developed and presented in Chapter 3, were used to further develop the uses of *U. lens* in aquaculture in Ireland. *U. lens* has been shown to be an ideal settlement inducer and initial food source for postlarvae *P. lividus* larvae.

Chapter 4: Investigation into the effect of dietary phosphorus enrichment on the growth and survival of Juvenile European Abalone *Haliotis tuberculata*. (Submitted to Aquaculture International: Under review)
Farm operators are faced with difficulties when juvenile abalone transfer through their feeding transitions. At some stage the abalone will have to switch from eating microalgal diets such as diatoms and move onto macroalgae or artificial formulated

feeds. This chapter examines effects on the growth and survival of juvenile *H. tuberculata* of a novel macroalgal-meal diet enriched with phosphorous, and compares the diet with the red macroalgae *Palmaria palmata.*

Chapter 5: General Discussion: The findings and contributions of the earlier chapters are synthesised in a general discussion on approaches to optimise invertebrate aquaculture. The importance, implications and limitations of the research are discussed, and recommendations for further work are presented.

Chapter II: Culture methods of live algal feeds for European aquaculture: optimising culture conditions for *Ulvella lens*

A version of this chapter was published as:

Colin Hannon, Rick A. Officer, Jean Le Dorven and John Chamberlain (2014). Culture methods of live algal feeds for European aquaculture: optimising culture conditions for *Ulvella lens*. Aquaculture International, Volume **22**, Issue **6**, Pages: 1813–1822.

A version of this chapter was presented as:

Poster Presentation at the European Marine Biology Symposium, N.U.I. Galway, Ireland, August 19-23, 2013.

Title: Developing Bioresources in European Aquaculture: Optimising Culture Conditions for *Ulvella lens*.

2.1 Abstract

The Japanese development of mass culture techniques for *Ulvella lens* during the 1980s for aquaculture has stimulated the development of rearing techniques for abalone and sea urchins in producing countries. However since the late 1980s there has not been any in-depth evaluation of culture methods for *U. lens,* nor the development of a new robust method for aquaculture operators. The use of this known inducer for settlement of veliger larvae has not been employed on a commercial scale in Ireland or in European aquaculture systems. The new methodology described here has produced a three-fold increase in the settlement of sea urchins (*Paracentrotus lividus)* and is recommended for adoption by the European aquaculture industry.

Keywords: *Ulvella lens;* Abalone; Sea urchins; Post larvae

2.2 Introduction

Ulvella lens is a green encrusting macroalgae that grows two dimensionally over surfaces by increasing the diameter of its disc shaped thallus. The algae can be induced to release zoospores over five consecutive days to provide a settlement substrate for swimming abalone larvae. Nielsen (1977) provides a basic culture method on a laboratory scale for collection of zoospores, but a reliable method of mass production of *U. lens* for use in aquaculture was not published until 1988 (Takahashi and Koganezawa 1988).

Whilst *U. lens* itself cannot support initial growth (Daume *et al*. 2000), its presence on settlement plates has been proven to induce increased settlement of veliger larvae (Daume and Ryan, 2004).

The use of *U. lens* in aquaculture as a settlement substrate for swimming veliger larvae has occurred since the 1980s (Takahashi & Koganezawa 1988; Ohshiro et al. 1999). U. lens has been used successfully in the enhanced induction of settlement in both urchins (Strongylocentrotus intermedius) and abalone (*H. discus hannai*). However it has taken over two decades for this known promoter of settlement growth and survival of urchins and abalone to see its way into commercial production in Ireland.

The culture of *U. lens* for use in abalone aquaculture, was initially established in Japan during the 1980s by Takahashi and Koganezawa (1988). Subsequently *U. lens* has been used in the cultivation of sea urchins and in abalone aquaculture (Ohshiro *et al*. 1999). Takahashi and Koganezawa (1988) found that cultures of adult *U. lens* sporophytes could be induced to release spores via controlling the light regime and temperature, these culture techniques are interlinked with outdoor atmospheric conditions, and have specific requirements, that are met only when the outdoor conditions suit.

However, the inherent variability in ambient environmental conditions makes reliable achievement of sporulation events challenging. Furthermore, duration of a production cycle from a zoospore release to young sporophyte can average 30 days or more.

For *U. lens* to release spores, the adult sporophytes need to be triggered or shocked into a sporulation cycle, the processes employed by Takahashi and Koganezawa, (1988) were adapted by Daume and Ryan, (2004), Dyck *et al*., (2011) to stimulate sporulation. The average production cycle of *U. lens* is 30 days from zoospore to juvenile sporophyte. Timing sporulation events incorrectly may delay the coating of settlement plates if sporulation is not successful.

U. lens has been utilised in many studies conducted on settlement of abalone larvae and growth of post larvae (Courtois de Viçose *et al*., 2012; Daume, 2007, 2003; Daume *et al*., 2004, 2003, 2000). Its use resulted in marked increases in the induction of settlement. This increase in settlement also occurs for other species such as the sea urchin (Agatsuma *et al*., 2006; Ohshiro *et al*., 1999; Rahim *et al*., 2004; Takahashi *et al*., 2002) and sea cucumber (Matsuura *et al*., 2009).

Daume and Ryan (2004) found that ready to settle abalone larvae preferred to settle on older conditioned coatings of *U. lens*, rather than on younger plants. It was also found that *U. lens* is a suitable substrate to improve settlement of the abalone *Haliotis laevigata*.

Two industry-based research programs found that use of *U. lens,* in conjunction with monospecific diatoms, allowed farm operators to improve and streamline production of juvenile abalone (Daume 2003; Daume 2007).

Use of *U. lens* to induce settlement of larvae and its use as a secondary diet for abalone greater than 3mm has been highlighted by industry-based research as an efficient and consistent method of producing seed abalone (Daume and Ryan, 2004).

This industry advice has not yet been adopted into full commercial production in Ireland, however it has been tested on a small scale (Leighton, 2008). *U. lens* has been tested on most commercially important species of abalone including *Haliotis tuberculata coccinea* in conjunction with *Ulva rigida* (Courtois de Viçose *et al*., 2012). Currently one farm in Europe (France Haliotis) employs *U. lens* in their production of seed abalone (Pers. Comms.).

U. lens is unable to support the growth and survival of abalone less than 3mm in length due to the animals ability to remove *U. lens* with its radula (Roberts *et al.,* 1999; Daume *et al.,* 2000; Daume and Ryan, 2004; Courtois de Viçose *et al.,* 2010). However when used in conjunction with a benthic diatom such as *Amphora* spp. it has been shown to provide adequate and sustained growth for animals 3-7mm in length (Courtois de Viçose *et al*., 2012; Qi-hua *et al*., 1997; Xing *et al*., 2007).

Since the development of Takahashi and Koganezawa (1988) techniques over two decades ago, there has not been any new investigation into cultivation methods of *U. lens* for aquaculture, and there has been no research conducted to transfer culture technologies to Irish conditions (Hannon *et al*., 2013). The aim of this study is to develop a greatly simplified method for production of *U. lens* that results in increased productivity for farm operators.

2.3 Materials & Methods

The initial stock culture of *U. lens* was purchased from the *Commonwealth Scientific and Industrial Research Organisation* (CSIRO) microalgal supply services, Hobart, Australia. The initial stock culture of one large petri dish with visible particles of *U. lens* was then sub cultured into 250ml flasks with sterile F/2 culture media.

As the settlement tanks in most commercial nurseries are outdoors or housed in structures that are affected by atmospheric conditions, controlling dark stages can prove to be difficult and inconsistent within larger culture tanks. Implementation of a new protocol was investigated to adapt algal rearing to cater for industry needs.

32 tanks were used in the trial, of which 16 tanks were coated with *U. lens* and the other 16 tanks were coated in a naturally occurring biofilm of diatoms.

2.3.1 Laboratory cultures

The autoclaved seawater was enriched with F/2 culture media (Guillard, and Ryther, 1962) Cell-Hi F2P (Varicon Aqua solution Ltd. Worchester, England) at 1ml per Litre of stock culture; therefore each 250ml flask received 0.25ml of nutrients. Culture media was selected on the basis that it is an industry standard and the initial stock culture was raised in F/2 at CSIRO, Hobart, Australia.

The flasks of the *U. lens* cultures were kept in total darkness for 14 days at approximately 10°C (Daume and Ryan, 2004; Dyck *et al*., 2011). After this period of total darkness the culture was then re-introduced to continuous light by placing under 4000°K cool white fluorescent tubes. Culture temperature was then increased to 20°C and maintained by placing the culture flasks into a temperature controlled bath for the duration of the sporulation event.

Initial spore release could be seen between 3-5 days after exposure to light, a light yellow haze in solution denoted a successful spore release. Sterile Glass slides were placed into each flask to provide a settlement surface for the zoospores. These glass slides were then used to scale up the cultures onto settlement plates.

In culture, the algae will release spores for five consecutive days, with the greatest spore release occurring on the 4th and 5th day providing the temperature remains at 20°C for the duration of the sporulation (Takahashi and Koganezawa, 1988).

After the sporulation event has been completed, it is important to remove the adult plates bearing adult sporophytes from the settlement tanks to avoid contamination of another unwanted species of algae, and to avoid mobile spores settling on the adult plates. When the sporulation event was completed, the adult plates were removed and lightly scrubbed to remove any settled germlings.

The adult sporophytes can be reused for spawning at a later stage if returned to filtered seawater below 10°C for a period of 10 days under natural light conditions, or until required (Takahashi and Koganezawa, 1988). The quantity of adult sporophytes on a settlement surface is dictated by the size of the settlement surface area, as *U. lens* grows two dimensionally over a surface, each adult sporophyte expands until the surface is covered or the sporophyte meet another adult sporophyte (Figure 2.1)

2.3.2 Commercial scale cultures

To ensure *U. lens* culture could be achieved on a commercial scale, other methods were investigated to obtain spore release in larger culture volumes. The method used by Takahashi and Koganezawa, (1988) is compared with the current authors method (Table 2.1).

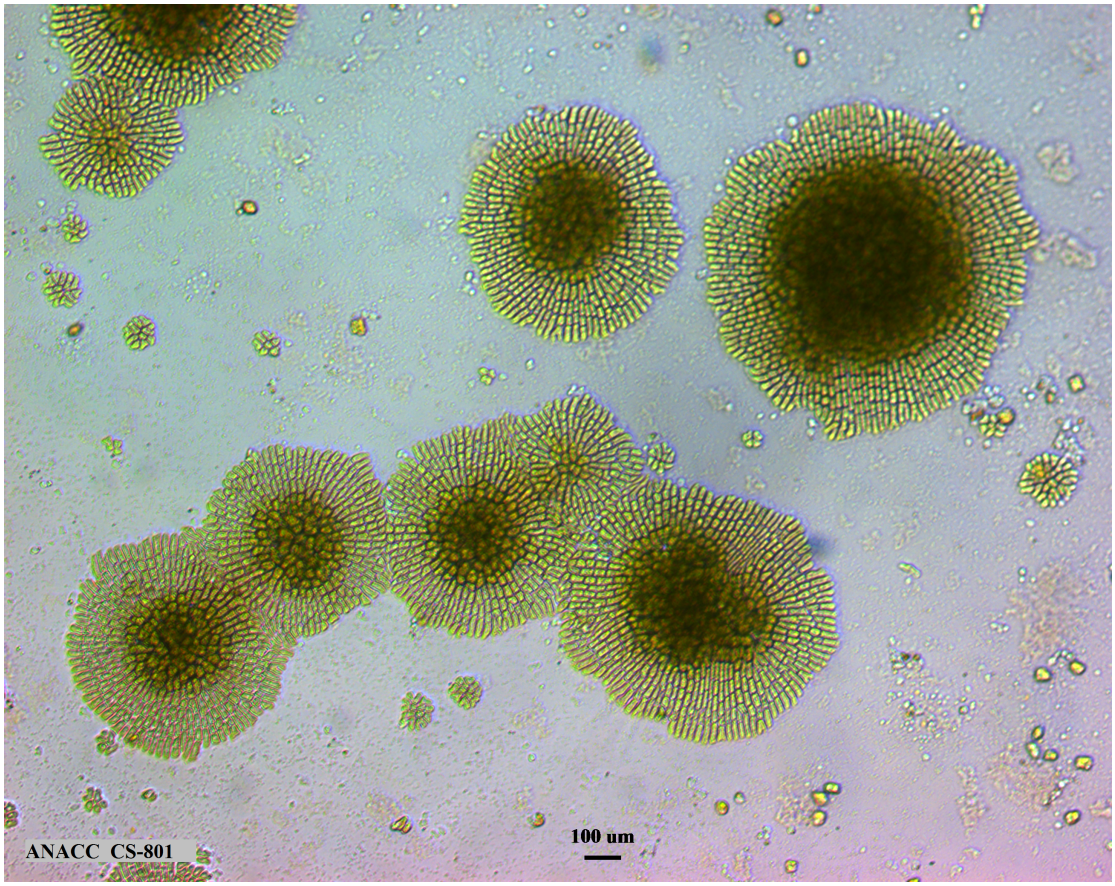


Figure 2.1. Discoid thallus of *U. lens,* Image Courtesy of Ian Jameson, ANACC (Australian National Algae Culture Collection), CSIRO, Hobart, Australia.

Method	Time (Days)	1	2	3	4	5	6	7	8	9	10	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Takahashi and Koganezawa (1988)		Phase 1: Maintain adult plates at 10°C for 10 days										Phase 2: Transfer adult plates to settlement tanks from storage at 10°C after 10 days	Phase 3: Maintain complete darkness for 14 days														Phase 4: Increase photoperiod, Nutrients and Temperature	Phase 5: Zoospore release				
Method	Time (Days)	1	2	3	4	5	6	7	8	9	10	10	11	12	13	14	15															
This Paper		Phase 1: Maintain adult plates at 10°C for 10 days										Phase 2: Transfer adult plates to settlement tanks from storage at 10°C after 10 days Phase 3: Increase photoperiod, Nutrients and Temperature	Phase 4: Zoospore release																			

Table 2.1. Comparison of time required to complete sporulation methods. Using Takahashi & Koganezawa (1988) method, if there is a failed sporulation event, the turnaround time is greater than 30 days including reconditioning the adult plates. Using the author's method reduced down time arises if the sporulation event fails. The dark phase was omitted from the authors' experimental trials due to observations of spore release from adult plates that had not gone through a dark phase.

Induction of *U. lens* was tested at different stages of the lunar cycle which showed no difference in settlement of zoospore. Adult plates were selected from the initial zoospore settlement in Boet Mor Seafoods Ltd. and maintained at 10°C in sterile filtered seawater under 12 hours (L: D) Light: Dark photoperiod.

Unwanted assemblages of diatoms proved a problem during initial trials but a control method was implemented using Germanium dioxide (GeO_2) at 0.04mg/liter, which breaks down the silica shell of diatoms, thereby killing them. Excluding sodium metasilicate (Na_2SiO_3) from the culture media greatly reduces unwanted diatom assemblages, as diatoms require a source of silicate and *U. lens* does not.

Culture tanks were set up with settlement plates and baskets. Seawater was chlorinated with sodium hypochlorite 10ml per 1000 liters (Figure 2.3) and de-chlorinated 24 hours later with sodium thiosulphate at 10g per 1000 liters. Once the water was sterilized chemically, 2ml/L of algal culture medium was added. One of the triggers that stimulates a spore release, is an increase in nutrient concentration. Initial trials using 1ml/L of nutrient supplementation proved inconsistent. Supplementation of concentration of nutrients to 2ml/L of F/2 achieved consistent, repeatable sporulation.

After the algal culture media was added the temperature was rapidly increased. This provides the second trigger for sporulation. Heating was achieved by placing two 300W aquarium heaters into each 1000L settlement tank. It is important to maintain the temperature of the settlement tank at 20°C as Takahashi and Koganezawa (1988) found that spore release was reduced at 25°C and non-existent at 30°C.

The photoperiod also needs to be increased to create a third trigger for sporulation. The current photoperiod of 12 hours (L: D) to 24 hours continuous light; this was implemented by hanging a 4000°K cool white fluorescent tube above the settlement tank.

Once the light, heat and nutrients have been increased then the adult sporophyte plates of *U. lens* can be placed into the settlement tanks. Minimal aeration of the settlement tank was applied to allow spore release to occur.

If the aeration is too high it is not possible to witness the zoospore release and the zoospores will be lost to the sides of the tank rather than coating the settlement plates.

During the mass zoospore release the mobile spores exhibit positive phototaxis (Nielsen, 1977) and can be seen by the naked eye. It is now important that aeration in the tank remain constant and at a low rate to allow the spores to settle evenly on the plates and to stop all the spores settling along the top of the tank.

Both methods used result in a release and settlement of zoospores, successful sporulation was gauged as a heavy coating on the plates, as a minor release of spores will not yield a coating on the tanks or plates sufficient to support subsequent settled abalone or urchins.

Dunmanus Seafoods Ltd. Durrus, Co. Cork, Ireland applied the authors method of culture for *U. lens* to their production of juvenile *Paracentrotus lividus*. Total settled numbers from tanks treated with *U. lens* and naturally occurring assemblages of diatoms (Figure 2.2).

2.4 Results

All attempts to achieve a sporulation of *U. lens* were successful and resulted in a mass sporulation coating each tank inside a 14-day period. No difference was noticed during different stages of the lunar cycle.

It is suggested by Sylvain Huchette of France Haliotis, (Pers. comms.) that adult sporophytes can be also be induced to release spores when in conjunction with a full moon, increased photoperiod, temperature and nutrients. Our investigation found this to be inconclusive as spore releases were achieved at different stages of lunar activity in 2010 and 2011 using the methodologies described.

Both methods resulted in the release of zoospores and the coating of settlement plates for production. Maintenance of the adult stock of *U. lens* is important, as is not allowing abalone to settle on the adult plates, as the juvenile abalone will graze *U. lens* off the plates.

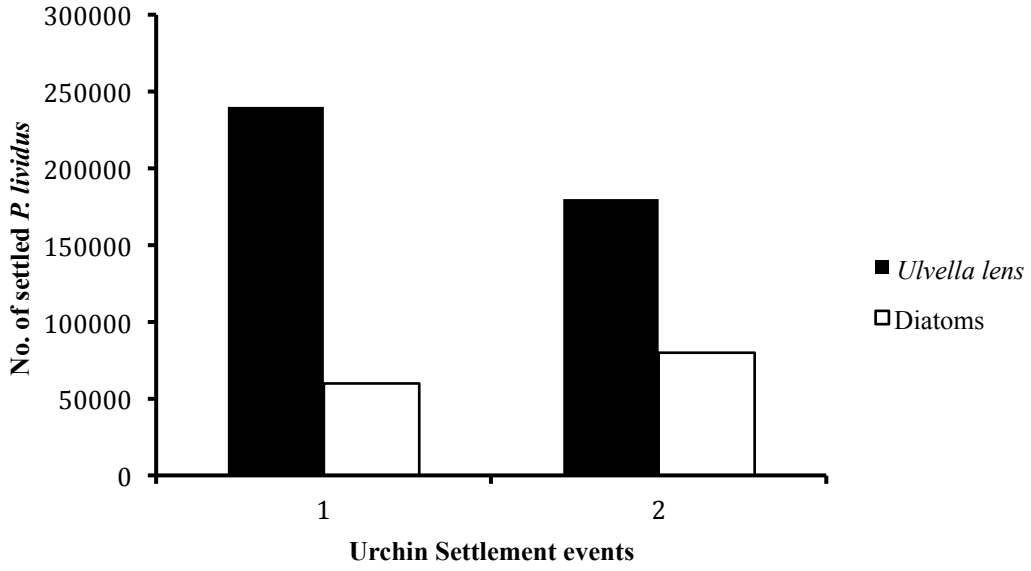


Figure 2.2.The total settled *P. lividus* numbers from two settlement events were collated. The *U. lens* settlement substrate induced three times the number of urchin larvae in the first settlement event. In the second settlement event *U. lens* more than doubled the induction rate compared to the natural assemblage of diatoms. Total settled numbers were taken from settlement tanks post settlement of *P. lividus* larvae.

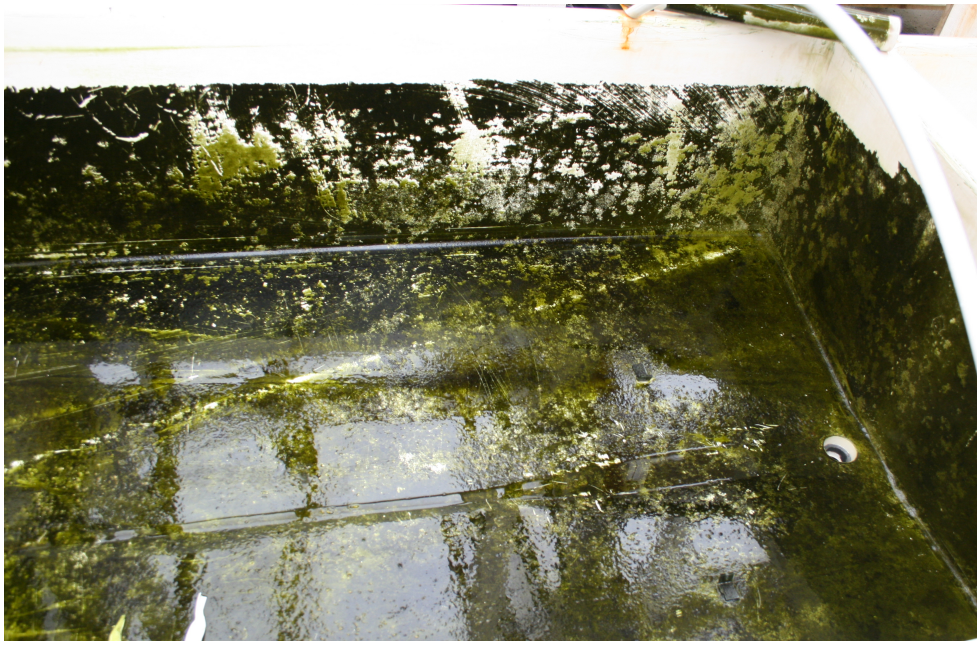


Figure 2.3. Older *U. lens* (Darker green) becomes recoated by recently settled zoospores (Light green).

2.4.1 Evaluation of culture techniques

Both methods used resulted in a release and settlement of zoospores. Whilst dealing with smaller cultures it is important to keep free of contaminants, as this is the initial stock culture.

Reducing the time needed to achieve successful sporulation by removing the dark stage results in a saving for farm operators and reduces the downtime on equipment and facilities. It is possible to attempt two sporulation of *U. lens* inside 30 days; if a sporulation is unsuccessful the farm operator can rerun a sporulation without losing one full month of work.

No difference in settlement of zoospores was noticed at different induction times as the results are based on a successful release or no release of zoospores. Once adult sporophytes are given sufficient recuperation time (10 days at 10°C), it is possible to reuse the adult plates to attain a subsequent successful spore release (Takahashi and Koganezawa, 1988).

2.5 Discussion

The study developed a greatly simplified method for production of *U. lens* that results in increased productivity for farm operators. This study also transferred technologies from an established producing area to Ireland's developing aquaculture industry.

The culture of *U. lens* has been investigated previously on a minor scale and is mentioned in (Leighton 2008). However commercial culture of *U. lens* has not been established in abalone production of prior to 2011 in Ireland.

In the wider European aquaculture industry there is the scope for expanding the use of *U. lens*. Currently *U. lens* is used as a part of the commercial production of juvenile abalone *H. tuberculata* in France (France Haliotis).

Until now there has not been a reliable, reproducible method for the production and use of *U. lens* in aquaculture. The main advantage of using the current method is the reduction of production time, thus allowing the farm operator to increase productivity and also reduce wasted time and equipment if a sporulation event does not take place.

Investigation of the sporulation protocol (Figure 2.1), indicated that it was possible to induce a release of zoospores without including the dark stage used by Takahashi and Koganezawa (1988). Whilst the methods described by Takahashi and Koganezawa (1988) are ideal for establishing and scaling up cultures, the methods described here are more amenable for larger cultures once adult sporophytes have been established on culture plates.

Due to the encrusting nature of *U. lens* it is possible to remove unwanted diatoms or other algae, as it requires extensive abrasive scrubbing and effort to remove *U. lens* from the settlement plates once coated. Therefore unwanted algae can be removed without damaging adult sporophytes.

The avoidance of the dark rearing period is an important improvement in the *U. lens* culture techniques (Table 2.1). Keeping a large enough quantity of adult sporophytes in complete darkness for 14 days may prove difficult. This requires extra tanks and space meaning that these facilities become unavailable for spat production, thus may slowing and diminishing production of the primary crop.

The adapted methods successfully achieved induction of zoospore release of *U. lens*. The dark stage required by Takahashi & Koganezawa (1988), Daume and Ryan, (2004) and Dyck *et al*., (2011) has been removed thus reducing duration and complexity of the production cycle of *U. lens* for aquaculture (Table 2.1).

Control of the conditions for the initial adult sporophyte plates is critical to successful production. A change in temperature or concentration of culture media will result in a failed sporulation. Maintaining these plates in 10°C filtered natural seawater for a period of 10 days allows the re-induction of the adult sporophytes to again release zoospores.

These methods have been implemented in the cultivation of *U. lens* at Connemara Abalone Teo. as part of their 2011 production. Connemara Abalone Teo. are now using the method to generate the principal settlement substrate for abalone. The success has shown that techniques for the culture of *U. lens* are repeatable and represent a significant transfer of technology into the Irish aquaculture industry resulting with the uptake by two commercial aquaculture enterprises.

This technology has also been established at Dunmanus Seafoods Ltd. as part of the commercial production of the purple urchin *P. lividus*. Dunmanus Seafoods have reported a three-fold increase in the settlement of *P. lividus* on plates of *U. lens* using culture methodologies (Figure 2.2). During two different settlement events of *P. lividus* at Dunmanus Seafoods, the current methodology was employed and produced a considerable increase in settlement of *P. lividus* when tested against an assemblage of naturally occurring diatoms.

It is important to control the settlement of zoospores and the spawning events as uncontrolled events can reduce the efficiency of the algae by coating settlement surface with new germlings that were previously colonized by older more beneficial sporophytes (Figure 2.3) (Daume 2003).

This shows improvement in the Irish aquaculture industry's adoption and technology transfer from one region to another. The methodologies described here have established protocols that are now readily being adopted by industry.

The main aim of this research was to establish a greatly simplified method for the repeatable reproduction of zoospore release of the macroalgae *Ulvella lens* for use in aquaculture.

The method detailed here has been proven by successful replication of these methods on three different aquaculture sites, and it's now being employed on two aquaculture enterprises as part of their commercial production. The outcomes of this direct research is the reduction of down time for settlement facilities and an increase of productivity without the need for expansion of facilities.

2.5.1 Acknowledgements

We thank Cathy Johnston of CSIRO Microalgae Supply Service for the supply of algal stock cultures and advice. We would also like to thank Ian Jameson of ANACC for the detailed images of *U. lens*.

Chapter III: Evaluation of the efficacy of algal conditioned substrates for inducing settlement of *Paracentrotus lividus* larvae

A version of this chapter has been submitted for publication in Aquaculture Research: Colin Hannon, Rick A. Officer, and John Chamberlain (*Under Review*) Evaluation of the efficacy of algal conditioned substrates for inducing settlement of *Paracentrotus lividus* larvae. Journal: Aquaculture Research.

3.1 Abstract

The commercially important sea urchin *Paracentrotus lividus* is of increasing interest for aquaculture in Europe. However suitable larval settlement substrates remain untested. Inconsistent and low settlement rates are currently achieved at the nursery stage by commercial hatcheries. In the present study settlement substrates conditioned with the monospecific diatom *Amphora* spp. and the green encrusting macroalgae *Ulvella lens* were tested for *P. lividus*. Settlement rates of competent urchin larvae were significantly higher on *U. lens* ($P < 0.05$), than on *Amphora,* or when both settlement substrates were mixed. When competent larvae were given a choice between algal settlement inducers, three times as many larvae settled on *U. lens* conditioned substrates, than on those conditioned with *Amphora* ($P < 0.05$). The control, which had no conditioned biofilm, did not achieve settlement. The consistent settlement rates achieved of 50% of competent larvae using *U. lens* exceeds the rates achieved by commercial operators. The results also suggest that the use of *U. lens* as a settlement substrate for *P. lividus* can be readily transferred for industry needs.

Key words: *Paracentrotus lividus; Ulvella lens; Amphora*; Diatoms; Settlement cues; Postlarvae; Settlement Substrates; Technology transfer.

3.2 Introduction

The edible purple sea urchin *Paracentrotus lividus* is a sub-tidal species, which occurs below the mean low water mark in tidal rock pools and reefs to depth of 10-20m (Heffernan, 1999; Lawrence, 2007). The species has a wide distribution extending from northwest Scotland, along the west coasts of Ireland, France and Spain to the eastern Mediterranean and North Africa.

The species is commercially fished in Ireland however the fishery declined in the 1980s (Byrne, 1990). At its peak in 1976 the fishery exported 350 metric tonnes from the south and west coasts of Ireland, but rapidly declined thereafter with minimal harvests up until the mid 1990s (Andrew *et al*., 1995; Hur *et al*., 2002). Presently there are no reported harvest data available for Ireland.

P. lividus is a well known commercially important species, in demand on markets around Europe for its high quality roe (Cook *et al*., 2007). This demand has generated interest in Europe in the commercial culture of the species (Watson *et al*., 2004).

Increasing demand extending beyond traditional European markets to markets in Asia has renewed industry drive for commercial culture of these economically important echinoderms. Efforts to establish other commercial hatcheries around Europe, have been hampered by inconsistencies in production (low settlement rates and incorrect settlement cues), which remain a key bottleneck.

There is increasing interest in the aquaculture of *P. lividus* due to its high commercial value and marked overexploitation in European fisheries (Lawrence and Lawrence, 2014). Research has indicated that the species is suited to various holding conditions (Gosselin and Jangoux, 1996) and diet provision (Cook *et al*., 2007; Lawrence, 2007) however reliable hatchery production remains key to future commercial development.

Inconsistent settlement rates in urchin larvae and mortality result from handling are the two main production constraints with the culture of sea urchins (Dale *et al.*, 2009).

Methods developed to maintain different settlement substrates on traditional settlement plates aim to extend the time the postlarvae spend in the nursery system prior to the handling involved in moving them to sea, or to weaning systems. Refinement in these processes aims to reduce mortality and increase production.

An opportunity exists to transfer technology that is successfully used in abalone aquaculture and other cultured species of urchins to the cultivation of *P. lividus* (Hannon *et al*., 2013; Takahashi *et al*., 2002). Hannon *et al*. (2014) developed methods for the culture of *U. lens,* which reduced culture time of the encrusting macroalgae, which induced the settlement of *P. lividus.* However very little research has been conducted on preferred settlement substrates for this commercial species of sea urchin.

3.2.1 Settlement cues

Larvae of invertebrates such as abalone and sea urchins are sensitive to induction or settlement cues in the environment (Morse, 1985) and in culture (Gosselin and Jangoux, 1996; Mos *et al*., 2011; Takahashi *et al*., 2002). Different settlement cues will have differing effects on the induction rates of invertebrate larvae due to predisposition and preferences to settlement cues (Hannon *et al*., 2014).

Extensive research has been conducted on the effect of induction cues on the metamorphosis and settlement of larval sea urchins primarily due to their use in larval development studies for over 100 years. Researchers have found that different settlement cues will give different induction rates (De La Uz *et al*., 2013; Kitamura *et al*., 1993; Pearce and Scheibling, 1991,1990,1994). However there is no literature available on the effect of *Ulvella lens* on the metamorphosis and settlement of *P. lividus,* nor on preferred settlement cues for this species of sea urchin.

The use of available and industry standard settlement cues such as *U. lens* has aided the development and intensification of juvenile abalone production in Japan and Australia (Daume, 2007, 2003; Takahashi and Koganezawa, 1988).

U. lens is a green encrusting macroalgae which forms a discoid thallus (Hannon *et. al.*. 2014). The use of *U. lens* as a settlement substrate in abalone aquaculture has been extensively established (Daume, 2003; Daume *et al*., 2004; Hannon *et al*., 2013; Takahashi and Koganezawa, 1988), however abalone post larvae are unable to graze the encrusting macro algae before they are greater than 3mm in shell length (Daume, 2003; Dyck *et al*., 2011). Prior to the use of *U. lens* in abalone aquaculture, it was used as a settlement substrate for the Japanese sea urchin *Strongylocentrotus intermedius* (Takahashi and Koganezawa, 1988; Takahashi *et al*., 2002) and for sea cucumber *Apostichopus japonicus* (Matsuura *et al*., 2009), suggesting that several invertebrate species prefer *U. lens* as a settlement substrate.

U. lens itself has been proven to work on a commercial scale for the settlement of more than one invertebrate species but uptake and application by industry has been slow (Hannon *et al*., 2014). The successful application of this settlement substrate to the culture of *P. lividus* may encourage expansion of the commercial culture of this important species.

Here we evaluate *U. lens* as a settlement substrate for *P. lividus* comparing it against *Amphora* spp. in order to determine the effect on settlement and metamorphosis. *Amphora* spp. are a common settlement substrate for abalone and commonly used as a settlement substrate in commercial aquaculture due to its availability and ease of culture (Daume *et al*., 2000), and have been used as a settlement cue and initial diet for the edible sea urchin *Lytechinus variegatus* (Lawrence, 2007; Lawrence and Lawrence, 2014). Prior to this research neither *U. lens* nor *Amphora* spp. had been tested as a settlement substrate for *P. lividus*.

3.3 Materials & Methods

Spawning, larval rearing and settlement trials were conducted at the Dunmanus Seafoods hatchery, Dunmanus, Bantry, Co. Cork, Ireland, between April and September 2013.

3.3.1 Algal culture & Settlement substrates

The initial stock culture of *U. lens* (Culture strain-801) and *Amphora* spp. (Culture strain-521) were purchased from the CSIRO microalgal supply services, Hobart, Australia. *Amphora* spp. (Culture Strain-521) is currently being amplicon sequenced by Australian National Algal Culture Collection in CSIRO. The settlement substrates and live algal feeds selected for the experiment were cultured on site. Swimming urchin larvae require feeding for the duration of the larval period and were fed *Phaeodactylum tricornutum* daily (Gosselin and Jangoux, 1996).

The initial larvae stocking density was held at one larva per ml and were fed 2.5-3L (cell count of 10,000 -12,000 cells per ml) of *P. tricornutum* per day/ 200L larval bin. Over feeding was avoided to maintain water quality and to mitigate mortality of urchin larvae.

P. tricornutum and *Amphora* spp. were grown under 18 hours light and six hours dark and received Cell-Hi F2P nutrient media (Varicon Aqua solution Ltd. Worchester, England) at 1ml/L of stock culture. As *Amphora* spp. and *P. tricornutum* are species of diatoms, sodium metasilicate (Na_2SiO_3) was added at 1ml/L to the culture media to enable formation of the diatom tests.

3.3.2 Substrate Conditioning

The settled and metamorphosed post larvae settled on either *Amphora* spp. and on *Ulvella lens*. Adult sporophytes of *U. lens* were sporulated using methods described by Hannon *et al*. (2014). The *U. lens* germlings were conditioned at 16°C in 1µm filtered seawater and treated with Ultra Violet (U.V.) light. The *U. lens* germlings were held under ambient light conditions for 30 days prior to settlement of *P. lividus* larvae.

The diatom *Amphora* spp. was conditioned (cultured on plates) for 14 days at 16°C in 1μm filtered and U.V. treated (sterilised) seawater under ambient light conditions prior to the settlement of competent larvae. All settlement substrates received F/2 nutrients for the duration of the conditioning period at 1ml/L. Diatoms also received 1ml/L of silicates along with the culture media.

3.3.3 Broodstock Selection

Broodstock urchins with a test diameter greater than 50mm were selected from wild stocks in subtidal rock pools nearby Dunmanus Seafoods Ltd. during April 2013. The broodstock were held in a conditioning system under ambient light and sea temperature. The urchins were fed *ad libitum* twice per week. The diets consisted of macroalgae, *Palmaria palmata* and *Ulva* spp.

The urchins were assessed for fecundity on a monthly basis. Randomly selected urchins were sacrificed and samples of the gonad were examined under binocular microscope for presence of eggs or sperm (gametogenesis). The broodstock were considered ripe at the end of July 2013. Egg sizes ranged from 65-80 microns, there was no variation in colour of the eggs.

3.3.4 Spawning

Urchins were selected on the 20th August 2013 and removed from the conditioning unit and washed and rinsed in sterile seawater to remove any fecal debris and left out of the water for one minute to drain. Spawning containers (approx. 150ml) were then filled with seawater filtered to 1 μ m and treated (sterilised) with U.V light. Container openings smaller than the test diameter of the urchins were used to prevent the urchins entering the containers.

The urchins were induced to spawn by injecting 0.5M Potassium Chloride (KCl) solution, into the body cavity. This method is widely used to induce spawning in sea urchins, however it results in high mortality of broodstock post spawning.
The treatment is applied to the urchins by injecting 1-2ml of KCl solution into the coelomic cavity. The dose given was dependent on the size of the animal. Injection of KCl was made between the bands of spines, between the mouth and test by pushing the needle gently though the peristomal ring (area of tissue surrounding the mouth) at a 30-

degree angle into the coelomic cavity. The urchins were then shaken vigorously for 30 seconds, to circulate the KCl solution around the coelomic cavity. The urchins were then placed on the containers upside down (Figure 3.1) (mouth facing upwards).

Figure 3.1. Male urchin releasing sperm from the gonophores, containers were kept completely full to aid the release of gametes.

Sperm and egg release usually began within 30 minutes, usually with the males beginning to release sperm first (Figure 3.1). During this spawning cycle, all five females released eggs in different amounts and all three males also released sperm. This resulted in the release of approximately six million eggs in total. Once the urchins begin to spawn they can not be separated into sexes, as the eggs are orange in colour and the sperm is white. Egg counts were conducted using a Sedgwick rafter slide to estimate the amount of eggs in 1ml of water. This was repeated five times to calculate an average. All eggs released were sieved through a 180µm sieve to remove any debris and then pooled to ensure viable and even fertilisation.

The egg solution was then divided between five 1L jugs; 2.5ml of the concentrated sperm was then added to each jug. As the male urchin releases sperm, it sinks to the bottom of the jar. This concentrate was collected with a pipette to avoid diluting it with excess seawater. The eggs and sperm were not disturbed for one minute to allow for even fertilisation. After one minute a sample was taken to assess fertilisation. After the formation of the fertilisation envelope or the formation of a polar body, the eggs were deemed to be fertilised (Figure 3.2). The fertilised eggs were then sieved once more through a 60-micron sieve and transferred into egg trays.

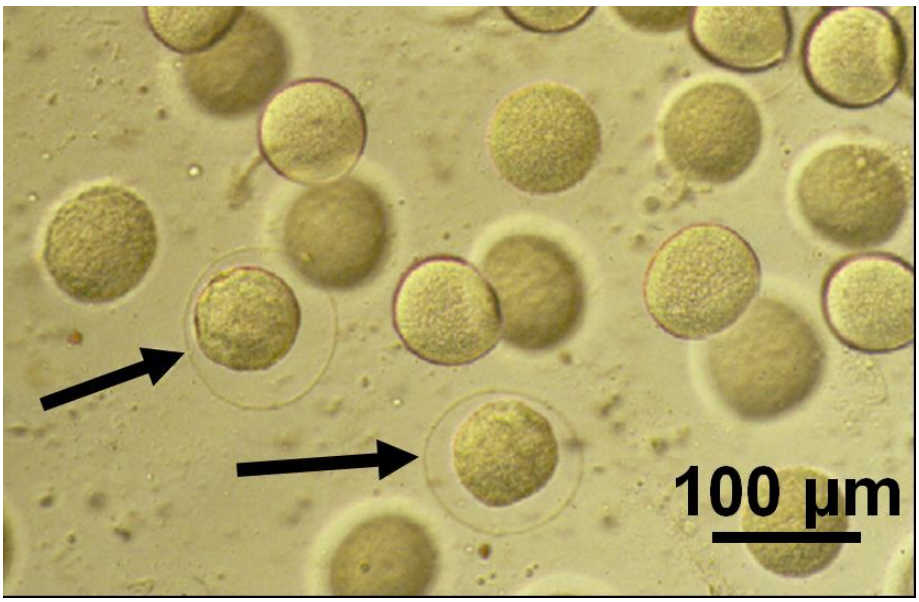


Figure 3.2. Successful fertilization is deemed by the presence of the fertilisation envelope, surrounding the egg (arrowed). This membrane inhibits polyspermy and can be formed in less than 1 minute. Image courtesy of Dr. Maeve Kelly (Scottish Association of Marine Science, Oban, Scotland)

3.3.5 Larval Rearing & Competent Larvae Grading

After 24 hours, swimming larvae became visible. Siphoning off the swimming larvae into the larval bins avoids the transfer of damaged eggs and membranes leftover after hatching. The larval bins used were 220L in volume and were flat bottomed.

Once the larvae were transferred to the larval bins light air flow was applied to circulate the water. Having too high an airflow forces the larvae to float to the surface and adhere to the sides of the bins. In the first 3-4 days the airflow was maintained to a single trickle of bubbles just to break the surface tension. This was increased gradually, and by day 10-11 the flow rate was kept constant to prevent larvae sinking to the bottom as they increased in size.

The water was exchanged every 3 days by sieving the larvae; the sieves used were 60 μ m for the first two changes followed by 120 μ m for the next three water changes and finally 180 μ m for the last water changes due to the increase in size of the urchin larvae. Progressing to each larger size allows for the less developed animals to be discarded whilst siphoning out the water. The water temperature was maintained by placing a 300W-heating probe into the 220L bin. Temperature was kept at 16°C for the length of the larval period of 21 days. The larvae were under ambient light conditions for the duration of the cycle.

The larvae on day 21 were deemed competent when the widest part of the rudiment reached 320 micron. The larvae were then passed through a 450 μ m sieve to ensure that all the smaller less developed larvae were removed for this settlement trial.

3.3.6 Experimental design

The experiment evaluated the settlement of *P. lividus* larvae on different substrate treatments under controlled conditions. The substrate treatments evaluated were:

1. *Amphora* Spp. (Amp);
2. *U. lens* (UL);
3. *U. lens* + *Amphora* Spp. (UL+Amp), and
4. Control: No substrate conditioning.

Eight replicates of each treatment were each contained in white PVC containers of 1L volume during the trial. The settlement substrates (treatments) were coated onto cut sections of PVC settlement plates measuring 2.5cm by 2.5cm prior to settlement of larvae; two of these plates were placed into each replicate container with their respective treatment. Sections of PVC plates with no biofilm were used as a control in sterile seawater with no possible inducer or settlement cue. Larvae were also given a choice between *U. lens* and *Amphora* spp. (Treatments) to evaluate a preference when given a choice between these two settlement inducers. This experiment also used eight replicates with a choice of each of the treatments in each of the eight-1L containers.

100 competent graded larvae were selected from a petri dish using a stereoscopic microscope and added to each replicate only. Settlement was assessed every 24 hours. After 48 hours the settlement experiment was terminated as the majority of the larvae had metamorphosed. Settlement did not occur within the first 24 hours. For each replicate, the total number of settled metamorphosed postlarvae and swimming larvae were counted using a stereoscopic microscope.

3.3.7 Statistical Analysis

Statistical analysis was carried out using the Minitab 16 statistical software package. All data were tested for homogeneity of variance and normal distribution, percentage of settled larvae were analysed. One-way ANOVA was carried out to assess the influence of settlement substrate on the total settled numbers of urchin larvae. Post-hoc analysis was carried out using Tukeys test (HSD) to assess the differences between each treatment. A Chi-Square test was also used to test the difference in settlement rates when the larvae were given a choice between *U. lens* and *Amphora* spp. only.

3.4 Results

There was a significant statistical difference in settlement rates between all the treatments ($P < 0.05$) (Table 3.1). *Amphora* spp. induced the overall lowest number of individual postlarvae and mean settlement of (Figure 3.3).

U. lens achieved the highest settlement of metamorphosed postlarvae with the largest mean settlement over all the replicates (Figure 3.3). Mixing the settlement substrates did have a positive effect on settlement rates when compared with *Amphora* on its own, $P < 0.05$ (Table 3.2). When larvae were given a choice of substrates, there was still a significantly higher settlement of individual postlarval urchins on *U. lens* than the mixed substrates (Figure 3.3). The control was not conditioned with a biofilm and did not achieve settlement at all. Therefore it is not plotted or displayed in the Figures and Tables. This shows that *P. lividus* larvae require a settlement cue to induce settlement.

Significantly higher numbers of postlarvae settled on *U. lens,* with a maximum of 65 individuals and the lowest of 32 for the same treatment (Figure 3.3). The percentage of settled postlarvae displayed in Figure 3.3, shows a mean figure of 50% settlement induced by *U. lens,* 29% for *Amphora* + *U. lens* and 7% for *Amphora.*

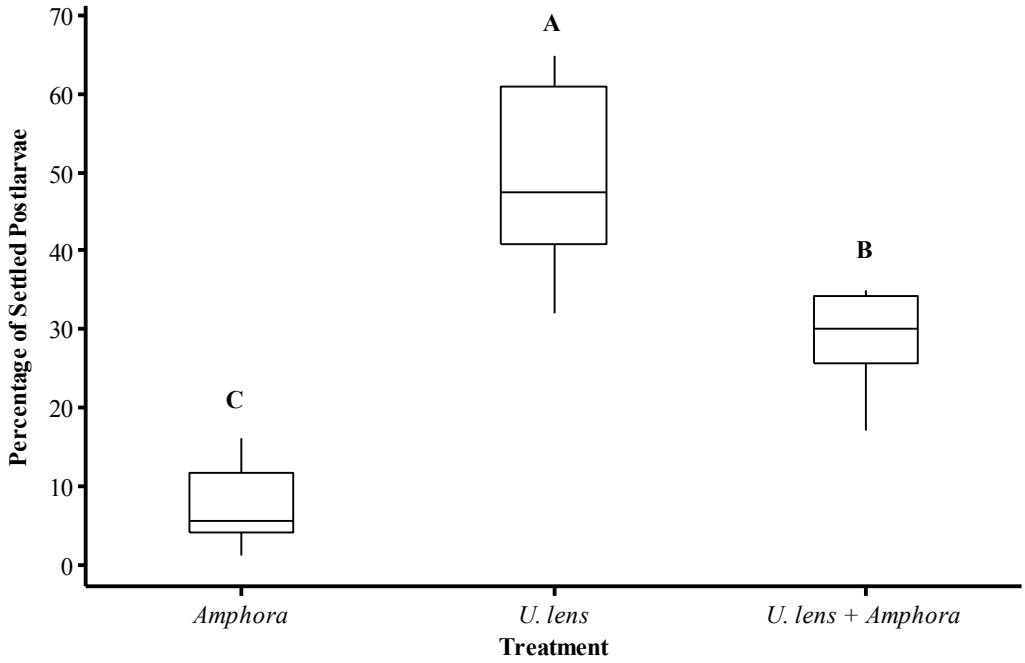


Figure 3.3. Box-Plot comparing the settlement achieved on the different treated settlement substrates. There are significant statistical differences between the settlement rates of each treatment; the *U. lens* treatment achieved the highest number of settled post larvae ($P < 0.05$) (Table 3.1). The letters above the bars indicate the results of the Tukey HSD. Letters not shared between bars indicate significant differences between treatments.

There is a significant statistical difference between the settlement levels of each treatment with the *U. lens* treatment achieving the highest number of settled post larvae ($P < 0.05$) (Table 3.1).

Source	**DF**	**SS**	**MS**	**F**	***P***
Treatment	2	0.72271	0.36135	56.94	0.05
Error	21	0.13328	0.00635		
Total	23	0.85598			
S = 0.07966	**R-Sq** = 84.43%		**R-Sq (adj)** = 82.95%		

Table 3.1 Results of one-way ANOVA indicating the highly significant effect ($P < 0.000$) of settlement substrate on the number of settled larvae. The greatest difference in settlement was between *U. lens* and *Amphora* spp. ($P < 0.05$) (Figure 3.3).

		Test		Contribution
Substrate	**Observed**	**Proportion**	**Expected**	**Chi-Sq**
Ulvella lens	29	0.5	19.5	4.62821
Amphora	10	0.5	19.5	4.62821
N	**DF**	**Chi-Sq**		***P*-Value**
39	1	9.25641		0.002

Table 3.2. Output of a Chi-Square test for larval choice, between *Amphora* spp. and *U. lens* solely. On average, 3 times as many larvae settled on *U. lens* than on the monospecific diatom *Amphora* spp. ($P < 0.002$). When given a choice between *Amphora* spp. and *U. lens* a higher number of larvae settled on the *U. lens* plates than the *Amphora* spp. (Figure 3.3), ($P < 0.05$) (Table 3.2). There was no settlement within the first 24 hours. However by 48 hours nearly 50% of the larvae metamorphosed and settled on *U. lens*.

3.5 Discussion

Significant difference between settlement rates was observed between all the settlement substrates in this trial (Table 3.1). Settlement was significantly higher on *U. lens* when compared with the mixed substrates and *Amphora* spp. (Figure 3.3).

On mixed substrates settlement rates were lower than on pure *U. lens* conditioned surfaces (Figure 3.3). However mixed substrates achieved higher settlement rates than plates treated with *Amphora* spp. on its own (Figure 3.3).

When competent larvae were given a choice between the green encrusting macroalgae *U. lens* and *Amphora* spp. nearly 3 times as many postlarvae were observed to settle on the *U. lens* conditioned surface than on those conditioned with *Amphora* spp. (Table 3.2). Settled metamorphosed postlarvae were counted in all replicates with the majority of settled individuals settling on *U. lens* (Figure 3.3). *U. lens* may have had a beneficial effect on the settlement of larvae on the *Amphora* spp. plates due to its presence in the same container, however the settlement on the *U. lens* plates was significantly higher (Table 3.2).

Postlarval *P. lividus* were observed to graze *U. lens* once settled and metamorphosed. This could be due to the Aristotle's lantern and the urchin's ability to mechanically remove the encrusting macroalgae. Other invertebrate species such as abalone, are unable to graze *U. lens* until they are greater than 3mm in shell length which can be up to 3 months post-settlement, therefore showing the suitability of *U. lens* for urchin aquaculture as an initial food source. Abalone graze *U. lens* by rasping their radula along surfaces removing material, the adhesion strength of *U. lens* can not be overcome until the abalone is greater than 3mm in length (Daume *et al*., 2000; Dyck *et al*., 2011). The difference in the mouthparts between abalone and sea urchins dictates the animals ability to utilise a food source.

Postlarval urchins were observed efficiently grazing areas of *U. lens* and utilising it as an initial food source. Not only can *U. lens* act as a settlement cue for swimming urchin larvae, it is also utilised by postlarvae as a initial food source (Takahashi *et al*., 2002).

Therefore *U. lens* may be a very suitable settlement substrate for *P. lividus* as they are able to feed directly on it earlier in their life history compared to abalone.

Commercial hatcheries settling juvenile sea urchins predominantly rely on naturally occurring biofilms which achieve mixed and inconsistent results (De La Suz *et al*., 2013). The application of *U. lens* in commercial aquaculture has widely been adopted for commercial abalone aquaculture (Hannon *et al.,* 2014), however it has not been tested prior to this on *P. lividus*.

Direct application of *U. lens* to the commercial *P. lividus* production at Dunmanus Seafoods achieved increased productivity without having to change existing settlement tanks, plates or settlement facilities. *U. lens* can be coated directly on to settlement surfaces such as standard corrugated P.V.C plates and fiberglass tanks.

The settlement substrates used in this evaluation are currently used in invertebrate aquaculture, however as they have not yet been incorporated in the commercial urchin aquaculture industry in Europe. Our results indicate significant differences in settlement between the treatments *U. lens* and *Amphora* spp. (Figure 3.3).

3.5.1 Industry implications

Previous development of commercial culture methods of *U. lens* for Europe's aquaculture industry recommended direct transfer of technology to commercial sea urchin aquaculture (Hannon *et al*., 2014). The settlement trial presented in this study has shown *U. lens* to be an important settlement substrate for *P. lividus* (Figure 3.3).

The methods of Hannon *et al.* (2014) were applied to commercial production of *P. lividus* at Dunmanus Seafoods Ltd. Previously settlement rates of this species of urchin on the substrates tested here were unknown. The use of *Amphora* spp. in this study proved to have relatively little utility for industry needs when compared with *U. lens.*

U. lens is a highly successful inducer of *P. lividus* larval settlement and is an important advancement in the culture of this commercial sea urchin species. The highly inductive nature of settlement cues *U. lens* emits and its use commercially in Japan since the 1980s has made this settlement cue an ideal substrate for the culture of abalone and sea urchins (Takahashi *et al*., 2002).

This is the first evaluation of settlement inducers that includes *U. lens* for this species of urchin *P. lividus*. The findings are readily transferrable to echinoderm aquaculture as they share similar life histories with other commercially important invertebrates (Hannon *et al*., 2014; Matsuura *et al*., 2009).

Industry application of these results within commercial *P. lividus* hatcheries, can increase productivity and reduce costs, by increasing the production capacity of commercial aquaculture enterprises without having to change existing nursery facilities or equipment.

Whilst these results demonstrate advancement of commercial sea urchin aquaculture, achieved through transfer of technologies established in invertebrate aquaculture, the long-term use of *U. lens* requires further research to establish post-settlement survival rates of *P. lividus* at commercial scales and under commercial conditions.

3.5.2 Acknowledgements

We thank Dr Maeve Kelly for the detailed image of the fertilisation envelope (Figure 3.2). We also thank Dr Matthew J. Slater for his comments during the preparation of this manuscript.

Chapter IV: Investigation into the effect of dietary phosphorus enrichment on the growth and survival of juvenile European abalone *Haliotis tuberculata*

A version of this chapter has been submitted for publication:

Colin Hannon, Rick A. Officer, Declan Hanniffy, (*Under Review*) Investigation into the effect of dietary phosphorus enrichment on the growth and survival of juvenile European abalone *Haliotis tuberculata*. Journal: Aquaculture International

4.1 Abstract

Sustaining the growth of abalone aquaculture globally requires farm operators to satisfy the specific diet requirements of the particular abalone species grown. Components of commercial aquaculture feeds usually contain animal protein in the form of fishmeal. Rather than adding generic animal protein foodstuffs, formulated diets may be more effective when supplemented with particular substances of metabolic use to the abalone. The inclusion of animal proteins may have physiological demands on the growth and survival on different species of abalone as formulated feeds change the colour of the shell bands and the components of the feed may not exist in the natural diet of abalone. Inclusion and enrichment of phosphorus in mixed macroalgal-meal diets for juvenile abalone *Haliotis tuberculata* was investigated. Macroalgae is the natural diet for juvenile and adult abalone and can be limiting in bioavailable phosphorus; increasing the available phosphorus content in the diet of juvenile *H. tuberculata* increased growth rates compared to *Palmaria palmata* (P <0.05). Two levels of enrichment were used, 0.9 and 1.5% total available dietary phosphorus. There was 100% survival in all treatments during the trial. Growth (shell length) was higher on the enriched diets 0.9 & 1.5% phosphorus; *P. palmata* produced the least growth of all the diets. Weight loss was measured in all treatments. The development of diets containing components that exist in the natural diet of abalone has implications for farm operators, as there is increasing importance for traceability of dietary components in abalone feed.

Keywords: Abalone; *Haliotis tuberculata*; Feeding transitions; Phosphorus; Macroalgae.

4.2 Introduction

Weaning of juvenile abalone from their initial diatom diet to macroalgae changes their feeding behaviour (Courtois de Viçose *et al*. 2012; Kawamura *et al*. 1995). This feeding transition results from lack of nourishment when the diatom diet can no-longer support growth (Johnston *et al.,* 2005). Longer foraging trips begin the weaning process when the juvenile abalone become cryptic and move on to larger foodstuffs. Behavioural changes during the weaning process are thought to occur as abalone move on to larger food sources dominated by macroalgae (Takami 2003).

At this stage in the abalone growth cycle, farm operators may introduce harvested naturally occurring macroalgae, and/or introduce formulated artificial feeds (Hannon *et al*. 2013). Due to seasonal changes in the nutrient content of macroalgae and the costs associated with its collection, some operators prefer to use artificial diets, provided that they can provide adequate growth coupled with reduced operating costs (Daume *et al*. 2007).

Previous research has established the basic nutrient requirements of juvenile abalone (Mai *et al*. 1995; Mgaya & Mercer 1995; Bautista-Teruel *et al*. 2003; Bautista-Teruel *et al*. 2011; Gómez-Montes *et al*. 2003) and identified that the timing of a dietary switch is crucial to farm outcomes (Huchette *et al.,* 2003; Pang *et al.,* 2006; Courtois de Viçose *et al.,* 2012). If the animal cannot dislodge, graze or digest the diet, implementation of a switch in dietary regime will negatively effect survival (Kawamura *et al*. 2001; Kawamura *et al*. 1995).

Components of extant formulated artificial diets may be non-palatable to abalone and are sometimes absent from their natural diets. In particular, the inclusion of animal proteins in formulated feeds may increase trypsin activity; a metabolic reaction to the indigestibility of animal proteins by juvenile abalone, representing an energy burden that can reduce the food conversion ratio (Johnston *et al.,* 2005).

Other commercially grown invertebrate species such as *Paracentrotus lividus* are capable of utilising animal proteins in artificial feeds as they are omnivorous in nature, however *P. lividus* is predominantly algivorous and share similar predispositions to macroalgal diets as abalone (Fernandez *et al*. 1998; Lawrence 2007). It may be possible that urchins will also achieve increased growth when given feeds with components existent in their natural diet which has been shown for abalone (Naidoo *et al.,* 2006).

Abalone are algivorous by nature and animal protein is not a component of their natural diet (Leighton 2008; Hannon *et al*. 2013; Mai *et al*. 2001). However, inclusion of fishmeal in the diets of juvenile abalone can increase growth rates (Chao *et al*. 2010; Bautista-Teruel *et al*. 2003; Viana *et al*. 1996). However as fishmeal is a controlled substance within Europe (EC Regulation 999/2001 & EC Regulation 183/2005), regulations restrict the species that may be used (O' Mahoney *et al*. 2014). As the majority of fishmeal and fish oil is derived from offal and offcuts of processed fish, determining the quality of this component and its uses in the abalone aquaculture feed industry is crucial.

Components of formulated feeds used during the abalone production cycle may also have an effect on the marketing of abalone for the commercial enterprises. Abalone fed some artificial formulated feeds show the colour in their new shell growth and shell band colour (Gallardo *et al*. 2003). This obvious indication of farmed origin can negatively affect the marketability of the product.

Viana *et al*. (1996) and Guzmán & Viana (1998) used abalone viscera silage as the animal protein source, which poses other more ethical problems than solutions to the animal protein component in formulated feeds for abalone. Traceability and declaration of the derived animal protein is important to commercial growers. Contaminated feed containing infected abalone by-products or wastes has been suggested as the vector for introduction of Ganglioneuritis (herpes like virus) into the Australian abalone industry (Gavine *et al*., 2009). A similar disease has been observed in the Taiwanese industry but confirmation that it is the source of the disease in Australia is inconclusive (Gavine *et al*., 2009; Hooper *et al*., 2007).

Commercial considerations are also important as fishmeal is the most expensive component of the feed (Fleming & Hone 1996; Guzmán & Viana 1998; Johnston *et al*. 2005). Obviating this expense through the use of a diet only containing components existing in the natural diet of abalone represents a commercial opportunity for farmers.

Naidoo *et al.* (2006) showed that mixed macroalgal diets were an effective substitute for commercially available feeds and formulated feeds. O'Mahoney *et al.* (2014) reported the lack of information or research on mixed macroalgal-meal diets. Hannon *et al*. (2013) also identified an opportunity for European feed producers to formulate a mixed macroalgal-meal diet for abalone species cultured in Europe as specific macroalgal derived feeds do not exist for *H. tuberculata* & *H. discus hannai.*

Abalone have nutrient requirements to maintain growth and cellular function (Tan *et al*. 2002). Abalone primarily fill their nutrient requirements through the in take of food (Coote *et al*. 1996). However some feeds can be lacking in certain bioavailable nutrients such as phosphorus (Sales *et al*. 2003).

Phosphorus has an important role in cellular function and production of several key enzymes, and is directly involved in energy producing cellular reactions (Sales *et al*. 2003). Supplementation with phosphorus may enhance growth as it is limiting in the diet of abalone (Coote *et al.* 1996; Tan *et al*. 2001; Chaitanawisuti *et al*. 2010).

Few studies have been conducted on the dietary requirement for phosphorus relative to calcium in the diet of abalone (Coote *et al*. 1996; Tan *et al*. 2001). Calcium was found not to be significant as a dietary supplement; abalone can obtain sufficient calcium directly from the water (Cenni *et al*. 2010). Thus the addition of calcium in the form of $CaCO_3$ can be controlled in the culture conditions (Recirculation system) rather than through their diet. Flow-through systems do not require the addition of $CaCO_3$ as calcium will not be depleted, as the water is not in a closed system.

The rearing of abalone in recirculation systems has intensified the culture of abalone in some countries (Leighton, 2008). However the lack, or depletion of calcium in such closed systems, can cause stress, behaviour changes and degradation of the animal's shells, more commonly known as Shiny shell (Cenni *et al*. 2010).

Investigations of the ratio of phosphorus and calcium in the diet of two abalone species *Haliotis laevigata* and *H. discus hannai,* (Coote *et al*., 1996; Tan *et al*., 2001) found an increase in growth resulted from the addition of phosphorous. Additional dietary calcium intake had no affect on growth as abalone attain their calcium requirements from the surrounding water (Cenni *et al*. 2010). The supplementation of phosphorus in macroalgal-meal diet may prove beneficial to the growth of juvenile abalone as the components exist in their natural diet and may provide an alternative to weaning and on-growing feeds containing fishmeal and other animal proteins.

This research investigates the effect of differing levels of phosphorus enrichment in macroalgal-meal diets on the growth of juvenile *H. tuberculata*. The focus of the trial is on the addition of phosphorus in the diet of juvenile *H. tuberculata* as phosphorus availability can be growth-limiting factor.

4.3 Materials & Methods

The feeding trial was conducted on site at Abalone Chonamara Teoranta, Rossaveal, Co. Galway, Ireland.

4.3.1 Abalone Collection & Maintenance

Hatchery produced juvenile *H. tuberculata* were collected from the on-growing unit and graded. 120 juveniles between 30-40mm in shell length were selected from the cohort. Each abalone's total shell length (SL) was measured to the nearest 0.01mm using digital callipers and weighed (Wt) using an electronic balance to the nearest 0.01g. The abalone, initially stocked at 38.7 ± 0.16 mm (n = 120) shell length, and 8.6 ± 0.16g (n = 120) live weight (Mean ± SE), were randomly divided between the 12 experimental tanks (Table 4.2).

Abalone were held in a flow through system (13°C±1°C) and under an ambient light regime. Tan *et al*., (2002a) and Tan *et al.*, (2002b) experienced poor growth due to fluctuation of temperature (9.8°C - 26.4°C).

Holding the juvenile abalone in a flow through system mitigated against depletion of calcium in the form of $CaCO_3$. Maintaining $CaCO_3$ concentration is recommended by Cenni *et al*., (2010), as depletion of calcium in a culture system can cause changes in behaviour and feeding.

The flow through system delivered 2L of seawater per minute into each 25L tank. The incoming water was filtered to 5µm and treated with ultraviolet light. Each 25L tank received aeration via a centrally placed air-stone. Filtered treated water entered from the opposite corner away from the outflow standpipe, which controlled the height of water in the tank.

The abalone spat were starved during an acclimatisation period of 7 days prior to the introduction of the treatments. This was conducted to ensure any mortality due to handling and stress occurred prior to introduction of the experimental diets.

Abalone were fed three times per week *ad libitum*, uneaten food and faeces were removed before feeding. Tanks were emptied completely once per week to coincide with the collection of biometric data of the juvenile abalone.

The trial utilised a commercial mixed-macroalgal meal produced by Ocean Harvest Ltd. (Galway, Ireland) and sodium alginate, to act as a binder (Oxoid Ltd. Basingstoke, Hampshire, England). Ocean Harvest Ltd. formulated the mixed macroalgal-meal diets used in this trial and measured their available phosphorus content.

Components of the feed used in the trial provided by Ocean Harvest cannot be disclosed due to commercial constraints on the macroalgal-meal (commercial mix). Experimental feeds only contained ingredients that exist in the juvenile abalone natural diet. *Palmaria palmata* was used as a comparison diet for the trial, as the abalone were reared on it.

Monobasic potassium phosphate (KH_2PO_4) (Fisher Scientific U.K Ltd, Loughborough**,** England**)** was used for phosphorus enrichment as it is readily soluble and has a high bioavailability (Tan *et al*. 2002; Chaitanawisuti *et al*. 2010).

The un-enriched basal diet had a total of 0.15% (Base) available phosphorus. Juvenile abalone were given feeds with 2 levels of enrichment 0.9 % (Moderate) and 1.5 % (High) total available dietary phosphorus. The *P. palmata* diet had a total available phosphorus of 0.19% (Table 4.1).

Treatment	**Component**	**Available P (mg/100g)**	**P (%)**	**Addition of P (g/100g)**	**Final Conc. P (%)**
Base	Commercial mix	150.0	0.15	0.00	0.15
Moderate	Commercial mix	150.0	0.15	0.75	0.90
High	Commercial mix	150.0	0.15	1.35	1.50
Macroalgae	*Palmaria palmata*	187.0	0.19	0.00	0.19

Table 4.1. Phosphorus availability in the treatments used during the trial

4.3.2 Experimental design

The feeding trial lasted 4 weeks. There were four treatments (diets), with three replicates (Tanks) per treatment. 10 Abalone per tank 30-40 mm in shell length were distributed amongst the rearing system, which consisted of 12 independent tanks using a completely randomised design.

4.3.3 Sampling & Data Collection

Collection of biometric data (length & weight) was conducted on a weekly basis; all abalone from each replicate per-treatment were measured and weighed. Survival (% survival) was assessed via dead shell counts on a weekly basis during water exchange and feeding. Daily increment in shell length (DISL μm/day) for each treatment was calculated at the end of the trial for each treatment.

DISL (μm/day) = [Shell Length final (mm) – Shell Length Initial (mm)]/ (No. Days) X 1000

4.3.4 Data Analysis

Biometric data of abalone growth were analysed using MINITAB 16. Normal distribution of the data and Sphericity were confirmed. Repeat measures ANOVA was carried out to assess differences in growth rates due to each treatment. Univariate analysis of variance and post hoc (Tukeys HSD) tests were applied to test the mean abalone interval sizes per treatment.

4.5 Results

100% of the juvenile *H. tuberculata* survived. Each treatment had a different level of available dietary phosphorus (Table 4.1), which produced different growth rates, with moderate enrichment producing the highest growth rate (Figure 4.1). There was no significant difference in growth in any of the treatments over the duration of the trial (ANOVA: df = 3, S = 1.428 R-Sq = 0.48%, R-Sq(adj) = 0.00% $P > 0.05$), however the difference in growth between the treatments was highly significant ($P < 0.05$) (Table 4.2). All treatments exhibited weight loss in the feeding trial (Figure 4.1).

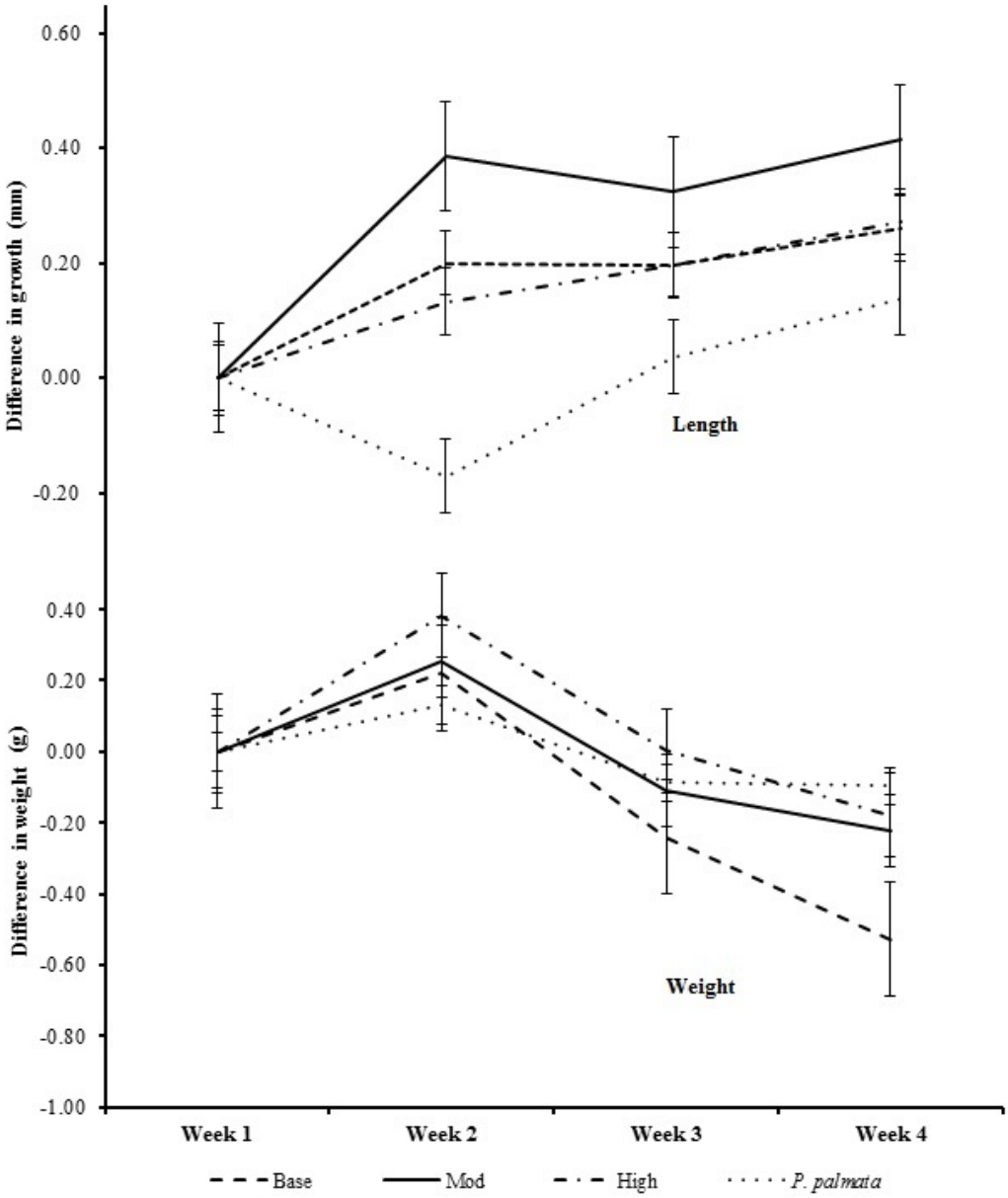


Figure 4.1. Treatment effect by week on wet weight and length of juvenile abalone fed differing levels of phosphorus over a 4-week period. Difference in weight of juvenile abalone over the duration of the feeding trial, all abalone in all treatments showed increased growth in total shell length. There was no significant difference in growth between the weeks, however there were significant differences between the treatments over the feeding trial (Table 4.2) (n = 3).

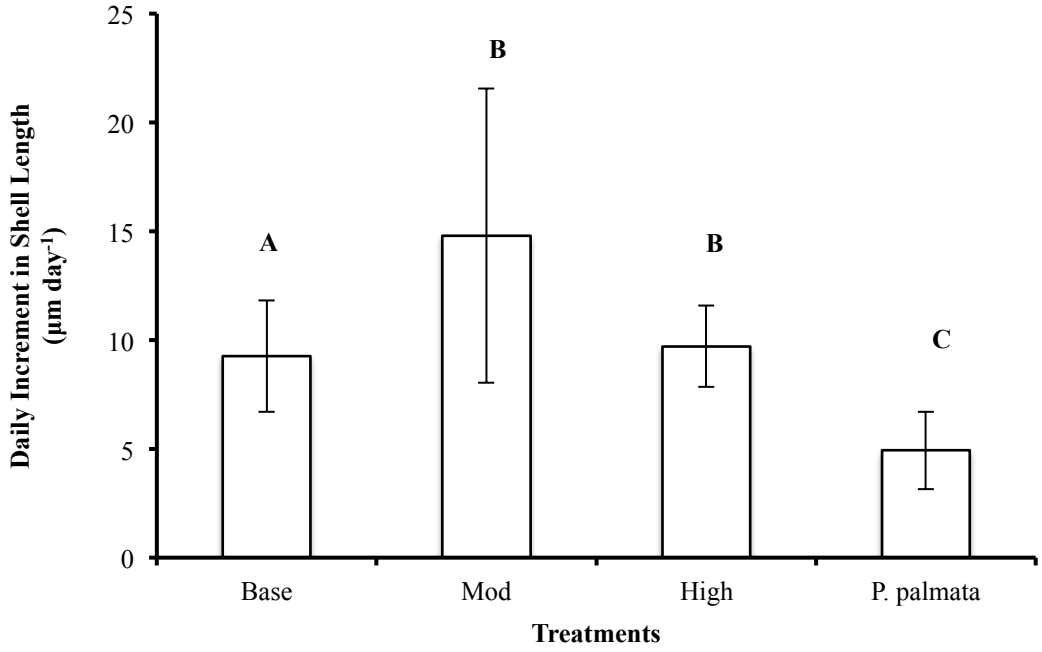


Figure 4.2. Daily increment in shell length (DISL) calculated in µm day $^{-1}$ of juvenile *H. tuberculata* per treatment. The highest incremental growth rate was attained by the moderate enrichment (P 0.9%) followed by high enrichment (P 1.5%). *P. palmata* had the lowest growth per day. The error bars indicate the standard error and the letters above the bars indicates the results of the Tukeys HSD test. Letters not shared between bars indicate significant differences between treatments.

Source	**DF**	**SS**	**MS**	**F**	***P***
Treatment	3	6.8813	2.2938	10.60	0.05
Week	3	0.4649	0.1550	0.72	0.550
Treatment*Week	9	0.2467	0.0274	0.13	0.999
Error	32	6.9225	0.2163		
Total	47	14.5153			
S = 0.465111		**R-Sq** = 52.31%		**R-Sq(adj)** = 29.95%	

Table 4.2. Result of repeated measures analysis of variance indicating a significant effect of the treatments on the growth (shell length) of the abalone during the feeding trial ($P < 0.05$), there was no significant effect on growth between the weeks or interaction between treatment and weeks ($P > 0.05$).

4.6 Discussion

This research investigated the effects of addition of supplementary phosphorus on the growth and weight of juvenile *H. tuberculata*. All juvenile abalone in the trial grew in shell length (Figure 4.1). There was significant difference in measured growth between the treatments (Table 4.2).

Although all treatments exhibited weight loss, this did not effect survival. The *P. palmata* treatment measured the least amount of weight loss, however this weight loss followed a similar profile to the experimental diets (Figure 4.1). Daily incremental shell length (DISL) was used to measure the difference in growth rates between the treatments. The moderate (0.9%) phosphorus enrichment attained the highest growth-rate per day (Figure 4.2). The high level enrichment 1.5% and the base treatment 0.15% Phosphorus also had a higher growth rate than the macroalgae *P. palmata.*

Coote *et al*., (1996) used an average of 0.68 % total phosphorus to enhance growth of *H. laevigata*. Tan *et al*., (2001) recommended a total phosphorus level of 0.64-0.65 % for *H. discus hannai*. Tan *et al*., (2002a) recommended that the total level of available dietary phosphorus for *H. discus hannai* should be 0.9 – 1.1%, which is slightly above recommendations previously made by Tan *et al.* (2001).

The addition of 0.9 (Moderate) and 1.5% (High) phosphorus in this trial was consistent with, and slightly above the dietary recommendations made by Tan *et al*., (2002a). Our results suggest that *H. discus hannai* and *H. tuberculata* share similar preferences for phosphorus availability.

The highest growth rate was measured at the moderately enriched level of 0.9% available phosphorus, consistent with the recommendations made by Tan *et al*., (2002a) (Figure 4.2). This indicated that there is an optimal enrichment range for *H. tuberculata* as there is for *H. discus hannai*, which requires further investigation.

The diets used in experiments by Coote *et al*., (1996) may have been deficient in available phosphorus because of the soybean component. Phosphorus is bound in the form of phytate in soybeans, thus the addition of supplementary phosphorus in Coote *et al*., (1996) diets may not have overcome this mild phosphorus deficiency.

Coote *et al*., (1996) suggested further investigation into phosphorus bound in the form of phytate. However phytate (phytin phosphorus) is unavailable to animals with simple stomachs because they lack the enzyme phytase in the gastrointestinal tract. This suggests that abalone will be unable to absorb phosphorus bound in phytate (Sales *et al*. 2003), making phytate enrichment futile.

Coote *et al*.'s (1996) findings were considered when the components of the experimental diets were selected for this study. Components with high levels of unavailable phosphorus such as soybeans were not included. As a result our research focused on differing levels of available phosphorus in components naturally occurring in the animals' diet (Macroalgae), predicated on the assumption that abalone respond better to components actually found in their natural diet.

The experimental diets in this research were observed to lose stability in water after 8-12 hours post feeding, thus not allowing the juvenile abalone to efficiently feed on them for a longer period of time due to this solubility in water. However weight loss cannot be solely attributed to the solubility of the experimental feeds in water, as a similar weight loss profile was measured on the *P. palmata* diet (Figure 4.1). Further investigation is required to identify affordable palatable binders to increase stability in water.

It is common practice to use inorganic phosphorus as a supplement in abalone feeds (Sales *et al*. 2003). Our use of monobasic potassium phosphate increased the shell growth of juvenile *H. tuberculata* (Figure 4.3). Investigating phosphorus content of feeds for abalone may allow feed manufacturers to increase the available phosphorus content using natural components rather than components that are non existent in the natural diet of abalone.

We observed that abalone fed the enriched treatments required more effort to remove from the experimental tanks during the collection of biometric data when compared to those fed with *P. palmata.* This observation suggests an effect of increased phosphorus strengthening the gripping behaviour of juvenile *H. tuberculata*.

Phosphorus can be limiting or in low concentrations in some macroalgae (Lobban & Wynne 1981). By using macroalgal-meal, it would be possible to increase the available phosphorus content by mixing more than one species of macroalgae with higher concentrations of phosphorus to increase the total phosphorus availability. Phosphorus levels vary from species of macroalgae to the time of the year, as nutrient profiles changes with the seasons (Werner & Dring, 2011).

Our findings suggest similar dietary requirements between *H. discus hannai* and *H. tuberculata* for available phosphorus, at levels similar to these recommended by Tan *et al*., (2002a). Enriching the diet of *H. tuberculata* with supplementary phosphorus appears to increase shell growth. Using a mixed macroalgal-meal diet proved to be more beneficial to growth when compared to *P. palmata*.

4.6.1 Acknowledgements

We thank Cindy O' Brien from Abalone Chonamara Teoranta for the use of the facilities and supply of juvenile *H. tuberculata* for the feeding trial. We also thank Michael Griffin for his help in the initial experimental set up.

Chapter V: General Discussion

5.1 Overview

The culture of abalone and sea urchins in Ireland began in the 1970s and late 1980s, where large scale production of both abalone and sea urchins was first commercialised in Europe (Leighton, 2008; Watson *et al*., 2004). Today, through increased production, expansion, investment, research and development, invertebrate aquaculture is no longer considered a novel concept but a vital industry in Ireland and Europe.

Commercial culture and production of abalone and sea urchins in Ireland is faced with the same limitations and barriers that are interlinked with the natural life histories of the species grown. Farm operators have to overcome these constraints by firstly identifying production barriers, and secondly implementing robust culture methodologies from known producing countries where the commercial culture of abalone and sea urchins is on an economically viable scale.

The research presented here investigates the status and culture constraints of commercially important invertebrates in Ireland. The novel application and implementation of culture technologies aimed to overcome production barriers for both abalone and sea urchins in Ireland, and was undertaken with direct industry collaboration in the research. This thesis aims to optimise Irish abalone and sea urchin aquaculture through a focus on four key research objectives:

1 To identify opportunities to overcome production constraints of industry expansion for abalone aquaculture;
2 To develop new culture methods of the commercially important encrusting macroalgae *Ulvella lens* for the Irish and European sea urchin and abalone industry;
3 To investigate the efficacy of *U. lens* as a settlement cue for *P. lividus* with the aim of industry implementation and application;
4 To investigate the development of a novel macroalgal-meal feed for *H. tuberculata* and investigate the effect of bioavailable phosphorus enrichment.

Each chapter outlined below addresses a key research objective with the overall aim of identifying and resolving particular production constraints experienced by commercial growers.

5.1.1 To identify opportunities to overcome production constraints of industry expansion for abalone aquaculture

Factors constraining the development of an intensive invertebrate aquaculture industry in Ireland needed to be identified prior to the implementation of transferable technology to overcome production barriers that inhibit industry expansion (outlined in Chapter 1).

Culture of abalone in Ireland has led the development of the industry in Europe through the importation of both European and Japanese abalone (*H. tuberculata* & *H. discus hannai*). The expansion of the Irish industry was evident as there were more commercial abalone farms than the whole of Europe combined (Chapter 1; Browne *et al*., 2008), however economic contraction and lack of stability in the industry has had a knock on affect on the whole abalone industry globally (Cook and Gordon, 2010).

Production constraints in Ireland are; economic, biological and technical. By not applying available information on current culture and on-growing methods, expansion of the industry is far below forecasted figures quoted by responsible authorities. The FAO stated that by 2008 that production of abalone in Ireland would have reached 25 metric tons (FAO, 2004) however the industry never reached its potential with current production in the EU stagnating at 3 metric tons (Cook, 2014).

Procurement of growing licenses in an economically viable time frame and supply of reasonably priced juveniles are two of the primary barriers to the development of a profitable industry here in Ireland (Soler Vila, 2008). Limiting factors to industry growth (outlined in Chapter 1) identify the leading causes of reduction in expansion of the industry in Ireland as:

- High energy and labour costs;
- Licensing constraints on establishment of new enterprises;
- Legislation on importation of broodstock and artificial feeds;
- Research providers and industry are not linked.

Similar limiting factors and production barriers have been experienced by enterprises in producing countries during initial development (Daume, 2003; Hahn, 1989; Nie and Wang, 2004). Once a critical mass of production can be reached in Ireland, it will allow for enterprises here to be self sustaining and profitable.

Chapter 1 outlined and identified solutions to the industry barriers and constraints experienced by Irish farm operators. The industry in Ireland can overcome some of these technical challenges by implementation of available information and industry standards from producing countries rather than a trial and error approach taken by some farm operators.

5.1.2 Inconsistent settlement rates

Inconsistent settlement rates for larval abalone were identified internationally as a major constraint to increased production of juveniles (Daume, 2003). The identification of this industry barrier lead to several commercial based research reports by the Australian Fisheries Research and Development Corporation (FRDC) on early life histories of juvenile Greenlip abalone *H. laevigata* (Daume, 2007, 2003).
Both the abalone and sea urchin industry experience inconsistent settlement rates (Daume *et al*., 1999; Lawrence, 2007). These common limitations are due to the shared similarities and preferences to settlement cues between abalone and sea urchins. Inconsistent settlement rates can be resolved by implementation of suitable settlement cues, and initial food sources such as benthic diatoms and *U. lens* offering an improvement to increase production and expansion of these industries
(Chapter 2 and 3).

The Irish industry has been slow to apply this research that highlights the dis-connection between research and industry. Suitable settlement substrates available to the Irish industry are limited due to lack of resources here and specific knowledge gaps in the industry. These knowledge gaps are identified in Chapter 1, and form part of Chapters 2 and 3.

The limitations experienced during Chapter 1 focus primarily on the lack of available information from Irish and European agencies on the production figures of abalone. This is crucial information for producers in both Ireland and Europe, and for the global expansion of the industry. Findings in Chapter 1 identified knowledge gaps in the production of abalone.

These are similar to the knowledge gaps identified in the production of sea urchins (Chapter 3) due to shared similarities and preferences to settlement substrate such as *U. lens* (Chapters 1 and 3).

The review of technical challenges in Chapter 1 identified the key research themes for subsequent investigation during this study. These addressed the unproven reliability of settlement, and deficiencies in weaning and on-growing.

5.1.3 Unproven reliability of settlement cues

Farm operators predominantly rely on naturally occurring diatom assemblages to induce settlement in abalone and sea urchins larvae however these assemblages will produce inconsistent unreliable results. Settlement can be induced at differing rates depending on the cue used, however early initial feeding rates have been shown to be influenced by species, shape and size of the diatom (Chapter 1; Chen, 2007; Courtois de Viçose *et al*., 2012; Daume *et al*., 1999; Lawrance, 2007).

The identification of preferred settlement cues for cultured abalone and sea urchins in Ireland is documented in this thesis (Chapters 1, 2, and 3). The development of a robust and repeatable time saving culture method for *U. lens* (outlined in Chapter 2) allowed for the direct transfer of *U. lens* mediated settlement induction to industry thus resolving issues with inconsistent settlement for abalone. *U. lens* was identified as an important settlement cue for abalone larvae (Chapter 1) however it remained untested for the native sea urchin *P. lividus* until now (Chapter 3).

5.1.4 Weaning and on-growing

Farm operators are faced with limited options when it comes to weaning and on-growing of juvenile invertebrates. This represents a barrier to increased production with a distinct lack of suitable feeds available to the industry (Chapter 1 and 4). Chapter 1 and O'Mahoney *et al*., (2014) highlighted a niche for a feed producer to develop feeds for abalone that only contain components existent in their natural diet.

Abalone are algivorous. Therefore animal protein, naturally non-existent in their diet, causes the animal to compensate for its inclusion in artificial diets by increasing trypsin activity (Johnston *et al*., 2005). Chapter 4 investigated the use of a macroalgal meal based diet enriched with bioavailable phosphorus for use in the Irish abalone industry. Phosphorus was identified as a limiting component in the diets of abalone (Tan *et al*., 2002a; Coote *et al*., 1996; Sales *et al*., 2003).

5.2 To develop new culture methods of the commercially important encrusting macroalgae *Ulvella lens* for the Irish and European sea urchin and abalone industry;

Unreliable and inconsistent settlement of larval invertebrates (Outlined in Chapters 1 and 2) for commercial growers is experienced in the absence of uptake of industry advice from commercial research in producing countries. *U. lens* was identified in Chapter 1 as a recommendation for increased juvenile abalone production in Ireland.

It is unclear why the use of *U. lens* in abalone and sea urchin culture was not established earlier in Ireland. One reason could be lack of available stock cultures to industry and a lack of dissemination of culture methods. *U. lens* has been used as the industry standard for the cultivation of abalone and sea urchins in Japan since the 1980s (Ohshiro *et al*., 1999; Takahashi and Koganezawa, 1988). *U. lens* was transferred into the Australian abalone industry which has since experienced increased production due to its implementation and integration into abalone aquaculture (Daume, 2007; Dyck *et al*., 2011).

The most available and cited method developed by Takahashi and Koganezawa, (1988) was the first account of a repeatable method of mass sporulation of this green encrusting macroalgae. However, the method developed by these authors does not integrate well into production conditions here in Ireland, due primarily to inherent variability of atmospheric conditions and the requirement for long periods of controlled photoperiod and temperature (Chapter 2). Furthermore the method developed by Takahashi and Koganezawa, (1988), also requires long periods of downtime for settlement equipment and facilities (up to 30 days) during the dark phase of the culture cycle (Chapter 2).

This long dark phase period identified the need for a shorter culture cycle that would allow for less downtime of important spat production facilities and equipment.
The method and findings developed as part of this thesis in Chapter 2 removed the need for a 14-day dark period resulting in a time saving in total of 15 days. This time saving if needed, could allow a farm operator to run two successful sporulation cycles of adult sporophytes in the same time taken to run Takahashi and Koganezawas, (1988) method once. A succession of failed sporulation events could result in delayed spat production and consequent increased cost for farm operators.

A timesaving for a commercial grower also generates economic savings, as there is a greater utilisation of important settlement facilities. *U. lens* can induce high numbers of veliger larvae to settle, in some cases up to 90% (Daume, 2003). This allows for more juveniles to be produced without having to increase the size of settlement facilities (Chapter 2 and 3).

The identification of *U. lens* in Chapter 1 as an important settlement inducer for the Irish abalone industry and the development of repeatable timesaving method in Chapter 2 for its culture is an advancement for the industry. This is a key industry constraint identified and resolved using available information and technology from producing countries, the application of a newly developed culture method for *U. lens* resulted in its direct uptake and implementation by industry here in Ireland (Chapter 2).

Conducting research on site within commercial aquaculture enterprises introduces limitations: commercial imperatives preclude expensive empirical investigation. Because such experimentation has not been undertaken by industry a new method for the production of *U. lens* remained to be identified. Preliminary results showed contamination of diatoms and unwanted algae in initial large-scale cultures, reducing the effectiveness of the sporulation and also the adhesion of newly settled germlings. Overcoming these limitations within industrial settings ensured greater credibility and assured the commercial enterprises of the effectiveness of the newly developed method. The application of *U. lens* as a settlement cue for abalone represents a significant technological advance if implemented by the aquaculture industry and a prime example of technology transfer providing solutions from one species to another.

5.3 To investigate the efficacy of *U. lens* as a settlement cue for *P. lividus* with the aim of industry implementation and application;

P. lividus, the edible purple sea urchin is a valuable commercially harvested invertebrate in Ireland. Due to the decline of harvestable stocks, focus turned to the culture of *P. lividus* to fill demand from the traditional markets of London and Paris (Watson *et al*., 2004). Both of these markets favour this species of urchin for its high quality roe (Cook and Kelly, 2007).

The first commercial sea urchin hatchery in Europe, Dunmanus Seafoods Ltd. in West Cork commercialised the production of juvenile *P. lividus*. Little information exists on preferred settlement substrates for *P. lividus* when compared to abalone culture. Both abalone and sea urchins have similar production constraints during settlement and on-growing. They also share preferences to commercially used settlement cues such as diatoms and *U. lens* (Lawrence, 2007; Ohshiro *et al*., 1999; Takahashi and Koganezawa, 1988).

Inconsistent and low settled numbers of post larval *P. lividus* experienced by hatcheries, prompted this investigation into the use of *U. lens* as a settlement cue and initial food source for the *P. lividus* industry (Chapter 3). *U. lens* has been applied successfully for other species of urchins such as *S. intermedius* (Takahashi *et al*., 2002), however until now remained untested for *P. lividus* (Chapter 3).

Newly developed culture methods for *U. lens* (Chapter 2) were applied to commercial scale production of juvenile *P. lividus* and were examined on an experimental scale to establish settlement rates over 48 hours (Chapter 3). Competent *P. lividus* larvae were induced to settle using *U. lens* coated on P.V.C plates, the diatom species *Amphora* spp. was used for comparison. When urchin larvae were given a choice, 3 times as many larvae settled on *U. lens* than *Amphora* spp. *U. lens* successfully induced on average 50% settlement and metamorphosis over all the replicates. This is an important result for farm operators as settlement numbers can be increased through implementation of just one change in juvenile production procedures. This one change can also increase productivity without having to increase the amount of settlement facilities through inducing more urchin larvae to settle (Chapter 3).

Prior to this research *U. lens* had never been utilised as a settlement cue for *P. lividus*. The valuable research presented in Chapter 3 highlights not only the successful transfer of technology from one growing region to another, but also from one invertebrate species to another.

The paucity of information on the preferred settlement cues and substrates for *P. lividus* was itself a limitation to progress. *U. lens* is known to induce settlement in other sea urchin larvae however its efficacy remained untested for our native species. Initial issues with the experiment were the competency and size of the larvae in each replicate which required the experiment to be run a second time. This problem was overcome by grading the larvae prior to selection through a 450µm sieve removing less competent larvae.

The results achieved in Chapter 3 could be progressed further through experimentation on a larger scale and investigation of post settlement survival and growth. The implementation of *U. lens* at Dunmanus Seafoods Ltd. has increased the production of this valuable echinoderm without having to increase farm size and overhead costs which are high for any new venture within this industry. Given that, the application of the techniques developed in this thesis offers other small-medium producers low cost expansion options during a time of economic growth following austerity in Ireland and Europe.

5.4 To investigate the development of a novel macroalgal-meal feed for *H. tuberculata* and the effect of bioavailable phosphorus enrichment.

The feeding transitions of the abalone have been well documented with the intensification of commercial aquaculture (Pang *et al*., 2006; Takami, 2003). This intensification of abalone aquaculture led to the development of formulated feeds that in some cases contain components that have been shown to increase growth (Gómez-Montes *et al*., 2003). However some of these components are derived from sources that do not form part of the natural diet of abalone (Naidoo *et al*., 2006).

Through investigation of production constraints in Chapter 1, a mixed macroalgal-meal diet was identified as a niche for a European feed producer to develop a weaning and on-growing diet for *H. tuberculata* & *H. discus hannai.* O'Mahoney *et al*. (2014) also identified the need for a mixed macroalgal-meal diet for the abalone industry in Europe.

The macroalgal-meal diet identified in Chapter 1 would contain only components found in the abalone's natural diet. As the main component of the macroalgal-meal diet was macroalgae, it could be transferred to the culture of sea urchins also, as they also exhibit an algivorous predisposition. Chapter 4 investigated differing levels of bioavailable phosphorus on the growth and survival of juvenile *H. tuberculata* based on production barriers identified in Chapter 1.

Juvenile *H. tuberculata* responded to all treatment with differing levels of available phosphorous. The basal (0.15% phosphorus) diet, an un-enriched mixed macroalgal-meal diet produced a higher growth than the abalone fed *P. palmata* (0.19% phosphorus) solely. The highest growth rates were measured on the 0.9 and 1.5% available phosphorus with 0.9% phosphorus producing the highest growth rate over all. The findings from chapter 5 show that juvenile *H. tuberculata* respond well to increased levels of bioavailable phosphorus.

Further research is required to increase the stability of the experimental diets in water, as this will allow the animals greater time to feed before the feed stability is lost. Feed stability was seen as a limitation to this study, however the animals responded well to the treatments. As a range for bioavailable phosphorus has been identified for *H. tuberculata* further research on a larger scale could be envisaged possibly investigating different shapes and sizes of diets.

As abalone feed via a rasping motion of their radula providing an edge or simulate the form of *Laminaria* spp. may allow for greater feed intake. Predominantly the feeds for abalone are pelleted; i.e. one pellet feeds one abalone. Increasing the surface area of the diet may allow for numerous animals to feed at the one time as they do when fed long strips of macroalgae. This research can be carried out for more than one species of invertebrate as abalone and sea urchins also share both the same preferences to macroalgae and the same problems when fed pelleted food.

5.5 Limitations to the study and future investigation

Some of the Chapters share the same limitations as the research was conducted onsite with commercial aquaculture enterprises. The main limitations to the research questions presented in Chapter 1 was the lack of production information from state and regulatory bodies in Ireland and Europe. As funding for abalone enterprises is broken up into two sources (BIM and Údarás na Gaeltachta) it was not possible to attain information on the total amount invested into the industry in Ireland. Information on available markets and routes to market for producers was also difficult to procure. The importance of the production constraints identified in Chapter 1 identified and resolved the first research question. There was also a distinct lack of information on routes to markets for Irish producers or even what markets existed for farmed abalone from Ireland. This in particular needs to be identified to allow for an expansion of the industry. The information gathered on production barriers identified the need for further investigation with each of the research chapters developed to address these research objectives.

Future investigations would require an economic examination of the industry to identify possible routes to market for the animals produced by the industry, both market sized and juveniles. These are two avenues of investigation needed for the expansion of the industry. Firstly identification of markets for animals should occur prior to production, rather than producing the animals and subsequently finding that the market is unattainable, or that the market price is below the production cost. Secondly the implementation and dissemination of industry advice (outlined in Chapter 1) is imperative to alleviate production barriers hampering both the Irish and European abalone industries.

Poor uptake of the application of *U. lens* by commercial invertebrate aquaculture operators (Chapter 2) is directly linked to firstly; the availability of information on the culture of *U. lens* and secondly; the availability of stock cultures in Europe. As this study was embarked upon in conjunction with the abalone and sea urchin industry this also brought its own range of difficulties. Most significant was the low transfer of knowledge between commercial operations in Ireland.

By making small changes to their techniques, it was possible to increase the potential for increased production. These gaps in knowledge had previously limited the expansion of this industry both in Ireland and Europe.

Further investigation could be conducted on extracting the chemical cue from *U. lens* that induces settlement for the aquaculture industry as a whole. This process would need to identify a material that inhibits *U. lens* germlings adhering to its surface allowing the macroalgae to be harvested. Adoption of *U. lens* in the wider European industry could increase production that in turn would increase supply of abalone and sea urchin spat from Ireland.

Limitations were also experienced in Chapter 3, mainly due to lack of available information on the effectiveness of *U. lens* on the settlement of *P. lividus*. The experimentation in Chapter 3, along with that of the other chapters, was conducted onsite at aquaculture enterprises. Due to the commercial nature of different companies, the culture of *U. lens* needed to be proven prior to implementation on both an experimental and large-scale basis. As part of Chapter 2 an investigation took place to assure the enterprise of the efficacy of *U. lens* as an induction cue prior to application in Chapter 3.

A further limitation in Chapter 3 that also has a possibility of future research is the investigation of the effect of *U. lens* on the post settlement survival and growth of postlarval *P. lividus*. Due to time constraints and financial pressures it was not possible to investigate at that time. Proposed future research would include a larger scale trial to investigate growth and survival post settlement, and to establish when *U. lens* becomes limiting on growth of *P. lividus* spat.

The trial conducted in Chapter 4 also experienced technical challenges; primarily the stability of the formulated feeds in water. In the current formulation the diets are neither directly transferable to industry nor suitable at the moment. Further research is needed on increasing the available phosphorus content through the inclusion of different macroalgae rather than the inclusion of monobasic potassium phosphate or other bioavailable compounds. Continued investigation is also required on the feed binder to increase the longevity and stability in water.

Future investigation would ideally trial the diets in a form with greater longevity on both abalone and sea urchins as a weaning and on-growing diet. Differing shapes and sizes of the feeds would also be tested. Predominantly feeds are in a pelleted form which in some cases do not suit either abalone or sea urchins, which favor flat surfaces with an edge, ideal replicating the shape and form of their natural diet of macroalgae.

5.6 Conclusion

The abalone and sea urchin industry is growing with the establishment of new hatcheries, farms and species around Europe. This increase of industry activity requires greater numbers of spat of both abalone and sea urchins. Irish growers are in a prime position to capitalise on this increase in demand. Similar production methods and predispositions between abalone and sea urchins allows for their integration into a production system, without changing existing equipment.

The importance of the research conducted as part of this thesis has also developed tools for the industry to overcome production barriers. Firstly, there are now cultures of *U. lens* in Europe available to the industry. Secondly; there is a robust method for the culture of this settlement cue for the industry. The development of novel macroalgal feeds may facilitate increased growth without the use of animal proteins.

This original research has had a measurable effect on commercial enterprises in Ireland (Chapters 2 and 3). Production barriers have been overcome through the direct application of research and technology transfer from producing regions, rather than by perpetuating a trial and error approach. Production constraints identified in the Irish invertebrate industry are also similar to those experienced in the European industry. Overcoming production barriers and identifying methods of increasing production like the application of *U. lens* to the Irish invertebrate aquaculture industry is a prime example of willingness of the industry to adopt and implement advice form research once disseminated.

References

Agatsuma, Y., Seki, T., Kurata, K., Taniguchi, K., 2006. Instantaneous effect of dibromomethane on metamorphosis of larvae of the sea urchins *Strongylocentrotus nudus* and *Strongylocentrotus intermedius*. *Aquaculture*, 251(2–4), pp.549–557.

Andrew, N.L., Agatsuma, Y., Ballesteros, E., Bazhin, A.G., Creaser, E.P., Barnes, D.K.A., Botsford, L.W., Bradbury, A., Campbell, A., Dixon, J.D., 1995. Status and management of world sea urchin fisheries. Ocean. Mar Biol Annu Rev 40, 343–425.

Bautista-Teruel, M.N., Fermin, A.C. & Koshio, S.S., 2003. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: animal and plant protein sources. *Aquaculture*, 219(1-4), pp.645–653.

Bautista-Teruel, M.N., Koshio, S.S. & Ishikawa, M., 2011. Diet development and evaluation for juvenile abalone, *Haliotis asinina* Linne: Lipid and essential fatty acid levels. *Aquaculture*, 312:172–179

Browne, R., Deegan, B., Watson, L., Mac Giolla Bhríde, D., Norman, M., Ó'Cinnéide, M., Jackson, D., O'Carroll, T., 2008. The Status of Irish Aquaculture 2007. *Report compiled by MERC Consultants*, p.144.

Byrne, M., 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Mar. Biol. 104, 275–289. doi:10.1007/BF01313269

Cenni, F., Parisi, G., Scapini, F., Gherardi, F., 2010. Sheltering behaviour of the abalone, *Haliotis tuberculata* L., in artificial and natural seawater: The role of calcium. *Aquaculture*, 299(1-4), pp.67–72.

Chaitanawisuti, N., Sungsirin, T. & Piyatiratitivorakul, S., 2010. Effects of dietary calcium and phosphorus supplementation on the growth performance of juvenile spotted babylon & *Babylonia areolata*; culture in a recirculating culture system. *Aquaculture International*, 18(3), pp.303–313.

Chao, W.-R., Huang, C.-Y. & Sheen, S.-S., 2010. Development of formulated diet for post-larval abalone, *Haliotis diversicolor supertexta*. *Aquaculture*, 307(1-2), pp.89–94.

Cook, E.J., Hughes, A.D., Orr, H., Kelly, M.S., Black, K.D., 2007. Influence of dietary protein on essential fatty acids in the gonadal tissue of the sea urchins *Psammechinus miliaris* and *Paracentrotus lividus* (Echinodermata). Aquaculture 273, 586–594. doi:10.1016/j.aquaculture.2007.10.032

Cook, E.J., Kelly, M.S., 2007. Enhanced production of the sea urchin *Paracentrotus lividus* in integrated open-water cultivation with Atlantic Salmon *Salmo salar*. Aquaculture 273, 573–585. doi:10.1016/j.aquaculture.2007.10.038

Cook, P., A., & Gordon, R., H., 2010. World Abalone Supply, Markets, and Pricing. *Journal of Shellfish Research*, 29(3), pp.569–571.

Cook, P., A., 2008. The international abalone industry - Historical perspectives and future prospects. *Journal of Shellfish Research*, 27(4), p.999.

Cook, P.A., 2014. The Worldwide Abalone Industry. Mod. Econ. 05, 1181–1186. doi:10.4236/me.2014.513110

Coote, T.A. Hone, P.W., Kenyon, R., Maguire, G.B., 1996. The effect of different combinations of dietary calcium and phosphorus on the growth of juvenile *Haliotis laevigata*. *Aquaculture*, 145(1–4), pp.267–279.

Courtois De Viçose, G., Viera, M., Bilbao, A., Izquierdo, M., 2010. Larval Settlement of *Haliotis tuberculata Coccinea* in Response to Different Inductive Cues and the Effect of Larval Density on Settlement, Early Growth, and Survival. J. Shellfish Res. 29, 587–591. doi:10.2983/035.029.0306

Courtois de Viçose, G., Porta, A., Viera, M.P., Fernández-Palacios, H., Izquierdo, M.S., 2012. Improving nursery performances of *Haliotis tuberculata coccinea*: Nutritional value of four species of benthic diatoms and green macroalgae germlings. *Aquaculture*, 334–337(0), pp.124–131.

Dale, Trine, Siikavuopio, Sten I. Aas, Kare., 2009. Roe enhancement in sea urchin: effects of handling during harvest and transport on mortality and gonad growth in *Strongylocentrotus droebachiensis*. J. Shellfish Res. 1235–1239. doi:10.2983/0730-8000(2005)24[1235:REISUE]2.0.CO;2

Daume, S., Brand-Gardner, S., Woelkerling, W., 1999. Preferential settlement of abalone larvae: diatom films vs. non-geniculate coralline red algae. Aquaculture 174, 243–254. doi:10.1016/S0044-8486(99)00003-4

Daume, S. & Ryan, S., 2004. Nursery culture of the abalone *Haliotis laevigata*: larval settlement and juvenile production using cultured algae or formulated feed. J Shellfish Res 23:1019–1026

Daume, S., 2003. Early life history of abalone (*Haliotis rubra, H. laevigata*) : settlement, survival, and early growth, Perth, W.A.: Dept. of Fisheries, p 110.

Daume, S., 2006. The role of bacteria and micro and macroalgae in abalone aquaculture: a review *Journal of Shellfish Research*, 25(1), pp.151-157.

Daume, S., 2007. Improvement and evaluation of greenlip abalone hatchery and nursery production: final FRDC report—Project 2003/203. Fisheries Research and Development Corporation, Department of Fisheries, North Beach, p 160

Daume, S., Davidson, M., Ryan and, S., Parker, F., 2007. Comparisons of rearing systems based on algae or formulated feed for juvenile greenlip abalone (*Haliotis laevigata*). *Journal of Shellfish Research*, 26(3), pp.729–735.

Daume, S., Huchette, S., Ryan, S., Day, R. W., 2004. Nursery culture of *Haliotis rubra*: the effect of cultured algae and larval density on settlement and juvenile production. *Aquaculture*, 236(1-4), pp.221–239.

Daume, S., Krsinich, A., Farrell, S., Gervis, M., 2000. Settlement, early growth and survival of *Haliotis rubra* in response to different algal species. *Journal of Applied Phycology*, 12(3), pp.479–488.

Daume, S., Long, B. & Crouch, P., 2003. Changes in amino acid content of an algal feed species (*Navicula* Spp.) and their effect on growth and survival of juvenile abalone (*Haliotis rubra*). *Journal of applied phycology*, 15(2), pp.201–207.

De La Uz, S., Carrasco, J.F., Rodriguez, C., Anadon, N., 2013. Metamorphosis, growth and survival of early juveniles of *Paracentrotus lividus* (Echinodermata: Echinoidea): Effects of larval diet and settlement inducers. Cah. Biol. Mar. 54, 691–695.

Dyck, M., Roberts, R., & Jeffs, A., 2011. Assessing alternative grazing-tolerant algae for nursery culture of abalone, *Haliotis iris*. *Aquaculture*, pp.62–68.

Edwards, M. & Watson, L., 2011. Cultivating *Laminaria digitata* - Aquaculture Explained No. 26. Irish Sea fisheries Board, (26), p.72.

FAO, 2004. Fishery and Aquaculture Country Profiles, Ireland. Available at: http://www.fao.org/fishery/countrysector/FI-CP_IE/en.

FAO, 2007. Fisheries and Aquaculture Dept., 2007. *The state of world fisheries and aquaculture, 2006*, Rome: Food and Agriculture Organization of the United Nations.

FAO, 2010. The State of World Fisheries and Aquaculture. *Aquaculture*, (February), p.197.

Fernández-Boán, M., Fernández, L., Freire, J., 2012. History and management strategies of the sea urchin Paracentrotus lividus fishery in Galicia (NW Spain). Ocean Coast. Manag. 69, 265–272. doi:10.1016/j.ocecoaman.2012.07.032

Fernandez, C., Boudouresque, C.F. & others, 1998. Evaluating artificial diets for small *Paracentrotus lividus* (Echinodermata: Echinoidea). *Echinoderms: San Francisco.* Balkema, Rotterdam, pp.651–656.

Fleming, A.E. & Hone, P.W., 1996. Abalone aquaculture. *Aquaculture*, 140(1-2), pp.1–4.

Gallardo, W.G. & Buen, S.M.A., 2003. Evaluation of mucus, *Navicula*, and mixed diatoms as larval settlement inducers for the tropical abalone *Haliotis asinina*. *Aquaculture*, 221(1-4), pp.357–364.

Gallardo, W.G., Bautista-Teruel, M.N., Fermin, A.C., Marte, C.L., 2003. Shell marking by artificial feeding of the tropical abalone *Haliotis asinina* L. juveniles for sea ranching and stock enhancement. *Aquaculture Research*, 34(10), pp.839–842.

Gavine, F.M., Doroudi, M., Ingram, B.A., Victoria, F., 2009. Development of management strategies for herpes-like virus infection in abalone. Fisheries Victoria.

Gómez-Montes, L., García-Esquivel, Z., D'Abramo, L.R., Shimada, A., Vásquez-Peláez, C., Viana, M.T.,2003. Effect of dietary protein: energy ratio on intake, growth and metabolism of juvenile green abalone *Haliotis fulgens*. *Aquaculture*, 220(1-4), pp.769–780.

Gordon, H R & Cook, P A, 2004. World abalone fisheries and aquaculture update: supply and market dynamics. *Journal of Shellfish Research*, 23(4), pp.935–939.

Gordon, N., Neori, A., Shpigel, M., Lee, J., Harpaz, S., 2006. Effect of diatom diets on growth and survival of the abalone *Haliotis discus hannai* postlarvae. *Aquaculture*, 252(2-4), pp.225–233.

Gordon, R., H., & Cook, P., A., 2001. World abalone supply, markets and pricing: Historical, current and future. *Journal of Shellfish Research*, 20(2), pp.567–570.

Gosselin, P., Jangoux, M., 1996. Induction of metamorphosis in *Paracentrotus lividus* larvae (Echinodermata, Echinoidea). Oceanol. Acta 19, 293–296.

Guillard, R.R., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. Can. J. Microbiol. 8, 229–239.

Guzmán, J.M. & Viana, M.T., 1998. Growth of abalone *Haliotis fulgens* fed diets with and without fishmeal, compared to a commercial diet. *Aquaculture*, 165(3-4), pp.321–331.

Hahn, K.O., 1989. Handbook of Culture of Abalone and other Marine Gastropods, CRC Press, Boca Raton, Florida,: CRC Press.

Hannon, C., Officer, R.A. & Dorven, J.L., 2013. Review of the technical challenges facing aquaculture of the European abalone *Haliotis tuberculata* in Ireland. *Aquaculture International*, 21(2), pp.243–254.

Hannon, C., Officer, R.A., Dorven, J.L., Chamberlain, J., 2014. Culture methods of live algal feeds for European aquaculture: optimising culture conditions for *Ulvella lens*. Aquac. Int. 22, 1813–1822. doi:10.1007/s10499-014-9784-4

Heffernan, M.L., 1999. A review of the ecological implications of mariculture and intertidal harvesting in Ireland. (Manual No. 7). Dúchas, The Heritage Service, Department of Arts, Heritage, Gaeltacht and the Islands, Dublin, Ireland.

Hu, C., Xu, Y., Wen, J., Zhang, L., Fan, S., Su, T., 2010. Larval development and juvenile growth of the sea cucumber *Stichopus* Spp.(Curry fish). Aquaculture 300, 73–79.

Huchette, S. M., Soulard, J. P., Koh, C. S., Day, R. W., 2004. Maternal variability in the blacklip abalone, *Haliotis rubra Leach* (Mollusca: Gastropoda): effect of egg size on fertilisation success. *Aquaculture*, 231(1-4), pp.181–195.

Huchette, S., 2010. Commercial Abalone Farming: Trial and error, Presentation from the Symposium on Sustainable Development of European SMEs Engaged in Abalone Aquaculture, October 2010, Aber-Wrac'h, Western Brittany.

Huchette, S.M.H., & Clavier, J., 2004. Status of the ormer (*Haliotis tuberculata* L.) industry in Europe. *Journal of Shellfish Research*, 23(4), pp.951–956.

Huchette, S.M.H., Koh, C. & Day, R.W., 2003. The effects of density on the behaviour and growth of juvenile blacklip abalone (*Haliotis rubra*). *Aquaculture International*, 11(5), pp.411–428.

Hur, S., Hunter, M., Hebert, K., Botsford, L., Johnson, C., Einarsson, S., Campbell, A., Ballesteros, E., Andrew, N., Gerring, P., Dixon, J., Bazhin, A., Juinio-Me, M., Barnes, D., Creaser, E., Bradbury, A., Agatsuma, Y., Kalvass, P., Miller, R., Moreno, C., Palleiro, J., Rivas, D., Robinson, S., Schroeter, S., Steneck, R., Vadas, R., Woodby, D., Xiaoqi, Z., 2002. Status and Management of World Sea Urchin Fisheries, in: Atkinson, R., Barnes, M. (Eds.), Oceanography and Marine Biology, An Annual Review, Volume 40. CRC Press, pp. 343–425.

Johnston, D., Moltschaniwskyj, N. & Wells, J., 2005. Development of the radula and digestive system of juvenile blacklip abalone (*Haliotis rubra*): potential factors responsible for variable weaning success on artificial diets. *Aquaculture*, 250(1-2), pp.341–355.

Kitamura, H., Kitahara, S., Koh, H.B., 1993. The induction of larval settlement and metamorphosis of two sea urchins, *Pseudocentrotus depressus* and *Anthocidaris crassispina*, by free fatty acids extracted from the coralline red alga Corallina pilulifera. Mar. Biol. 115, 387–392. doi:10.1007/BF00349836

Kawamura, T., Saido, T., Takami, H., Yamashita, Y., 1995. Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. *Journal of Experimental Marine Biology and Ecology*, 194(2), pp.189–199.

Kawamura, T., Roberts, R.D. & Nicholson, C.M., 1998. Factors affecting the food value of diatom strains for post-larval abalone *Haliotis iris*. *Aquaculture*, 160(1-2), pp.81–88.

Kawamura, T., Roberts, R.D. & Yamashita, Y., 2001. Radula development in abalone *Haliotis discus hannai* from larva to adult in relation to feeding transitions. *Fisheries Science*, 67(4), pp.596–605.

Kelly, M.S. & Owen, P.V., 2002. Growth of the abalone *Haliotis tuberculata* L. at Scottish sea temperatures. *Aquaculture Research*, 33(9), pp.729–733.

Lawrence, J.M., Lawrence, J.M., 2014. Sea urchins biology and ecology. Elsevier.

Lawrence, J.M., 2007. Edible sea urchins biology and ecology. Elsevier, Amsterdam; Boston.

Legg, T., 2010. Developing marketing data for abalone in Europe, Presentation from the Symposium on Sustainable Development of European SMEs Engage_in Abalone Aquaculture, October 2010, Aber-Wrac'h, Western Brittany.

Leighton, P., 2008. Aquaculture explained: Abalone Hatchery Manual, Irish Sea Fisheries Board, No. 25, P. 95

Lobban, C.S. & Wynne, M.J., 1981. The Biology of Seaweeds, Blackwell Scientific publications, Oxford.

Mai, K., Mercer, J.P. & Donlon, J., 1995. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. III. Response of abalone to various levels of dietary lipid. *Aquaculture*, 134(1-2), pp.65–80.

Mai, K., Wu, G. & Zhu, W., 2001. Abalone, *Haliotis discus hannai* Ino, Can Synthesize Myo-Inositol De Novo to Meet Physiological Needs. *The Journal of Nutrition*, 131(11), pp.2898–2903.

Malham, S.K., Lacoste, A., Gelebart, F., Cueff, A., Poulet, S. A., 2003. Evidence for a direct link between stress and immunity in the mollusc *Haliotis tuberculata*. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, 295(2), pp.136–144.

Matsuura, H., Yazaki, I. & Okino, T., 2009. Induction of larval metamorphosis in the sea cucumber *Apostichopus japonicus* by neurotransmitters. *Fisheries Science*, 75(3), pp.777–783.

Mgaya, Y.D., 1995. Synopsis of biological data on the European abalone (Ormer), *Haliotis tuberculata* Linnaeus, 1758 (Gastropoda: Haliotidae). Food & Agriculture Org. No. 156

Mgaya, Y.D. & Mercer, J.P., 1995. The effects of size grading and stocking density on growth performance of juvenile abalone, *Haliotis tuberculata* Linnaeus. *Aquaculture*, 136(3-4), pp.297–312.

Morse, D.E., 1985. Neurotransmitter-mimetic inducers of larval settlement and metamorphosis. Bull. Mar. Sci. 37, 697–706.

Mos, B., Cowden, K.L., Nielsen, S.J., Dworjanyn, S.A., 2011. Do Cues Matter? Highly Inductive Settlement Cues Don't Ensure High Post-Settlement Survival in Sea Urchin Aquaculture. PLoS ONE 6, e28054. doi:10.1371/journal.pone.0028054

Naidoo, K., Maneveldt, G., Ruck, K., Bolton, J. J., 2006. A Comparison of Various Seaweed-Based Diets and Formulated Feed on Growth Rate of Abalone in a Land-Based Aquaculture System. *Journal of Applied Phycology*, 18(3-5), pp.437–443.

Nicolas, J.-L., Basuyaux, O., Mazurie, J., Thebault, A., 2002. *Vibrio carchariae*, a pathogen of the abalone *Haliotis tuberculata*. Dis. Aquat. Organ. 50, 35–43.

Nie, Z.Q., Ji, M.F. & Yan, J.P., 1996. Preliminary studies on increased survival and accelerated growth of overwintering juvenile abalone, *Haliotis discus hannai* Ino. *Aquaculture*, 140(1-2), pp.177–186.

Nielsen, R., 1977. Culture studies on *Ulvella lens* and *Ulvella setchellii*. *British Phycological Journal*, 12(1), pp.1–5.

O'Mahoney, M., Mouzakitis, G., Doyle, J., Burnell, G., 2011. A novel konjac glucomannan-xanthan gum binder for aquaculture feeds: the effect of binder configuration on formulated feed stability, feed palatability and growth performance of the Japanese abalone, *Haliotis discus hannai*. *Aquaculture Nutrition*, 17(4), pp.395–407.

O'Mahoney, M., Rice, O., Mouzakitis, G., Burnell, G., 2014. Towards sustainable feeds for abalone culture: Evaluating the use of mixed species seaweed meal in formulated feeds for the Japanese abalone, *Haliotis discus hannai*. Aquaculture 430, 9–16. doi:10.1016/j.aquaculture.2014.02.036

Ohshiro, T., Nakano, S., Takahashi, Y., Suzuki, M., Izumi, Y., 1999. Occurrence of bromoperoxidase in the marine green macro-alga, *Ulvella lens*, and emission of volatile brominated methane by the enzyme. *Phytochemistry*, 52(7), pp.1211–1215.

Olin, P. & McBride, S., 2000. Abalone culture. In: Stickney, R.R. (Ed.), Encyclopedia of Aquaculture. Wiley, New York, USA, pp. 1– 7

Onitsuka, T., Kawamura, T., Ohashi, S., Horii, T., Watanabe, Y., 2007. Dietary value of benthic diatoms for post‐larval abalone *Haliotis diversicolor* associated with feeding transitions. *Fisheries Science*, 73(2), pp.295–302.

Pang, S., Zhang, Z. H., Boa, Y., Gao, S. Q., 2006. Settling abalone veliger larvae in a free-swimming microalgal culture. *Aquaculture*, 258(1), pp.327–336.

Pearce, C.M., Scheibling, R.E., 1994. Induction of metamorphosis of larval echinoids (*Strongylocentrotus droebachiensis* and *Echinarachnius parma*) by potassium chloride (KCI). Invertebr. Reprod. Dev. 26, 213–220. doi:10.1080/07924259.1994.9672420

Pearce, C.M., Scheibling, R.E., 1991. Effect of macroalgae, microbial films, and conspecifics on the induction of metamorphosis of the green sea urchin *Strongylocentrotus droebachiensis* (Müller). J. Exp. Mar. Biol. Ecol. 147, 147–162. doi:10.1016/0022-0981(91)90179-Z

Pearce, C.M., Scheibling, R.E., 1990. Induction of Metamorphosis of Larvae of the Green Sea Urchin, *Strongylocentrotus droebachiensis*, by Coralline Red Algae. Biol. Bull. 179, 304–311. doi:10.2307/1542322

Qi-hua, W., Shu-hong, W., Ming-jin, D., Mei, L., Ruo-fu, S., Ai-hua Cheng, 1997. Studies on culture conditions of benthic diatoms for feeding abalone I. Effects of temperature and light intensity on growth rate. *Chinese Journal of Oceanology and Limnology*, 15(4), pp.296–303.

Rahim, S.A.K.A., Li, J.-Y. & Kitamura, H., 2004. Larval metamorphosis of the sea urchins, *Pseudocentrotus depressus* and *Anthocidaris crassispina* in response to microbial films. *Marine Biology*, 144(1), pp.71–78.

Roberst, R.D., Revsbech, N.P. & Damgaard, L.R., 2007. Effect water velocity and benthic diatom morphology on the water chemistry experienced by postlarval abalone. *Journal of Shellfish Research*, 26(3), pp.745–750.

Roberts, R.D., Kawamura, T. & Nicholson, C.M., 1999. Growth and survival of postlarval abalone (*Haliotis iris*) in relation to development and diatom diet. *Journal of Shellfish Research*, 18(1), pp.243–250.

Sales, J., Britz, P.J., Viljoen, J., 2003. Dietary phosphorus leaching and apparent phosphorus digestibility from different inorganic phosphorus sources for South African abalone (*Haliotis midae* L.). Aquac. Nutr. 9, 169–174.

Soler Vila, A., 2008. Bureaucracy and technology problems behind low European abalone production. *Inshore Ireland*, 4(5).

Stott, A., Takeuchi, T., Koike, Y., Yamakawa, H., Imada, O., 2002. Using micro particle diets to replace diatoms for feeding postlarval abalone *Haliotis discus discus* (Reeve.). *Fisheries Science*, 68(5), pp.1088–1093.

Stott, A.E., Takeuchi, T. & Koike, Y., 2004. An alternative culture system for the hatchery production of abalone without using livefood. *Aquaculture*, 236(1–4), pp.341–360.

Takahashi, K., Koganezawa, A., 1988. Mass culture of *Ulvella lens* as a feed for abalone *Haliotis discus hannai*. NOAA Tech. Rep. NMFS 70, 25–36.

Takahashi, Y., Itoh, K., Ishii, M., Suzuki, M., Itabashi, Y., 2002. Induction of larval settlement and metamorphosis of the sea urchin *Strongylocentrotus intermedius* by glycoglycerolipids from the green alga *Ulvella lens*. Mar. Biol. 140, 763–771. doi:10.1007/s00227-001-0749-6

Takami, H., 2003. Feeding Ecology of an Abalone, *Haliotis discus hannai*, in Their Early Life Stages. In *Aquaculture and pathobiology of crustacean and other species*. Davis and Santa Barbara, California U.S.A, p. 180.

Takami, H. & Kawamura, T., 2003. Dietary changes in the abalone, *Haliotis discus hannai*, and relationship with the development of the digestive organ. *Japan Agricultural Research Quarterly*, 37(2), pp.89–98.

Takami, H., Muraoka, D. & Kawamura, T., 2003. When is the abalone *Hailotis discus hannai* Ino 1953 first able to use brown macroalgae. *Journal of Shellfish Research*, 22(3), pp.795–800.

Tan, B., Mai, K. & Liufu, Z., 2001. Response of juvenile abalone, *Haliotis discus hannai*, to dietary calcium, phosphorus and calcium/phosphorus ratio. *Aquaculture*, 198(1–2), pp.141–158.

Tan, B., Mai, K. & Zhi-guo, L., 2002a. Dietary phosphorus requirement of young abalone *Haliotis discus hannai* Ino. *Chinese Journal of Oceanology and Limnology*, 20(1), pp.22–31.

Tan, B., Mai, K., & Wei, X., 2002b. Availability of phosphorus from selected inorganic phosphate to juvenile abalone, *Haliotis discus hannai* Ino. *Chinese Journal of Oceanology and Limnology*, 20(2), pp.118–128.

Viana, M.T., López, L.M., García-Esquivel, Z., Mendez, E., 1996. The use of silage made from fish and abalone viscera as an ingredient in abalone feed. *Aquaculture*, 140(1), pp.87–98.

Viana, M.T., Correa, G., Lazo, J. P., Frías-Díaz, R., Durazo-Beltrán, E., Vasquez-Pelaez, C., 2007. Digestive physiology and metabolism of green abalone *Haliotis fulgens* from postlarvae to juvenile, fed three different diatoms. *Aquaculture*, 271(1-4), pp.449–460.

Walsh, M. & Watson, L., 2011. A market analysis towards the further development of seaweed aquaculture in Ireland, Dublin: Irish Sea Fisheries Board.

Watson, L, Stokes, A., 2004. Seahorses to Sea Urchins: The Next Big Splash in Irish Aquaculture, Irish Sea Fisheries Board.

Werner, A. & Dring, M., 2011. Cultivating *Palmaria palmata*, Aquaculture explained, No. 27, Irish Sea Fisheries Board, 76.

Xing, R., Wang, C., Cao, X., Chang, Y., 2007. Settlement, growth and survival of abalone, *Haliotis discus hannai*, in response to eight monospecific benthic diatoms. J. Appl. Phycol. 20, pp.47–53.