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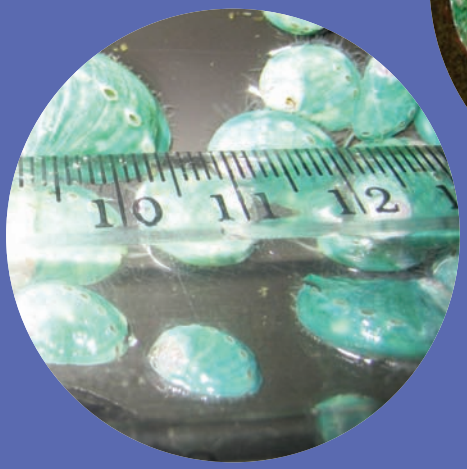
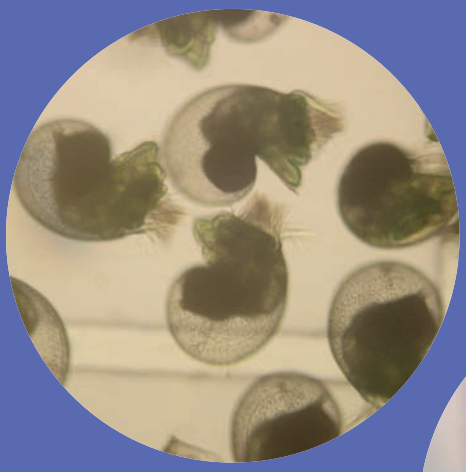
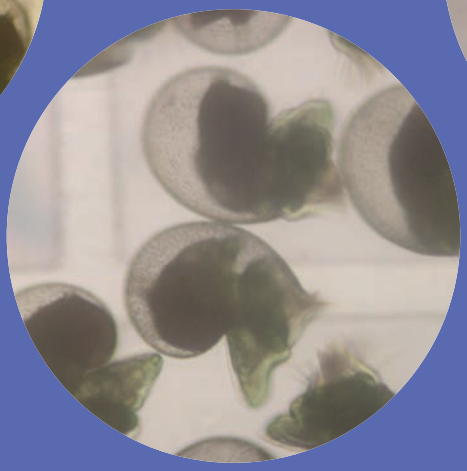
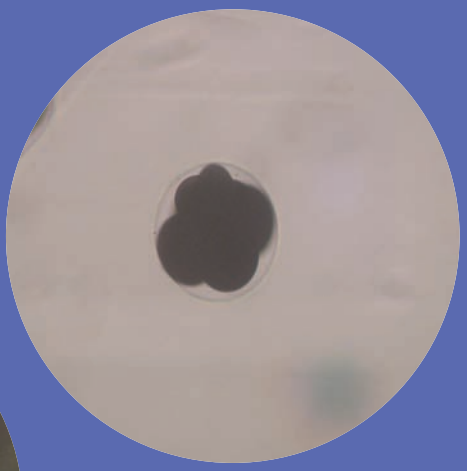
ABALONE HATCHERY MANUAL









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AQUACULTURE EXPLAINED

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ABALONE HATCHERY MANUAL

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Contents

Chapter 1: Introduction

1. 1. Background

Abalone are a very high value shellfish with prices of up 70€/kg commonplace. There are approximately 100 abalone species worldwide and of these about 25 are of commercial interest. Of these 25 approximately 16 species are cultivated, including one hybrid. Abalone culture is now practised in many countries including Japan, China, Taiwan, the United States, Mexico, Chile, Australia, New Zealand, Thailand, Korea, France and Ireland among others. The most important species commercially would be *Haliotis discus hannai, Haliotis diversicolor supertexta, Haliotis rufescens, Haliotis rubra, Haliotis laevigata, Haliotis iris, Haliotis australis and Haliotis midae.*

Most of the market demand for abalone is in Asia and from Asian ethnic communities in many of the larger conurbations throughout the world. As a result most commercial cultivation of abalone species is in Asia and abalone aquaculture and fishery harvests from other parts of the globe are to a large extent intended for these markets.

There are two abalone species currently being cultured in Ireland. These are the Japanese or Pacific abalone *Haliotis discus hannai* and the European abalone or Ormer, *Haliotis tuberculata.* Neither of these species is native to Irish waters, both being introduced to the country during the late 1970s and mid 1980's. *Haliotis tuberculata* is common in the Channel Islands and along the coast of Brittany and Normandy. It is also found in certain areas along the north coast of Spain. It was due to the similarity of the Irish coastline to those of the Channel Islands and Brittany that this species was introduced into quarantine at the Shellfish Research Laboratory, in Carna, Co. Galway during July 1976. These were conditioned and spawned and the resultant juveniles were given disease-free status in 1977. There were further importations during this period. Trials with this abalone were successful but the unavailability of large quantities of its preferred diet of dilisk or dulse (*Palmaria palmata*) at certain times of the year led to the introduction of the Japanese abalone *Haliotis discus hannai* during 1986. The preferred diet of this abalone is kelp and the Irish kelp *Laminaria digitata* is very similar to the Pacific kelp *Laminaria japonica*.

The Irish abalone industry has been slow to develop but in more recent years with the successful establishment of a number of commercial hatcheries around the south and west coasts, in addition to developments in land based recirculation technologies, there has been increased interest and investment in this sector. At the time of publishing there were four hatchery/production units and two ongrowing units. Most of the emphasis now in Ireland, is on the growout to market size being undertaken in land based recirculation

units. Unlike previous methods carried out at sea these technologies allow for accurate control over the environmental conditions being experienced by the abalone. In particular the ability to control temperature should considerably reduce the time taken to reach market size.

Total world abalone supply was 22,677 tonnes in 2002 (Gordon and Cook, 2004). This figure includes abalone supplied from fisheries, cultured abalone and an estimation of what is reaching the markets as a result of illegal fisheries or poaching. This figure for total abalone supply fluctuates only somewhat but the productivity from the supplying countries and the species can vary greatly. In 2002 Australia was the largest supplier contributing 6,224t of the total. This would be from both fishery and culture. China was the next biggest producer with 4,500t. Chinese production would be almost exclusively from culture. Production from China varies greatly. Disease has had a major impact here, at one stage production plummeted from 3,000t to 300t as a result of a viral infection. More recently production has again increased and much of this is due to culture of the hybrid pacific abalone. This abalone is a result of cross breeding Japanese *Haliotis discus discus* with Chinese *Haliotis discus hannai.* It is a very resilient abalone and grows faster than either of its parent species. Japan itself contributed 2,882t to the world supply and this was closely followed by South Africa, Mexico, New Zealand and Taiwan at 1,731t, 1,669t, 1,555t, 1,500t respectively. Taiwanese production is currently closer to 3,000t. There has been a marked trend in more recent years that the world supply is being made up of cultured abalone rather than those from fisheries. This is influenced by the closure of many major fisheries such as the U.S.A. and South Africa.

1. 2. General biology of the abalone

Abalone are marine gastropod molluscs and are considered to be amongst the most primitive group in this phyllum. At the other end of the molluscan scale are the highly evolved squid and octopus. Abalone are quite similar and closely related to limpets which are common in our intertidal areas. However abalone are, for the most part subblittoral, being found below the intertidal zone.

A characteristic of these gastropods are cleft or perforated shells in which a pair of gills are found. As in all molluscs there is a mantle cavity present, it being located directly under the shell pores. The gill cilia produce water currents in which the waste products from the gut and kidneys are removed via the cleft or slits. Abalone have a pair of gills but due to pronounced asymmetry of the shell the right gill is considerably smaller than the left. In the abalone the shell is generally flattened and there are a series of shell pores, normally 5-10, whose number varies with age and species. The pores develop as the shells grow and each one arises as a notch at the front margin of the shell and becomes sealed at the rear. In general the shell is flattened, has an asymmetrical spiral and a greatly enlarged body whorl creating a

very large aperture, allowing it to completely protect the muscular foot. The foot is the prized part of the abalone and is used in locomotion and feeding. It is a large flat creeping sole adapted for locomotion over a variety of substrata. It is heavily ciliated and has a dense concentration of mucous glands. The foot is highly muscular and capable of complex movement. This complexity of muscle movement and mucous secretions allow the abalone to glide across substrata.

At the anterior part of the foot is the head which is typically very snail like. The mouth is at the base of the head and has a scrapping organ or radula which is used for feeding. Between the shell and foot in the posterior part of the animal is the gonad which envelops the digestive gland or liver and together they form a large cone shaped appendage which is often referred to as the conical appendage.

Plate 1: Adult *Haliotis discus hannai* on feeding plate with *Laminaria digitata.*

Plate 2: Two *Haliotis tuberculata* broodstock on feeding plate

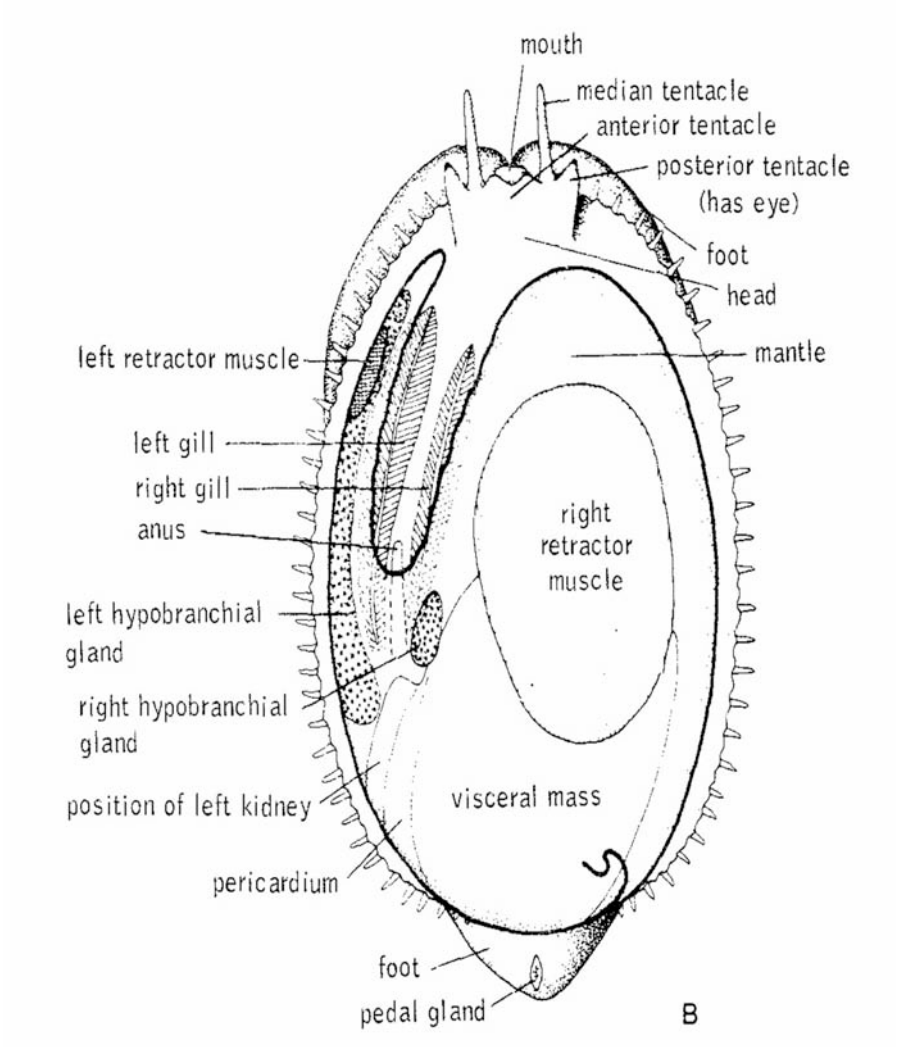


Figure 1. 1: Dorsal view of *Haliotis* with shell removed and mantle cavity exposed. From Barnes (1991).

Abalone are almost entirely algivores with various types of seaweed being their preferred diet. However, some non-vegetable foods such as hydrozoa, bryozoa, sponges and small crustaceans can occasionally be digested but these would be found on the seaweed. Newly settled juvenile abalone tend to feed on benthic diatoms and newly settled seaweed plantlets. The principal organs of feeding are the foot, the cephalic tentacles, the snout, the jaws and the radula. The foot is used to search for and hold the food and bring it to the mouth. For the most part abalone are considered to forage for food nocturnally or under the cover of darkness.

Abalone are dioecious, possessing either male or female reproductive organs. Sexing can be carried out by visual inspection if the foot and mantle are forced away from the right side of the shell to expose the conical appendage. Gonad colour is species dependant but in general, males tend to be from white to creamy beige in colour while the females tend to be darker, from dark green to greyish green to almost black. There is no sexual dimorphism of the shell structure enabling external differentiation.

It is thought that temperature and photoperiod are the two major factors influencing the development of the gonads. Spawning of the various species around the world coincides with local conditions. They are broadcast spawners with both male and female gametes released directly into the water column.

There is a tendency for males to release their gametes into the water column somewhat earlier than the females.

As abalone are molluscs the larval development cycle is similar to that of other familiar molluscs spawned in commercial hatcheries.

However the egg has an internal food supply provided by a yolk sac. Consequently the larvae do not need to feed on microalgae and as a result have a much shorter life cycle than say oysters, scallops or clams. As a result of the eggs being yolk laden they also tend to be relatively large. Typically an abalone ovum could be 180μm or more as compared to 60-90μm for typical bivalve larvae. In addition, there is a thick gelatinous coating surrounding the unfertilised eggs resulting in an overall diameter of 300-400μm.

After fertilisation the trochophore larva hatches out from the egg case. This stage is characterised by a band of cilia referred to as a protrochal girdle. This then develops into the veliger which is characterised by a heavily ciliated swimming band called the velum. During this stage the larvae undergo the first and more rapid stage of torsion whereby they rotate through 90˚. The second stage follows at a slower rate over the next few hours. A spiral shell and operculum have developed by this stage. By the end of the third day the larvae start to develop cilia on the sole of the foot and the first signs of settlement behaviour maybe observed. After 4-5 days (though much

more in some species) the benthic veliger stage is established and larvae can be seen making exploratory creeping movements associated with settlement.

1. 3. Principal abalone species of the world

Haliotis discus hannai
Known as Ezo awabi, the Pacific abalone or the Japanese abalone this is the most valuable abalone in terms of meat content. It is also the most studied and, until recently the most cultivated of abalone species. It is found from Siberia to China over to the western coast of Japan. It is considered a cold water species and can tolerate temperatures down to 5˚C for long periods of time. It has been cultured on a large scale in both Japan and China and has been imported into Chile, Iceland and Ireland among other countries as an aquaculture candidate. It has a dark shell with 5 or more raised pores. It is normally found at a water depth of 1m to 5m. It can grow to 20cm in length but is normally harvested at 9cm from the wild and cultured to between 5cm and 7cm.

Haliotis tuberculata
The European abalone or Ormer is the only commercial species in Europe. It is found around the Channel Islands south to the Normandy and Brittany coasts and along the Basque and Galician coasts of Northern Spain. It grows to a maximum shell length of 12cm and tends to be a brownish to pink colour. There are some sub-species found in the Mediterranean Sea, along the African coast and on the Canary Islands but these are smaller in size than *Haliotis tuberculata* and not of commercial value. They are currently being cultured in small quantities in France and Ireland and with a number of operations also being commenced in Spain.

Haliotis rufescens
The Red abalone is the largest abalone species worldwide and can grow almost 30cm in length and almost 2kg in weight. They have a very distinctive dull red shell colouration and are found along the west coast of the U.S. and into Mexico. They possess a high quality meat and are farmed intensively in both these regions. They grow rapidly and much research has been carried out on them. They are found from the intertidal to a depth of 20m.

Haliotis fulgens
The Green abalone, found between Santa Barbara and Baja California. It is quite a large abalone and there was an extensive fishery for them a few years ago. They have a particularly beautiful shell. Can grow up to 25cm shell length. They are found from intertidal to 20m depth.

Haliotis corrugata
The Pink, Corrugated or Yellow abalone. Again this is found in Southern California and was fished intensively in the past.

Haliotis cracherodii
The Black abalone is so called because of the dark coloured meat it possesses. Found from northern to southern California from the intertidal to a depth of about 6m. The shells tend to be quite smooth and it is thought that this if from their tendency to stack on each other and graze fouling from each others shells. They were not heavily fished due to the dark, unattractive colour of their meat. This abalone species was badly affected by Withering Foot Syndrome outbreaks in California.

Haliotis kamtschatkana
The Pinto abalone is found from Aleutian Islands in Alaska down to California. They are found intertidally in the northern part of their range and to a depth of 12m to 17m in the southern range. They were heavily fished and formed a large part of the diet of coastal native Indian tribes. Once thought to be very closely related to *H. d. hanna*i but not considered to be now.

Haliotis discus discus
Once called *Haliotis discus* the Kuro awabi or Black abalone is found around the coasts of Japan. It is a shallow water species being found most abundantly at 2m depth. While not being farmed in Japan it formed a large part of the Japanese wild fishery. The meat would be considered tougher than that of *H. d. hannai*.

Haliotis diversicolor supertexta
The Tokobushi is quite a small abalone with adults rarely exceeding 5cm. It is a warmer water species than many of the other Japanese species. While only making up a small portion of the fisheries catch it forms the major part of the massive Taiwanese abalone aquaculture industry.

Haliotis rubra
The Black-lip abalone is one of the three commercially important abalone species in Australia. It generally grows to about 10cm-15cm but some have been found up to 20cm. It has a high meat quality and very good growth rates.

Haliotis laevigata
The Green-lip abalone or mutton fish is the second most important species of Australian abalone. It grows to 12cm-14cm and has white flesh.

Haliotis asinina
The Donkey Eared abalone is found in northern and north western Australian coasts and also in the Philippines and Thailand. There is currently a large fishery for this species and trials into its culture are now being carried out. It is a small but very fast growing species that is used principally to supply the abalone cocktail market.

Haliotis iris

The Paua is the principal commercial species of New Zealand. It has a very iridescent shell that is strikingly beautiful. This species forms the bulk of the developing abalone aquaculture industry in New Zealand. There is also a significant jewellery and pearl industry associated with the shell. The meat is quite dark in colour and less valuable than that of other species unless it is put through a bleaching process.

Haliotis australis

The Yellow Foot Paua is the second commercial New Zealand species. It has a wide distribution around the coast and formed part of the fishery but is not cultured to any extent.

Haliotis midae

Perlemoen is the only commercial abalone found on the African continent. The populations are concentrated along the south west coast of Cape Province in South Africa. It can grow up to 20cm in shell length. It has a distinct white heavily ridged shell and was the basis of a large fishery in the region. It has been heavily poached and heavy restrictions have been placed on the fishery. South Africa has one of the most rapidly developing abalone industries worldwide.

Chapter 2: Conditioning of broodstock

2. 1. Introduction

In many countries involved in abalone production broodstock can be obtained readily from the wild. They can be dived for and brought to conditioning units in varying stages of ripeness. Conditioning period thereafter will vary depending on gonad state upon introduction into the conditioning unit and also the water parameters within the unit.

Unlike countries such as Japan, the U.S.A., Australia etc. in Ireland we are unable to bring our broodstock in from the wild. Instead our broodstock must be selected from stock being ongrown on the farm or from animals which have been selected specifically for this purpose. This is as a result of being unable to make fresh importations of broodstock due to recent tightening of legislation on fish movements in and out of the EU.

It is generally considered that temperature is the principal factor influencing gonad development in most abalone species. Food availability is also very important while the influence of photoperiod is limited.

2. 1. 1. Gonad index

The simplest way of assessing gonad development in abalones is by direct observation. This is carried out using a spatula or a blunt knife to prise back the mantle edge at the back right of the shell when the abalone is inverted. This gives the observer a visual or external gonad index as described below.

2. 1. 2. Visual or external gonad index

0 - Immature, sex indeterminate, digestive gland visible as grey brown mass.

1 - Sex determinable, gonad small, colouration, tip pointed.

2 - Gonad large, tip rounded but not swollen.

3 - Gonad very large, tip rounded and swollen, bulging at shell edge.

Using the above guideline on its own may not be totally reliable. While it should give a good general indication as to the reproductive condition of the animal it should be used in conjunction with data on conditioning temperature and also the number of days that the abalone has been exposed to such a temperature.

Conditioning Temperature	1,000°C - days	1,500°C - days
14°C	156	234
15°C	135	197
16°C	119	179
17°C	106	160
18°C	96	144
19°C	88	132
20°C	81	121
21°C	75	112

Table 2. 1. A guideline to conditioning times needed for *Haliotis discus hannai* in relation to water temperature.

2. 1. 3. Effective accumulative temperature

There has been much research on the conditioning of *Haliotis discus hannai*, in particular, over the years. This work established a Biological Zero Point (BZP) of 7.6˚C for this species. This is the theoretical minimum temperature at which gonad growth and development begins. Conditioning *H. d. hannai* at 18˚C is in effect contributing 10.4˚C (18 - 7.6) to actual gonad development. The difference between the biological zero and the water temperature is the effective temperature while the summation of this value during complete maturation is referred to as the Effective Accumulative Temperature (EAT). It is considered that *H. d. hannai* are fully mature at 1500˚C or more. This means that conditioning *H. d. hannai* at 18˚C would take a minimum of 144 days. However some of the abalone could be in condition before this period of time while conditioning for a longer period will yield better spawning results. Similarily using a 15˚C conditioning temperature would necessitate holding the abalone in the unit for 203 days while using 21˚C would involve conditioning for 112 days or about three and a half months.

Gonad maturation can be divided into three stages related to EAT for *Haliotis discus hannai:*

(a) Immature stage (0-500˚C-days) - the visual or external gonad index ranges from 0-3 as the gonad volume increases but spawning cannot be induced.

(b) Mature stage (500-1,500˚C-days) - the gonad index reaches 3. The spawning rate and quantity of gametes released rises with increasing EAT. Animals with an EAT between 1,000 to 1,500˚C-days are sufficiently ripe for hatchery production.

(c) Fully mature stage (>1,500˚C-days) - gonad development reaches its maximum level and there is a reliable high rate of spawning.

A general guideline as to the amount of conditioning time needed in relation to the water temperature for *H. d. hannai* is as shown in Table 2.1 above. As stated above these are guidelines. In any conditioning unit there are quite likely to be hiccups during this period. The temperature may drop for a while meaning that direct application or the EAT wont be entirely accurate. System malfunction or breakdown could also occur. In such circumstances a combination of the EAT and visual gonad index should be applied to ascertain correct spawning time.

N.B. In addition to the above factors it should also be considered that as *H. d. hannai* have been in Ireland for many years now, isolated from other populations, there is now the distinct possibility that their biological zero is no longer 7.6˚C.

Less work appears to have been carried out on the conditioning of *Haliotis tuberculata*. However French (Fleming, 2000) and Irish (Mercer, 1981) workers suggest that full condition (1,500˚C - days) can be achieved after 90 days at 18˚C. This would suggest that *H. tuberculata* has a lower biological zero point than *H. d. hannai*. In reality it does appear that *H. tuberculata* is easier to condition than *H. d. hannai* and this can be observed regularly on the ongrowing units where they can be seen in condition over the summer and indeed have often been observed to spawn in the ongrowing tanks. The BZP has only been calculated for about eight abalone species to date, including the Pacific Hybrid abalone.

2. 2. Selection of broodstock

The number of broodstock selected is determined by the projected seed output of the farm. If the farm is meeting just its own requirements, the number necessary may be quite small whereas if the farm is acting as a seed production unit for itself and other units, then more than likely many hundreds of broodstock would be required.

As mentioned already sources of broodstock are limited in Ireland. They must be selected from abalone being ongrown on the farm. A number of factors should be considered when selecting the broodstock.

They should be relatively large and fast growing animals. It is best to select young abalone between 50mm-70mm. In particular, younger females tend to produce healthier quality eggs. These animals should have a relatively thin shell free from much fouling. However record keeping of stock on the farm should indicate which abalone are the best performers.

It would also be advisable to choose a female to male ratio of 3 or 4:1. This is practised on most abalone farms due to the lower spawning response of the females making eggs the limiting factor.

The shells should be free of heavy fouling and in particular they should be free of Polydora infestation. Polydora is a mudworm which bores into the shell of the abalone. It causes pitting and blistering on the inside of the shell surface. Heavy infestation stresses the abalone, affecting their reproductive output and often killing them. There is also a risk of infecting abalone without worm infection in the conditioning unit.

2. 3. The conditioning unit

The specific aim of a conditioner is to provide fully gravid abalone for spawning purposes at particular times throughout the year. To achieve this, abalone must be supplied with a specific temperature regime and an abundance of high quality food. The conditioning unit is designed to do this and also to maintain high water quality during the process.
A wide variety of designs are used in different culture units throughout the world. Most farms tend to use flow-through designs while more recently there has been a trend towards complex recirculation conditioning systems.

2. 3. 1. A Basic conditioning unit

A very simple system which was successfully used to condition abalone at the Shellfish Research Laboratory in Carna consisted of a large sump of ca. 3,000 litres. A number of baskets were suspended directly into the sump. These contained shelters under which the abalone attached. A submersible Otter' type pump provided water circulation via spray bars through these baskets. Alternatively a series of air lifts could provide the same water circulation down through the basket. The system had a 1.5/2kW immersion heater used to maintain the temperature at ca. 18˚C. Ambient seawater entered the unit at about a litre per minute while it left the system via a stand pipe at the same rate.

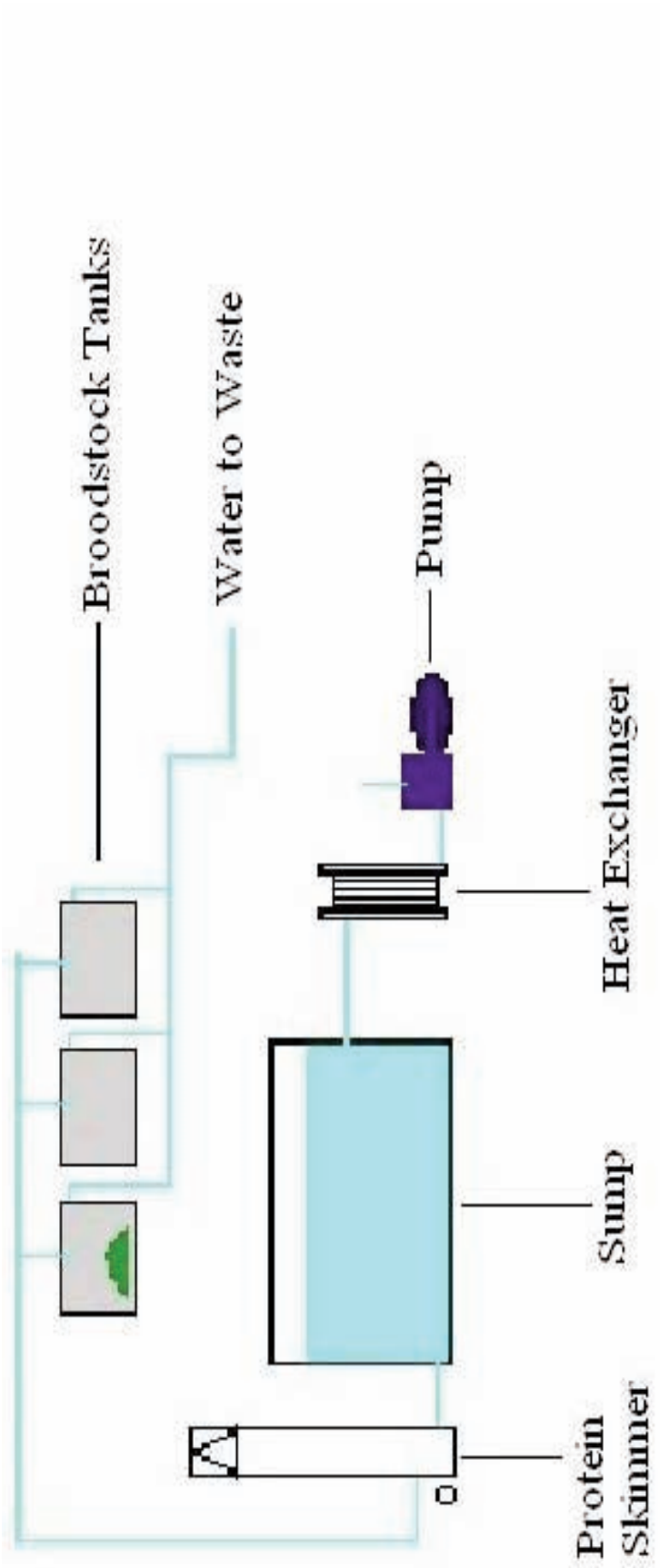


Figure 2.1: A flow to waste abalone broodstock conditioning unit. Not drawn to scale with only a few broodstock tanks shown.

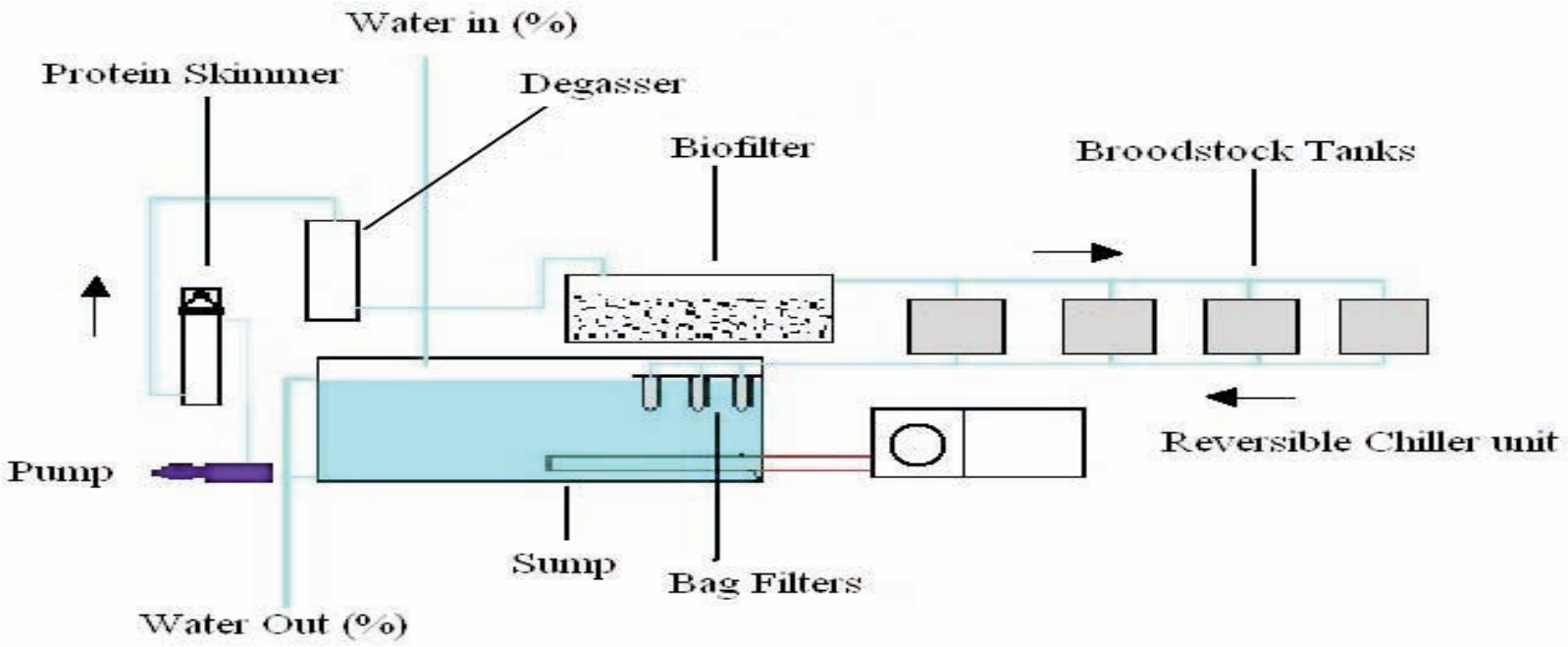


Figure 2. 2: A basic abalone recirculating broodstock conditioner.

This small flow through rate ensured that water quality was maintained at a high standard. This system could comfortably condition 50-60 large broodstock of either or both species simultaneously. As the waste from the baskets dropped to the sump/reservoir base it could be siphoned out. On occasions the baskets with broodstock inside could be moved to another tank with water at the same temperature while this tank was cleaned. This is a cheap and reliable method of conditioning abalone. The disadvantage of such a system is that mechanical failure e.g. pump breakdown or heater malfunction could result in mass spawning of broodstock, in particular if they are close to full maturity.

2. 3. 2. Flow through conditioning unit

The most common type of conditioner currently in use is a flow-through unit whereby ambient water is heated to the required temperature in a reservoir/sump (or in-line) before being delivered to conditioning containers with male or female broodstock present. The conditioning containers are generally 20-35 litre food grade plastic boxes. Each of these boxes holds 2-6 abalone this depending on the size of the animals. The quantity of boxes in the system will depend on the number of abalone to be conditioned. If 200 broodstock are being conditioned then at least 30-40 boxes will be required. One or two reservoirs with a capacity of 30-50% total system volume would be necessary. Water would be pumped from here to the individual boxes or alternatively it could be gravity fed if positioned above them.

Heating would be provided by an immersion heater in the reservoir or using a titanium in-line heater. There should be an exchange of 200ml-500ml per minute for each box. If there are 50 boxes this would necessitate an intake of 25 litres per minute of raw ambient seawater into the system.

Some hatcheries will have a protein skimmer or foam fractionator connected to the conditioning reservoir. This is to remove sediment or fine debris before it reaches the abalone in the conditioner. This is very much optional, being site dependant and also related to other filtration in use on the farm.

2.3.3 Recirculating conditioning units

Recirculating conditioning units are being constructed more frequently. They have many distinct advantages over flow-through units. They are cheaper to maintain than a flow-through system. This is mainly due to the fact that there should be less ambient seawater to be heated. Temperature can be maintained at a constant level very accurately. This permits accurate prediction of abalone reaching full maturity.

Such a unit was in operation at Rainbow Abalone and Pearls, New Plymouth, Taranaki, New Zealand. Basically, the water was carried from the boxes to a simple biofilter of limestone chips. From here the water is pumped up to a header tank via a reversible heater chiller unit. The header tank had a foam fractionator connected to it removing particulate debris. The water was then gravity fed to the brood tanks. There was an input of 6-10 litres of raw seawater per minute, in effect, making the system a semi-recirculation unit as opposed to a complete recirculation unit. This system was run at 15˚C as *Haliotis iris* is a colder water species than either *H. d. hannai* or *H. tuberculata*. At this time the broodstock were being fed artificial food and were spawning successfully. There were no problems encountered with this unit during this period.

Components of the recirculation may vary but there should be no UV included as this would act as a spawning stimulant.

However, recirculating systems do have some potential disadvantages. There is a risk that if some individuals within the system close to or at full maturity were accidentally triggered to spawn they could in turn stimulate many others into spawning with subsequent heavy gamete loss. There is also the risk of mechanical failure and the associated water quality issues. Water quality parameters should be monitored as in a recirculation grow-out unit. In general, if there are no mechanical problems with the system then there should be no water quality issues, as the stocking density in a broodstock recirculation unit is relatively very low as compared with growout systems.

As the farm may have a recirculating broodstock unit and may also have a recirculating or partial recirculating weaning system data on water quality, parameters are included.

2. 4. Water quality parameters

Dissolved oxygen or DO along with temperature, controls the metabolism of the abalone. While it forms 21% of atmospheric gas it is not particularly soluble in water and thus can be limiting for fish. Warm water fish species are more tolerant of lower oxygen levels than cold water species. A minimum DO concentration of 5mg/l is recommended for warm water species while for cold water species 6mg/l is recommended. It is accepted that almost all fish species being cultured perform best when DO levels are near saturation i.e. 100%. For abalone it is generally felt that growth is seriously curtailed if DO drops below 85%. All farms should have a DO meter for monitoring levels on a regular basis.

Carbon dioxide or CO_2 is highly soluble in water while only forming a very small part of the atmosphere. It enters the water by diffusion from the atmosphere and it is also produced by respiration and the biological oxidation of organic compounds. With increased CO_2 levels

within a system there is a tendency for the water to become acidic.

The oxygen carrying capacity of fish blood is reduced as the amount of CO_2 increases.

Proper degassing units in the system can offset such a situation. It is generally recommended that CO_2 levels are maintained below 9mg/l.

If oxygen conditions are adequate within a culture system then the build-up of nitrogenous compounds is the next limiting factor. The principal source of nitrogen in the system is from the protein content of the fish food which is in the form of amino acids. Ammonia is also produced by the decay of organic matter in the system. Nitrogen has many forms within the system but the principal forms of interest are gaseous nitrogen (N_2), un-ionised ammonia (NH_3), ionised ammonia (NH_4^+), Total ammonia (NH_3^+ NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). Nitrogen gas itself is considered for the most part to be harmless. It forms 78% of the atmosphere and will diffuse in and out of the system. If the water becomes supersaturated then there is a risk of the abalone getting gas bubble disease.

Parameter	Concentration
TAN	0-1.0mg/l
NH_3	0-0.025mg/l
NO_2^-	0-0.5mg/l
NO_3^-	0-50mg/l
DO	90-100%-6.5-8mg/l
Salinity	35p.p.t
pH	8-8.2
Alkalinity ($CaCO_3$)	200+mg/l

Table 2. 2: Ideal water quality parameters in an abalone recirculation unit.

Un-ionised ammonia (NH_3) is considered to be particularly toxic in fish culture. As these levels increase in the culture water, the fish loses its ability to excrete ammonia and as a result, levels build up in the blood and tissues causing severe damage. ionised ammonia (NH_4^+) is much less toxic and fish in general can cope with quite large concentrations of ammonia in this form. Many commercially available water quality testing kits do not distinguish between the two forms of ammonia but rather measure Total ammonia (NH_3^+ and NH_4^+). In general, the higher the pH of the water the greater the level of the toxic form NH_3, while the lower the pH the greater the level of ammonia in its ionised form.

Plate 4: *Ulva lactuca* or sea lettuce.

Plate 3: *Ulva lactuca* (bottom left) beside fronds of *Laminaria saccharina* and *Fucus sp*.

Plate 5: *Laminaria digitata* frond.

Plate 6: *Palmaria palmata* or dilisk.

When measuring ammonia as Total Ammonia or TAN, ideal levels would be less than 1mg/l while in its most toxic form, levels ideally should not exceed 0.025mg/l.

During the nitrogen cycle ammonia is converted to nitrite by nitrifying bacteria called Nitrosomonas. This nitrite (NO_2^-) is also extremely toxic to aquatic animals and levels within a system for abalone should not exceed 0.5mg/l. During nitrification nitrite is in-turn converted to nitrate (NO_3^-) by Nitrobacter, which is as such, non toxic unless present at very high concentrations.

The pH is a measure of the acidity or alkalinity of the system. Natural seawater tends to have a pH of 8-8.2 and this is the desired level within an abalone culture system. At neutrality or pH 7 there is an equal number of hydrogen ions [H^+] and hydroxyl ions [OH^-] in the water. Below 7 the [H^+] ion predominates and the water is acidic while above 7 the [OH^-] ion predominates and the water is basic or alkaline. Increased CO_2 production in a system will increase the [H^+] ions and as a result lower the pH. If the pH goes below 7.6 the calcium carbonate of the abalone shell starts to dissolve. This must be counteracted by adding one or more of a number of buffers which keep the pH at 8-8.2. To ensure this buffering capacity the alkalinity or concentration of $CaCO_3$ must be maintained at 200mg/l or higher.

Salinity should be maintained as close as possible to normal oceanic levels of 35 p.p.t.

A summary of ideal water quality parameters are summarised in Table 2.2.

Water quality levels should be checked at least once weekly and it is also worth considering sending a monthly or bimonthly sample to an independent water quality analysis company.

2. 5. General conditioning considerations

Initially a hatchery operator must establish how many broodstock are to be conditioned over the course of the year or indeed over a period of a few months. This will be directly related to the amount of seed required. The amount of seed produced will be related to the amount of settlement tank space available, the productivity of the tanks and also to the amount of weaning tank space that will be available thereafter.

Unlike many Southern Hemisphere countries spawning in Ireland tends to be seasonal. Generally these are carried out between Spring and late Summer. In South Africa, Australia and New Zealand many hatcheries will spawn throughout the year. In Ireland we are

influenced to a large extent by ambient seawater temperatures. Our minimum seawater temperature can quite often, in February, be as low as 6˚C on some parts of the West Coast. Considering a larval rearing temperature of 18˚C - 22˚C the variation is too great. Also during this period of the year it can be difficult to establish and maintain benthic diatom films on the settlement tanks and plates.

It is possible when conditioning to target a number of large spawnings during the spring and summer. The broodstock would be introduced accordingly. At 18˚C it will take at least 96 days for *H. d. hannai* to reach spawning capability while to enhance success they should be conditioned for another 1 1/2 months. Something similar would be required for *H. tuberculata*.

The hatchery manager will know what quantity of 5mm-10mm spat the settlement tanks are capable of producing. This is essential in predicting the number of broodstock and subsequent spawnings required. In addition to this some hatcheries are dependant on income from seed sales and as a result need more spawnings and more tank space for nursery culture of juveniles.

If the hatchery has demand for *H. tuberculata* then this will reduce the number of *H. discus hannai broodstock* that can be conditioned. It would seem advisable for hatcheries to operate more than one conditioning unit. Two identical flow-through type conditioners, a flow-through and a recirculating unit or a flow-through and a number of smaller type conditioners as described earlier would be ideal. Having more than one conditioning unit on the farm spreads the risk load of gamete loss due to accidental spawnings or systems failure. As with many other systems on the farm the conditioners should be on an alarm system. This would monitor temperature fluctuation and the water level in the sump.

Broodstock should be fed a balanced diet at least twice weekly. It is advisable to provide a mixture of macroalgae such as *Laminaria digitata, Palmaria palmata, Ulva lactuca* and *Porphyra umbilicalis* (see Plates 3-6). All seaweeds should be washed in freshwater before they are introduced into systems. This will remove or kill many unwanted organisms such as copepods. Artificial broodstock diets are now readily available and of a very high quality. These could be fed as a single diet or as part of a combination with seaweed species. It is important to monitor feeding of the broodstock. If the abalone are in a stress free environment they should continue to feed and grow at normal rates (as they would in a good grow-out system). It is important to remove uneaten or decaying food on a regular basis. If boxes are being used to condition the abalone then they should be siphoned at least twice a week. Some boxes have a central drain in the middle to facilitate this while vigorous aeration should contribute to a type of self cleaning by keeping some of the waste in suspension thereby facilitating its removal through the overflow.

Light does not seem to be a major factor in the conditioning of abalone species. Most units are run on a 12L:12D regime. Some are kept under continuous dark conditions. The actual timing of this is up to the operator but it is advisable to have the light period for at least a few hours in the morning to facilitate feeding, cleaning, monitoring etc. and not to have the dark phase coming on too late in the day to facilitate spawning. An ideal time would be lights off automatically at 10a.m. Very low light intensity should be used mimicking the abalones sub-tidal environment. Most commonly quoted figures vary between 150-350 Lux or 40-100 Watt globes. Red light is frequently used and it is advisable to use a low power head lamp when examining broodstock.

Water quality in the conditioning unit should be monitored regularly in conjunction with monitoring carried out on other parts of the farm and a water temperature log should be installed to monitor and record daily temperature.

It should be sufficient to check the gonad development on a monthly basis. A visual inspection should take a matter of minutes for each abalone. More regular monitoring can prove stressful and hinder development. Individuals or batches of broodstock should be monitored on the basis of spawning performance. Those not spawning regularly or releasing few gametes should be replaced by better performing stock.

An interesting technique employed by some South African hatcheries producing *H. midae* juveniles and also some New Zealand hatcheries producing *H. iris* is the spawning of broodstock on a year round basis. In such hatcheries they use wild broodstock which are normally between 150mm-200mm. The abalone are actually trained into this routine. The broodstock are held at a ratio 4:1 female to male ratio. After collection these animals are kept at 18˚C and monitored regularily. After an initial spawning attempt (successful or unsuccessful) the abalone are then spawned on an eight week cycle thereafter. Eventually they will reliably shed gametes on this bimonthly basis. This technique ensures year round supply of viable gametes for seed production. It is possible that this technique could be applicable to other species, even when cultured entirely on the farm.

It is also advisable to spawn abalone once early in the year, discard the gametes, and recondition. The gametes from the first spawning are often considered to be poor quality.

Chapter 3: Spawning

3. 1. Introduction

There are a number of different methods that have been used to induce spawning in abalone. These include gamete stripping, desiccation, thermal shock, ultraviolet light irradiated sea water and chemical induction using hydrogen peroxide. Gamete stripping is not used as it involves the sacrifice of the adults while desiccation and thermal shock methods are very unreliable. However these two methods are sometimes used in conjunction with the UV method of spawning to increase its reliability. In Ireland chemical induction with hydrogen peroxide has been almost exclusively used although one hatchery has now switched to using the UV method. This is probably due to it being used initially when the animals were imported and this method being repeated thereafter. In addition many hatcheries would not have had UV light facilities which could be utilised in spawnings.

3. 2. The hydrogen peroxide spawning method

When Hydrogen Peroxide or H_2O_2 is added to water (H_2O) the hydroperoxy free radical, HOO^- or the peroxy diradical, $^-OO^-$, are produced. The presence of these free radicals in the water is believed to be responsible for the induction of spawning.

This method of spawning induction is both cheap and reliable, assuming proper conditioning has been carried out. Reagent grade (30%) is recommended as weaker solutions are unstable and give unreliable results. Abalone to be spawned are sexed and placed in spawning vessels. The pH of the water is first increased to 9.1 using tris - (hydroxymethylamino) methane. This has a molecular weight of 121.14 and is added in a 2M solution at 6.6ml per litre of seawater in the spawning vessels. A working solution of Tris is made by adding 24.22g of the chemical to about 75ml of distilled water and topping this up to 100ml when it is fully dissolved. If for example twelve 10 litre buckets were being used for the spawning with each bucket containing 8 litres of water then 8 x 12 x 6.6ml = 633.6ml of solution are required. This would necessitate 169.54g of Tris being dissolved in distilled water to make 700ml of solution.

Thereafter 52.8ml of the solution would be added to each spawning vessel.

Tris is generally left with the abalone in the spawning vessels for 15 minutes. The reagent grade hydrogen peroxide is diluted down to a 6% working solution. To do this 20ml of the hydrogen peroxide is added to 80ml of distilled water. 3ml of this solution is added per litre of water in the spawning container. Water in the container is then thoroughly mixed. Usually the abalone are left in this solution mix for 2.5 hours though this time may vary with species, farm, country etc.

It is also advisable to carry out this procedure up to 30 minutes later on the males than on the females as generally the former spawn earlier and more readily than the latter. After 2.5 hours the solution is decanted from the containers and the abalone are thoroughly washed in isothermal water to remove any traces of the chemicals. This is essential as the chemicals will destroy the released gametes. The containers are then refilled with isothermal water and after 30 minutes to an hour and a half later the abalone should start to shed their gametes.

3. 3. Ultraviolet light spawning method

Despite being little used in Ireland this method is generally considered the best and most reliable method of spawning induction in the majority of abalone culture facilities worldwide. It is sometimes used in conjunction with desiccation of the abalone and also thermal shock. Low-pressure mercury type UV lamps are most commonly used and these generate a spectral wavelength of 254 nanometers that is very close to the recognised peak germicidal effectiveness of 265nm. Such lamps are suitable for use in spawning induction and all but the smallest wattage lamps could be used. It is recommended that there is a flow of 3-5 litres/hour through containers with broodstock for spawning. An advantage of using UV for induction of spawning is that it is completely harmless to the gametes. As a result water changes during the spawning process are not necessary. It is also possible to programme lights to come on at a particular time thereby controlling the timing of the process. It is considered that the ideal UV strength for spawning is 800 milliwatt hours per litre for *Haliotis discus hannai* and this has resulted in male gamete release after ca. 3 hours 15 minutes and female gamete release after 3 hours 45 minutes. *H. tuberculata* can release sperm in as little as 1 hour 15 minutes and eggs in 1 hour 30 minutes using UV stimulation (Le Dorven, pers. com.). Hone et al. (1997) recommend using UV at a minimum strength of 600-800 milliwatt hours per litre in Australian abalone hatcheries.

N. B. It is very important to never use the H_2O_2 method of spawning in combination with the U.V. method as they greatly stress the abalone. The abalone will shed few gametes and the small quantities that are released will not be viable.

Chapter 4: Larval development and larval culture

4. 1. Larval development

This period is considered to be the time between fertilisation and the initiation of metamorphosis. There are many developmental stages reached during this period and some of the principal ones are outlined.

After fertilisation the first polar body is released. Soon after this the second polar body is discharged close to the first. The next obvious stage is the first cleavage and thereafter cleavages are repeated until the gastrula stage has been reached. The gastrula has a distinct band of cilia referred to as the prototrochal girdle and a clump of cilia called the apical tuft is also formed. The gastrula will sporadically rotate within the egg membrane.

As the prototrochal girdle thickens rotation within the egg membrane increases. This movement weakens the membrane and with the aid of the apical tuft of cilia the embryo breaks free from or hatches out of the egg membrane.

This stage is referred to as the trochophore. After hatching it swims to the surface. At around this time initial shell secretion also begins.

With the development of a swimming ciliary band called the velum the larva is then considered to be a veliger. Shell development continues until it is close to the velum.

A larval retractor muscle then develops and an attachment for the visceral mass to the inside of the shell also forms. The retractor muscle will allow the veliger to withdraw into the shell as it develops and the shell increases in size. As shell development continues the foot mass protrudes through the open area of the shell.

The first 90˚ rotation of torsion occurs next. Torsion is an evolutionary adaptation by larval gastropod molluscs to move the position of the head/foot complex to offer it more protection from the shell and also to improve feeding and sanitation for adults. The head/foot complex or cephalo-pedal mass rotate between the region of the body covered by the larval shell and the 'waist'. This torsion process will continue at a slower rate thereafter until a 180˚ rotation has been completed.

In figure 4.1 Stages 1, 2, 3 and 4 (A, B and C) show fertilisation, discharge of the two polar bodies and first cleavage.

Stages 5-10 (D and E) show the second cleavage to gastrula stage.

Stages 11-13 (F and G) show the development of prototrochal cilia

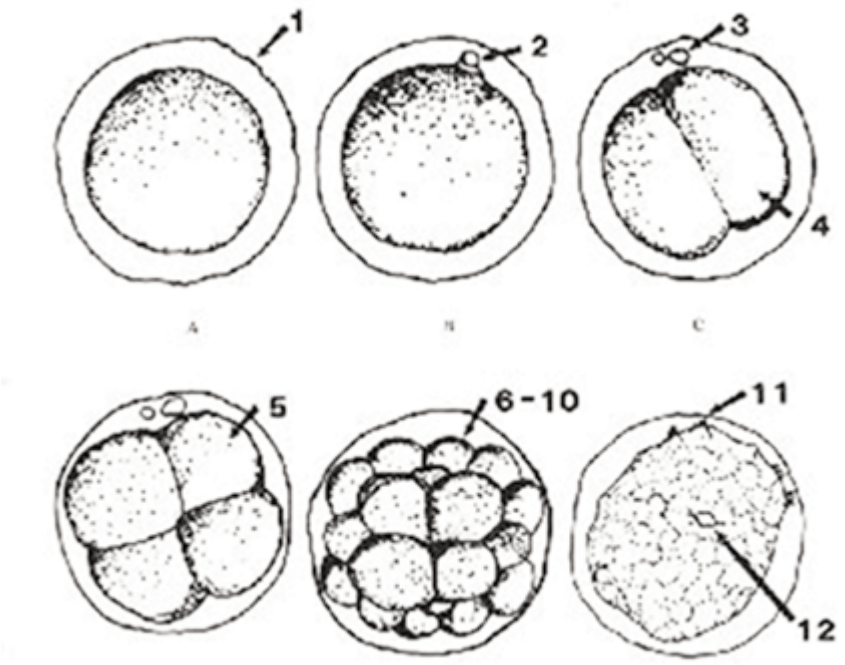
and prototrochal girdle. Stage 15 shows initial larval shell secretion.

Stages 16 and 17 (H and I) show development of the velum and formation of the retractor muscle respectively. Stage 18 (J) shows attachment of the integument to the larval shell. Stages 19-22 (K and L) show protrusion of foot mass, completion of shell development, the first 90˚ of torsion followed by the complete twisting of the cephalo-pedal mass through 180˚. Stage 23 (M) shows the formation of long spines on the metapodium or foot while Stage 24 shows the formation of the operculum. Stage 25 (N) shows the development of fine cilia on the foot while Stage 26 shows the formation of vertical grooves on the velum.

Stage 27 shows the appearance of the eyespot while Stage 28 shows the propodium on the front region of the foot. Stage 29 (O) shows development of the cephalic tentacle on which four tubules will later develop.

Stage 30 and 31 (P) show cilia development on the propodium and in the mantle cavity. Stage 33 shows the formation of the first cephalic tentacle while Stage 34 shows development of the otolith.

Stage 35 shows development of a short spine on the cephalic tentacle and Stage 36 shows first snout protrusion. Stage 37 shows the development of two tubules on the cephalic tentacle and Stage 38 shows ciliary formation on the roof of the mantle cavity. Stage 39 shows the appearance of the third tubule on the cephalic tentacle (indicative of ability to settle). Stage 40 shows the retractor muscle withdrawn into the mantle cavity while Stage 41 shows the development of the fourth tubule on the cephalic tentacle.



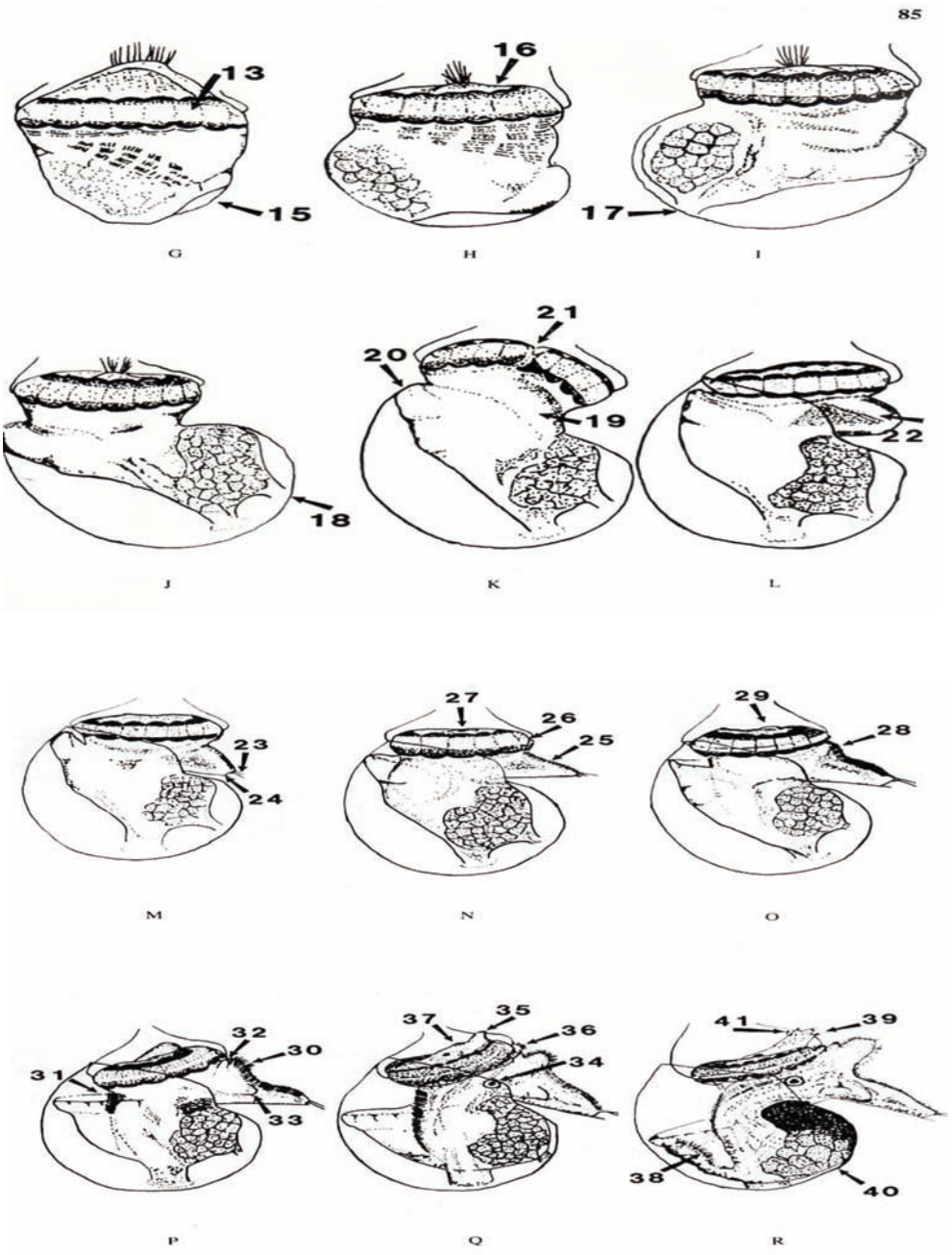


Figure 4. 1: Principal larval developmental stages of *Haliotis discus hannai* ; from Hahn (1989).

The development of an operculum is the next stage. This facilitates complete retraction of the foot into the larval shell with the operculum sealing the shell opening. At approximately the same time cilia start to develop on the sole of the foot. A groove develops in the velum, an eye spot develops and a podia called the propodium develops. A pair of cephalic tentacles develops on the head on which four tubules will later form. Cilia develop in the mantle cavity and start to beat. A pair of epipodial tentacles form on both sides of the foot under the operculum and at this stage the larva is capable of crawling on surfaces. An otolith is formed and the snout starts to protrude from under the velum. The larval retractor muscle attached to the larval shell draws the enlarged mantle cavity towards the back of the shell. Larval development is considered complete when the fourth tubercle

develops on the cephalic tentacle.

The above synopsis is described in more detail in Hahn (1989) and this in turn was previously described by Seki and Kan-no (1977). The description describes development for *H. d. hannai* and while many of the principal stages are similar to those of the larvae of other abalone species exact timing and stages do vary.

Haliotis tuberculata may take somewhat longer to develop through to settlement than *H. d. hannai.* Koike (1978) estimated that at 20˚C *H. tuberculata* larvae will take 108 hours for the ciliary process in the mantle cavity to develop. This precedes the formation of the third tubule on the cephalic tentacle at which time the larvae are considered competent to settle. At the same rearing temperature Seki and Kan-no (1977) observed that *H. d. hannai* took 76.2 hours to reach this stage.

4. 2. Larval rearing

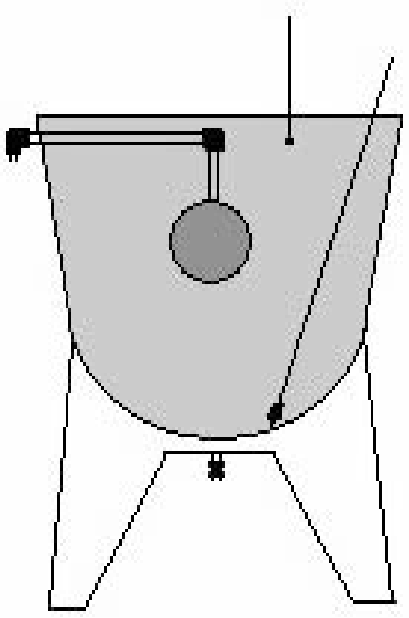


Figure 4. 2: Diagram of a typical larval flow through culture vessel showing 'banjo' sieve attached to overflow. Also shown are pair of airlines to top and bottom of water column and a central draining valve at the base of the vessel.

This section will deal with the handling of gametes after spawning right up until the larvae are ready for settlement in diatom coated tanks.

During the spawning process the males and females will release their gametes into separate spawning containers. If the abalone were washed properly in isothermal seawater after the removal of the Tris and hydrogen peroxide solutions then further intensive

washing should not be necessary. Sperm can be gently decanted in a number of pyrex beakers. Gentle decanting should prevent the transfer of faecal material to the vessel. This container should have sperm from as many of the males as possible. It is advisable to make up a solution with fresh sperm at intervals during the spawning process to ensure the freshest and strongest sperm is available throughout.

The eggs, which are negatively buoyant, can be siphoned gently using a narrow diameter tube through a pair of fine sieves. The size of the larger sieve should be such that it enables the eggs to pass through but will retain faeces or other debris. Ideally it should be 300μm as this will also allow 220μm diameter eggs to pass through undamaged. The collecting sieve below should be 50 - 90μm and is immersed in seawater. This sieve will retain the eggs. However it is advisable not to use a sieve any larger than this e.g. 120μm, as some of the egg diameters will not be much greater than this and will actually pass through the smaller mesh when being lifted out of the water in the spawning tray. This will damage and actually rupture many of them. Before being transferred from this sieve they are gently washed using the filtered seawater supply. Alternatively use the larger size mesh and let them pass directly into the fertilization container. For the fertilization process a quantity of very clean white hatchery buckets ca. 12-15 litres are recommended. These should be used for this specific purpose only and should be graduated by the hatchery operator to give an exact idea of water volumes used thereby making estimation of larval numbers quite easy. As batches of females seem to be approaching the completion of spawning siphon the eggs through the sieve(s) and collect in one to a few buckets. Fertilization is best carried out using a high density of sperm (20-30ml sperm solution). Allow a few minutes (maximum 15) for fertilization to take place. Then gently decant off the water from all but the bottom portion of the container and refill with isothermal seawater to remove excess sperm. This process can be repeated. When this rinsing process is complete allow the fertilized eggs to sink to the bottom of their containers. Before dividing between other containers gently wash/stir them on a 75μm sieve.

A layer of eggs on the bottom of each bucket is sufficient but normally a little more than this quantity will develop and hatch ok. However if a multiple layer of fertilized eggs settle on the bottom of the bucket there is a risk of oxygen depletion resulting in death of the embryos. The hatchery operator will know how many eggs are required but with the relatively large eggs of abalone 500,000-750,000 is probably enough.

In general fully mature female abalone will release between 100,000 and 500,000 eggs. However this amount is size, species and age related. Some larger broodstock may release larger quantities, ca. 1,000,000+, but this would be unusual.
A pipette sample of 1ml should be taken from the bottom of each

bucket after 1 hour, placed on a Sedgewick-Rafter counting slide and examined under the microscope. Eggs are checked for fertilization by the presence or absence of polar bodies and the first cleavage stage in the more rapidly developing embryos. If fertilization is adjudged to be poor at this stage (<50%) then fresh sperm can be added. This process is repeated until all of the females have shed their gametes. Buckets with developing embryos are then left in the hatchery overnight and in complete darkness.

By the next morning the hatch of trochophore larvae should be well underway. The vast majority of them will be swimming in the top layer of water. Many of them will be swimming in distinctive spiral whorls extending half way down the container which are characteristic of the trochophore stage. If not there has been a problem with the gametes, water quality or the temperature of the hatchery may have been too low. Twenty four hours after fertilization this hatch should be complete. In theory the slower and weaker larvae are the really late hatchers. The lights can be turned on as soon as the operator is ready to commence work. It is advisable to commence filling the larval rearing bins as early as possible. These are filled with 1μm filtered UV treated seawater. The temperature should not differ very much from that in the hatching buckets. This should be predetermined and the air temperature in the hatchery should be selected to maintain the water temperature at ideally 18-20˚C.

While the larval rearing bins are being filled the top 70-80% of the hatching trochophore larvae should be siphoned either into buckets containing some fresh filtered UV treated seawater. Alternatively the top 70-80% from a number of buckets could be gently siphoned onto a sieve immersed in water and thereafter distributed into buckets. When siphoning the buckets spray the sides with isothermal filtered UV water as the trochophores will get trapped on the sides of the bucket as the water level drops. A flexible plastic wash bottle is ideal for this purpose.

Normally the portion of water left in the hatch buckets is discarded. While there will be many hatched and unhatched larvae in this it will also contain dead embryos, egg cases and many other bacterial sources. However if the spawning is poorer than expected then these buckets could be refilled and a second hatch taken off at a later stage during the day.

After collection the larvae are counted to ensure even distribution among the rearing bins and also to monitor survival at the end of the rearing cycle. Three to five 1ml samples are taken and counted on a Sedgewick-Rafter counting slide. As the larvae are swimming quite strongly at this stage it may be necessary to put a drop of 10% formalin, killing them, onto the slide to facilitate counting.
When a total count has been estimated divide them evenly

between the rearing bins. It may not be practical to do this exactly as they should be transferred as soon as they have been counted. Unlike the larval stages of some other shellfish species stocking density is not such an important issue. The main reason for this is that the larvae are lecithotrophic. This means that they have their own yolk sac which supplies them with the nourishment needed to reach settlement and through their metamorphosis. Generally shellfish larvae are planktotrophic and to reach settlement they rely on phytoplankton as their food source. As a result this planktonic phase can last up to a few months for larvae at sea. Lecithotrophic abalone larvae are often ready to settle in as little as five days. These larvae do not require microalgae to be cultured or fed to them. There is no competition for food in the rearing bin and as such stocking density is not a major issue. A comfortable stocking density would be 5 larvae per ml of seawater in the culture container but they could easily be stocked at 10+ per ml

Initially abalone larval culture systems would have been 'static'. This is as used in oyster or clam culture whereby the bivalve veligers are reared in large culture containers and the water inside is emptied or siphoned out on a regular basis. While the water is being changed the larvae are retained on mesh sieves immersed in seawater. In most abalone culture facilities at present larvae are cultured in flow-through rearing systems.

Flow through larval rearing systems are quite similar in most ways to the flow through broodstock conditioning units already described. Indeed some facilities will have the larviculture water coming from the conditioning reservoir/sump with the addition of UV irradiation before the water enters the culture vessels.

A large sump or double sump of ca. 1,000 - 2,000 litres is required. This needs either an immersion heater or in-line titanium heater to elevate the water temperature to 18-20˚C.

This water is filtered to 1μm if possible and is UV treated before entering the culture vessels. The reservoir should have a float valve to indicate when fresh seawater needs to be introduced. A pump supplies the water to the culture tanks inside the hatchery room. The flow into each bin is regulated by a series of valves inside the hatchery and excess water is returned to the reservoir via a return valve.

Generally culture tanks have 300-500 litre capacity. They tend to be cylindrical at the top with either a conical or hemispherical base. At the base of the tank there should be a valve or bung to allow complete draining for larval removal and also cleaning. The flow through each tank is not calculated very scientifically but should really be up to a few litres per minute with a tank of 300-350 litres. The water exits the tank via a mesh of ca. 90-150μm. A 'banjo' sieve is commonly used. This is where the mesh is attached to a pvc pipe ring. Typically this

ring would be 25mm width and have a diameter of 20mm+. This should be completely submerged in the culture water to ensure the maximum surface area for larvae and mesh contact. The less the surface area the more likely clogging will occur with the risk of overflow and subsequent larval loss. Other designs such as piping with large slots or grooves removed and covered with mesh can also be used. These may in fact offer greater surface area than the 'banjo' sieve. There should be a rigid airline with airstone at its base supplying air to the bottom of the tank close to the bung or draining valve. A second airstone on or at the surface layer of water is optional but of benefit.

The trochophores are distributed as evenly a possible between the rearing bins. In reality 4-8 bins should be enough to cater for most spawnings. If larvae are stocked at a low level of 5 per ml of seawater and the bin has a 350 litre volume then this can hold 1,750,000 veligers. Four of these bins could hold 7 million larvae and six bins could potentially hold more than 10 million. This should represent enough larval holding capacity to accomodate any size spawning.

(Aeration can be for left off for the first 24 hours). If it is turned on it should be very gentle. This is due to the fact that the shell on the veliger is still developing and the agitation caused by aeration will lead to shell abnormalities. In particular, the retractor muscle and integument attachment to the shell do not form, thus leading to very heavy mortalities later in the larval cycle. On the second day after introduction to the tank the aeration is turned on gently. Using a flow- through system there is little labour or monitoring required. Check flow rates and temperature in the culture a few times daily.

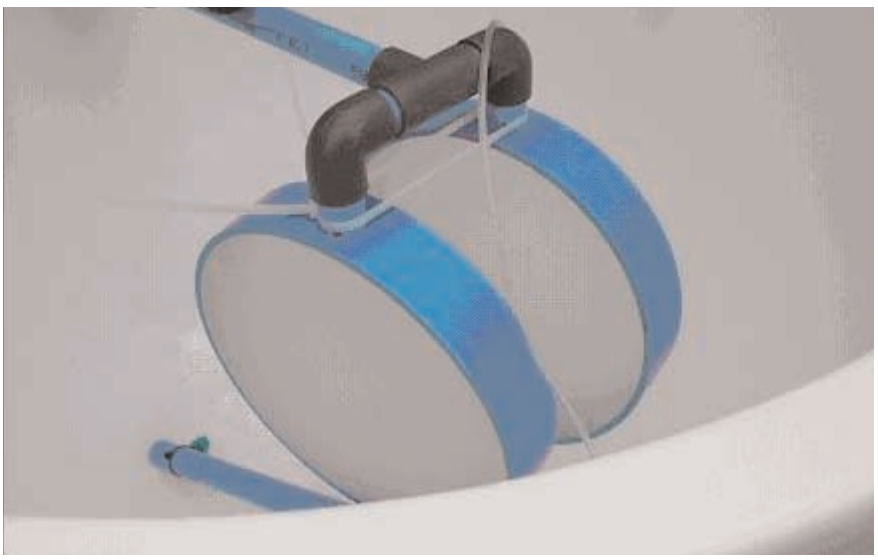


Plate 7: Double 'banjo' sieve in rearing bin at Mara Chleire Teoranta.

Check for 'dead spots' in the container. These are patches of dead larvae which tend to accumulate in dark clumps. These should be removed using a long narrow gauge siphon specifically for this purpose. The larvae can be checked microscopically throughout the cycle to monitor development but this is not even necessary. It may also be necessary to turn off the water, remove and clean the 'banjo' sieves occasionally. Alternatively a spare set of sieves can be used for this purpose. If conditions, in particular temperature, are normal during the rearing cycle the larvae of *H. d. hannai* and *H. tuberculata* will be competent and ready for settlement on the fifth day after spawning or the fourth day after their introduction into the larval rearing containers. *H. tuberculata* may take somewhat longer and could in fact be left until later on the fifth day. It is interesting to note that this time varies greatly with temperature and with species. *Haliotis midae* reared at 18˚C takes an extra day to reach settlement while *H. iris* conditioned at 15˚C and reared at 13-15˚C takes 9 days from fertilization to settlement.

Plate 8: Larval rearing bins used at Mara Chleire Teoranta.

Indications of competence to settle include 'crawling' or 'gliding' behaviour. The eye spot should be present already. The presence of the third or fourth cephalic tentacle tubule is considered indicative of imminent settlement but these are difficult to observe under the microscope. Another feature considered to indicate settlement capability is snout protrusion for *H. discus hanna*i at an EAT 925˚C-hr.

On the morning of settlement the larvae are either siphoned or drained onto 90-150μm sieves immersed in 1μm filtered UV treated seawater. They are gently washed into graduated buckets and counts are carried out. They are then distributed to the prepared settlement tanks at the desired settlement densities. Care should be taken to

gently wash stranded larvae down from the side of the tank as well as the 'banjo' sieve and support pipe.

Plate 9: Abalone larval rearing bins on flow through at Brandon Bay Seafoods.

4. 2. 1. Use of hatchout trays during larviculture

Hatch out trays are by no means a necessity in the abalone hatchery but are more indicative of a farm involved in a high level of production. They offer an easier way to handle the developing embryos between fertilization and transfer to the larval culture vessels. They also offer a method of selecting the earlier hatching stronger swimming trochophores that are theoretically the healthiest ones. They also ensure a continuous supply of water passing over the developing embryos. They are basically a flat rectangular tray of 70-80 litres. with a water intake at one end or side and a water exit at the opposite end or side. The intake is of filtered sterilized seawater and the out flow is into the larval bin over which it is fixed. There is a ridge or shelf either along one side or one end of the tray. This ridge determines the water level and when the water is turned on the water must exit over the entire length of this ridge. The fertilized eggs are spread evenly over the bottom of the tray. It is best not to move the eggs once cleavage has commenced as they are very fragile at this stage. Water enters the tray via a spray bar and after a number of hours is turned on. This would usually be turned off the next morning before the hatch is complete thereby excluding the slower developers.

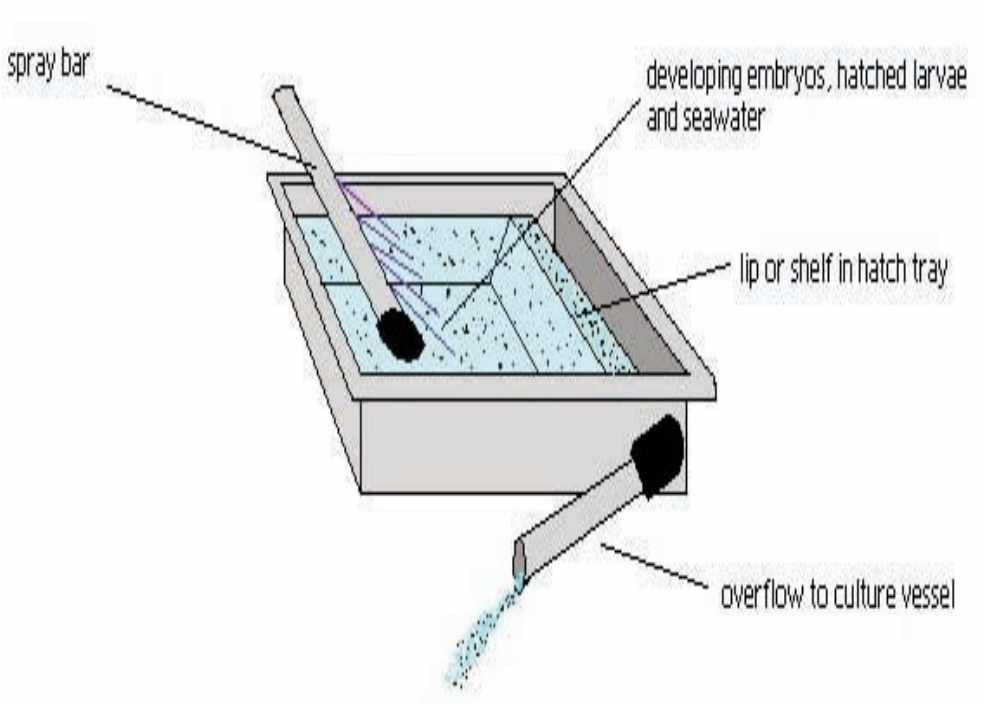


Figure 4. 3: Representation of hatch tray with developing embryos and swimming larvae overflowing to a larval rearing tank.

Chapter 5: Settlement

5. 1. Introduction

Settlement is the process of the veligers seeking out a suitable substrate and thereafter changing from the swimming larva into a benthic juvenile and undergoing the associated process of metamorphosis. It is considered that veligers are competent to settle when there is snout protrusion. While they are capable of settlement at this stage they are not actually capable of undergoing metamorphosis until some time later generally considered to be the appearance of the third tubule on the cephalic tentacle. For *Haliotis discus hannai* this corresponds to an EAT of 925˚C-hr for snout protrusion and 1030˚C-hr for the appearance of the third tubule on the cephalic tentacle or 74.6 and 85.5 hours after fertilisation at 20˚C. A veliger can settle on a substrate for a period and swim off again if the substrate is unsuitable. The process only becomes irreversible when the larva casts off the 24 cilia cells of the velum and metamorphosis begins. During metamorphosis there is further development on the cephalic tentacles, shell growth, radula formation, mouth and digestive tract development. Initially the mouth is only about 10μm but after about two days it increases to ca. 30μm. The gills do not develop in the juveniles until much later and 0_2 diffuses over the mantle surface.

5. 2. Settlement induction

There are considered to be a number of stimulants which can induce settlement and subsequent metamorphosis in abalone larvae. These are diatoms, mucous and GABA.

5. 2. 1. Diatoms

Diatoms are marine microalgae and can be both planktonic and benthic. Benthic diatoms are the principal source of food for newly settled juvenile abalone. While newly settled juveniles appear to be quite selective as regards to which diatom species they graze settlement induction does not appear to be as closely associated with particular diatom species. Diatoms secrete extracellular mucous and this is also believed to be utilised as a food source. It is believed while newly settled juveniles may in fact not be able to digest some diatom species they are capable of digesting their mucous.

5. 2. 2. Mucous

Mucous is secreted by the foot of juvenile and adult abalone. It remains attached to the substrata which they crawl across and is thought to be relatively insoluble. Larvae have been observed to settle in high densities on mucous trails in nursery tanks. It is

believed that the larvae can actually distinguish between different types of mucous secreted by the foot. They appear to favour mucous secreted when the adults or juveniles have been grazing. Newly settled juveniles are also believed to feed on this conspecific mucous trail and it seems to be a very good nutritional source at this stage. The abalone must be removed before settlement so as not to compete for food with newly settled juveniles. Some hatcheries use mucous trails in addition to coatings of benthic diatoms to encourage settlement of their abalone larvae although it does create additional labour in an already labour intensive process.

5. 2. 3. GABA

It has long been recognised that crustose red algae including *Lithophyllum spp*. and *Lithothamnion spp*. are associated with settlement of juvenile abalone. This is also the case for many species of juvenile sea urchins (Pearse and Scheibling, 1991). While they can induce settlement they are of little dietary value to the juveniles due to their calcerous nature. Morse et al. (1979) contended that a macromolecular substance present in these algae stimulated settlement and metamorphosis. They believed that this substance was closely related to GABA or γ- aminobutyric acid. This compound is a neurotransmitter found in the human brain and also in the tissues of higher animals. While it has since been proven that GABA is not the actual substance present in *Lithothamnion/Lithophyllum* responsible for the induction of metamorphosis it does have a similar affect as the compound. GABA does induce settlement of veligers if used at the correct time and concentration. However using concentrations that are too strong can be lethal. While used in some American and Mexican hatcheries for settlement and metamorphosis of *Haliotis rufescens* it is not considered necessary in the vast majority of hatcheries worldwide.

5. 3. Metamorphosis

This is the process whereby the free swimming veliger larva develops into a grazing benthic juvenile. There are a series of distinct stages in this process and they vary between species. However once metamorphosis has been initiated the process will be complete inside 24 hours. There is no feeding during this period and the entire process relies on energy from the larval yolk sac. One of the first stages is the casting off of the swimming velum. The mantle membrane separates from the larval shell and moves to the shell edge. Juvenile shell growth commences followed by initial mouthpart development, cephalic tentacle formation and radula formation. The digestive tract then develops and soon after the mouth becomes fully developed. The circulatory system also develops and the heart starts beating. The mouth is very small at this stage and they are capable of ingesting only very small diatoms ca. 10μm in length and they are also believed to ingest bacteria at this stage. When metamorphosis is complete the juvenile abalone have a shell length of ca. 350μm.

5. 4. The role of benthic diatoms in settlement and juvenile nutrition

Benthic diatoms are crucial in the culture of abalone. They are the food source for newly metamorphosed larvae and without them the juveniles will starve. Yolk reserves carry the larvae through settlement and metamorphosis but thereafter small digestible diatoms are required. This culture phase between settlement and weaning offers the potential to greatly increase or decrease survivorship.

The significance of the role of benthic diatoms in this are often greatly underestimated. It has been observed in many hatcheries worldwide that large scale mortalities can occur, in particular, between three and eight weeks after settlement (Leighton, 1989; Roberts et al.,1999).

Initial work carried out at the Shellfish Research Laboratory, Carna yielded quite low juvenile production rates. Juvenile or spat production rates are considered to be the amount of juveniles that reach ca. 5mm in shell length. At 5mm in shell length the abalone spat are considered to be quite hardy and can readily be transferred to weaning units or certain other ongrowing units. Settlement was generally carried out in 3,000 litre Fasttanks®. These are canvas PVC coated tanks and for settlement purposes white ones were used. White surfaces greatly facilitate and enhance diatom growth. Pairs of clear plastic corrugated plates were suspended into the tank and these also provided surface area for diatom growth and settlement. Settlement survival rates during these early stages of abalone culture in Ireland were very low, from as little as 0.5% up to no more than 5.0% maximum. This survival rate for the most part was influenced by the ability to provide diatoms to the juveniles.

Survival rates in Irish hatcheries have improved greatly on the above figures. The improvements can be attributed to three major factors;

- advances in benthic diatom layer production and diatom mass culture;
- use of Taiwanese settlement plate and basket systems;
- greater understanding and use of weaning system technology;

Since the initial work at the S.R.L. there is increased awareness of the use of natural light and its role in the development of diatom films. Settlement tanks for abalone larvae tend to be indoors either in greenhouse type structures that allow light through or in buildings that are impenetrable to light. In the case of the latter an alternative source of low intensity light must be provided to bloom

and sustain their culture. In the former, a type of mesh material can be used to control the amount of light reaching the settlement tank. It is crucial after the establishment of a benthic coating to maintain correct light intensity otherwise the diatoms will be replaced in succession by green microalgae and thereafter green macroalgal followed by other seaweed species. Once diatom coatings have been lost they are extremely difficult to replace. Roberts et al. (2000) recommends using light intensities between 20 and 100μEm^{-2} sec^{-1}. Bulbs to provide such intensity would be fluorescent tubes such as 'daylight ' and 'cool white which, would be of low watt (ca. 40 - 60) rating and these are typically used in the New Zealand V- type settlement tanks. In Irish hatcheries diatoms are encouraged to bloom naturally on the settlement tank and plates. This usually occurs between mid Spring and mid to late Autumn. This process can take from a fortnight to many weeks to occur. The length of time will be related to the amount of light and the concentration of diatoms in the water. This time can be shortened by the addition of nutrients to specifically encourage diatom growth (see chapter six). The coating will build up and increase in thickness with time.

In hatcheries in other countries diatom 'spikes' may be added to the settlement tanks to encourage the development of diatom films. This is where a highly concentrated amount of a cultured species is added on a one off basis, or, frequently over a period of time. Sometimes the 'spike' may consist of a cocktail of diatom species that are considered of benefit to the newly settled juveniles. However the actual effectiveness of this 'spike' seems to be limited and less likely to contribute to a diatom coating than natural light and nutrient additions.

It is generally agreed in hatcheries throughout the world that such coatings are excellent to encourage settlement and metamorphosis of juvenile abalone. However while such a coating contains a multitude of diatom species some of which will actively encourage settlement while others are an excellent source of food, not all species present will be of benefit. Benthic diatoms, while all being single celled and possessing a cell wall that is heavily impregnated with silica, vary greatly in their size, shape, structure and form. They have two complex structured valves connected by a series of structures called girdle elements. They are for the most part Pennate with the valves being elongated and oval while a second type are referred to as being Centric diatoms and generally have round valves and are almost cylindrical. Some are found as single cells on the substrate while others are in the form of colonies. Some of those attached are motile to varying degrees while others are firmly attached to the substrate. Some of those found in colonies cover a large surface area while others form upright growths. Kawamura et al. (1998) suggested a number of factors which determine the digestive efficiency of a diatom strain for post larval abalone:

a) Attachment Strength - some very tightly attached diatoms require considerable radular force to remove

them and are usually broken as a result. This greatly helps in the subsequent digestive process. Others with lower adhesive strength can actually pass through the gut alive and undigested.

(b) Structural Strength - the strength of the cell wall or frustule can also determine whether the cell wall is broken during grazing and subsequent digestion. It is of particular benefit to juveniles over 0.6mm that the cell wall is broken.

(c) Cell Morphology - cell size and/or stalk length can limit digestibility particularily in small post larval juveniles.

5. 5. Preparation of larval settlement tanks

In commercial abalone hatcheries throughout the world settlement tanks are generally at least a few thousand litres in volume and have racks or baskets of clear settlement plates. Design may differ but the principal is largely the same throughout. The settlement plates are either used from new or thoroughly scrubbed to remove any debris that may interfere with diatom growth. In some of the Southern Hemisphere hatcheries there is not as much emphasis on plate cleaning. These plates are scrubbed in saltwater and reused immediately to utilise existing diatoms and in particular the coating of *Ulvella lens* which coats the plates throughout the year. *Ulvella lens* is not a benthic diatom but an encrusting green macroalgae. It is quite tough and reproduces by the mass release of zoospores from the adult sporophytes. It is prevalent in many of the larger abalone producing countries such as the U.S., China, Japan, Australia and South Africa. While it may be present in Irish waters or at least a closely related species is present, it does not naturally form coatings on settlement plates in our hatcheries. While this is a disadvantage to Irish abalone producers it is not a limiting factor. In fact in these other abalone producing countries there is a tendency to allow or encourage diatom species to coat the plates in addition to the *Ulvella lens*. However, *Ulvella lens* is not as susceptible to collapse as are diatom coatings.

Cleaning used plates in Irish hatcheries can be extremely labour intensive. If the plates have been allowed to dry over a period removal of algal debris can be quite troublesome. It is best to leave plates soaking in water and then clean as soon as possible after removal of juvenile abalone. Dried crustose algal can also be difficult to remove. Calcerous *Spirorbis* tubes are also particularly difficult to remove. The hatchery operator must try to balance the amount of labour afforded to plate cleaning with the condition of the plate surface on settlement. More often than not a quick scrub on each plate surface with a soft brush or a washing with a power hose may be sufficient to remove excess debris and allow adequate diatom settlement thereafter. There are now commercial

plate cleaning machines available and these may be worth purchasing for hatcheries producing large quantities of seed.

In addition to using clean or new plates and baskets to provide surface area for benthic diatom attachment, it is advisable to use settlement tanks that are white in colour. The white colour reflects light and greatly enhances diatom formation. It also facilitates relatively easy observation of the settled spat.

Tank design varies greatly but in general they have a volume in excess of 1,000 litres. but in some hatcheries tanks as small as 500 litres are used.

The Taiwanese plate and basket system has become almost standard to Irish hatcheries. They have many advantages over the traditional suspended plate pairs that were initially used in Irish hatcheries. They provide a large amount of surface area as there are 20 plates per basket. They are compact and relatively easy to handle. It is generally accepted that the highest concentration of larval settlement occurs on the upper surface of the curve on the plate ridges. There are many ridges on each plate thereby increasing the specific area for larval settlement. A slight disadvantage of these plates is that as they are so close together in the basket it can be difficult for light to penetrate to the bottom of the tank thereby reducing the diatom coating. Rotation of the baskets through 180˚ (turn upside down) can alleviate this to an extent. If the settlement tank is large enough then it may not be necessary to pack the baskets very close together, in particular along the tank margin thereby facilitating better light penetration and resultant diatom growth. The Taiwanese settlement plates are quite expensive, currently costing ca. US$0.39 each, but they are reusable for long periods.

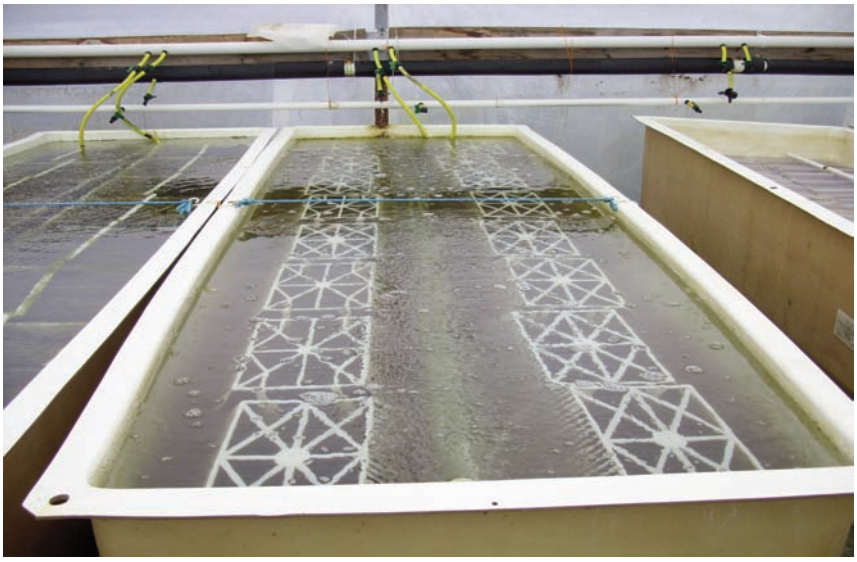


Plate 10: Blooming settlement tanks at Brandon Bay Seafoods. Note that the baskets are upside down.

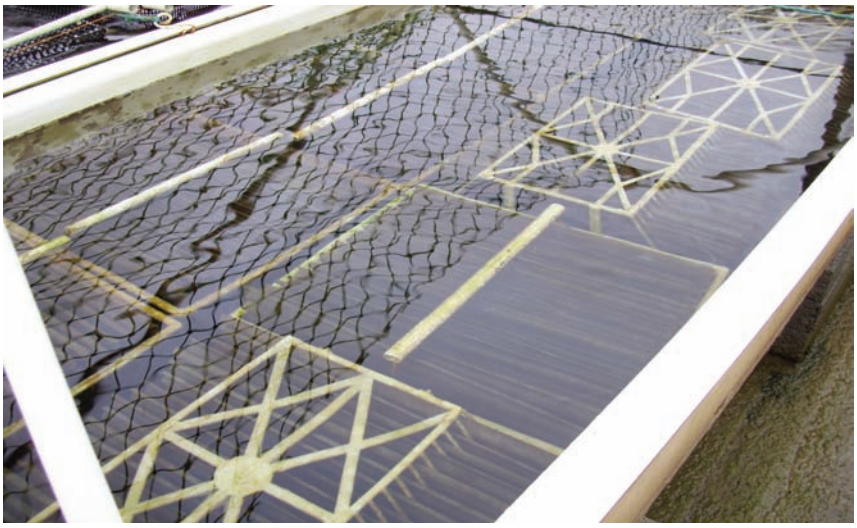


Plate 11: Plates being bloomed. Some are overturned.

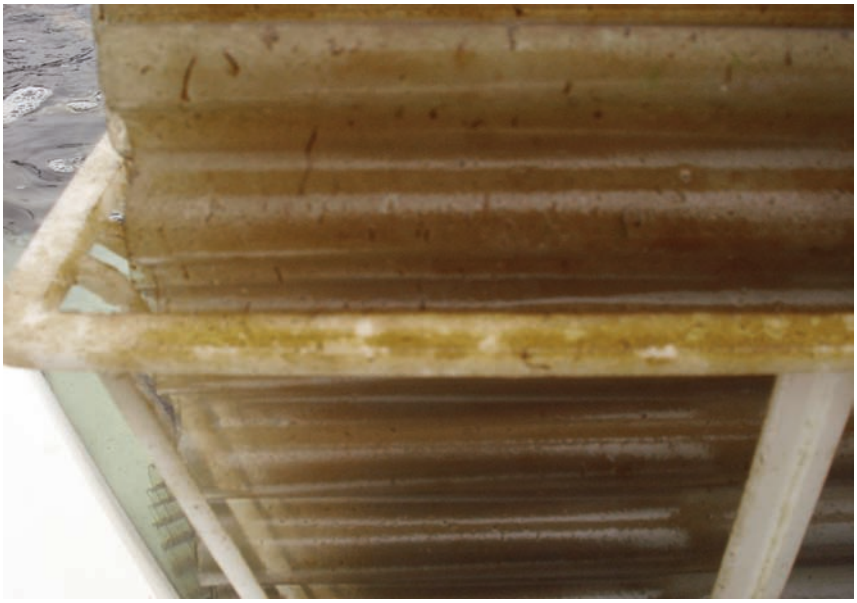


Plate 12: Plates and basket with heavy diatom coating prior to settlement.

5. 5 .1. Protocol for benthic diatom coating production in greenhouse settlement tanks

Thoroughly clean plates, baskets and tanks.

Place one or two layers of shading over tank and between top of greenhouse. Preferably have a layer close to the top of the greenhouse which is fixed and a second layer in close proximity to the tank which can be easily moved and fixed to control light intensity.

Have two airlines for each row of baskets in the tank. These should have fine pores (ca. 0.5mm) placed at intervals of a few centimetres. There should be a number of valves to regulate the air pressure entering the tank. In general the settlement tanks are just

deep enough to cover the baskets by a few centimetres. It is not practical to stack baskets for settlement purposes.

Fill tank with seawater filtered to10-20μm. This will allow natural diatoms to pass through the filter and act as an inoculum. There should be a minimum flow rate of 5 litres per minute through the tank.

Table 5. 1. Preparation of nutrient solutions for diatom bloom in settlement tanks.

Turn off water flow through tank each morning. Turn off or reduce air flow.

Add 30m - 50ml. of Solution A directly into the tank. Then add 30ml - 50ml of Solution B diluted in 0.5 - 1 litre of freshwater into the tank. The Sodium metasilicate solution will clump in the tank if it is not mixed with freshwater. This will reduce its effectiveness as a nutrient.

The air and water supply should be returned to normal rate after 3-4 hours.

Repeat daily until a light brown colour becomes visible to naked eye on the plate and tank wall surfaces. This should take 2-4 weeks, depending on light intensity, water temperature and density of diatoms in the incoming water. This repeated process of turning off the water and adding nutrients is actively encouraging the bloom of benthic diatoms in the tank.

Continued addition of nutrients is optional thereafter.

Use of the mesh shading layer closest to the tank is also optional. However if there is any appearance of green microalgae on the top of the water layer, baskets or plates then shading should be maximised immediately.

Solution A	100g Sodium Nitrate ($Na\ NO_3$)
	100 ml of liquid plant nutrient (LGP, Baby-Bio etc)
	Make up to 1 litre with distilled / de-ionised freshwater
	Prepare at least 5 litres of solution
Solution B	100g-350g Sodium Metasilicate ($Na_2\ SiO_3$)
	Make up to 1 litre with distilled / de-ionised freshwater
	Prepare at least 5 litres of solution

As the diatom film develops it is advisable to turn the rack of plates upside down to get a coating on the bottom portion so as to increase settlement and feeding area for the larvae.

Timing of benthic diatom layer production should be planned to

coincide with spawning.

5. 5. 2. Transfer of larvae from hatchery to settlement tanks

In general the competent veliger larvae are transferred on the fifth day after fertilisation assuming that the rearing temperature has been maintained at 18-20˚C, although this varies with abalone species. The settlement tanks should be prepared as described above. It is best to have a thin, single layer, no more than a 'dusting' on the plate and tank surfaces. Heavier coatings tend to become 3D and heavily matted. There is a risk of the newly settled juveniles being 'smothered' by such a heavy growth. This type of growth can also leave anoxic areas on the plate surfaces resulting in juvenile mortality. However if tanks have reached this condition then proceed with the settlement nonetheless.

The temperature variation between the larval rearing containers and the settlement tanks should be the same or at most varying by just a few degrees. During peak summer conditions they will be quite similar while at other periods the settlement tank temperatures may need to be raised using immersion heaters or warm water from a boiler heating system.

The larvae should be examined for 'crawling' and 'gliding' behaviour on the morning of transfer. This is observed under the microscope as the larvae using its protruding foot to move short distances while in contact with the slide surface.

The larvae can be either drained or siphoned into ca. 90μm sieves immersed in water. If draining via the valve at the bottom of the tank ensure the flow is not strong enough to damage the veligers when coming in contact with the mesh surface. When removing the larvae it is essential to have a trickle of filtered seawater to wash them down from the 'banjo' sieve and walls of the culture vessel.

This can be via a narrow hose or tube from the larval water supply or by using a plastic wash bottle. Failing to do this can result in the loss of up to 50% of larvae as they readily adhere to the surface of the rearing bin at this stage.

When a tank has been drained or siphoned the larvae should be gently washed into a graduated bucket. From this known volume 3 to 5 aliquots should be taken and counted under the microscope using a Sedgewick-Rafter or similar counting slide. It may be necessary to use a drop of 10% formalin solution if they are particularly active. Counting is necessary to estimate the total number of larvae to be distributed between available tanks.

Experienced hatchery operators will know the amount of larvae to put into their settlement tanks and they will also know what spat yield to expect at a specific time after settlement. It is difficult to recommend exact larval numbers per tank but more often than not

a specific tank design has a production capacity above which it can rarely exceed.

The hatchery operator should be looking to establish the minimum larval quantity necessary to achieve this figure. Thus if the nursery tank normally produces between 15,000 - 25,000 juveniles after four months then it is better to put in 250,000 larvae to attain this as against putting in 500,000 for the same result.

Water and air are turned off and the water level is dropped slightly. They are left off until later that day when settlement has been observed. They are then turned on at low levels.

Many Japanese hatcheries culturing *Haliotis discus hannai* recommend adding 1,000-4,000 larvae per m^2. This would then yield between 100-700 juveniles per m^2 at an average of 5mm shell length (Roberts, 2000). This is in fact not dissimilar to figures of 30-60 spat per plate observed in Irish hatcheries when they have a shell length between 3mm-10mm. For the above figures per m^2 or per plate to be achieved it is assumed that there is maintenance of a reasonable coating of diatom film over the period. However diatom films can be overgrazed through the addition of too many larvae, as a result of poor growth conditions or from copepod build up in the tank. They are also very sensitive to environmental changes within the tank. Rapid temperature fluctuation, loss of aeration or loss of water supply can result in complete collapse of the layer. Apart from this resulting in no food for the juveniles there will also be a detrimental loss in water quality and both can contribute to heavy mortality.

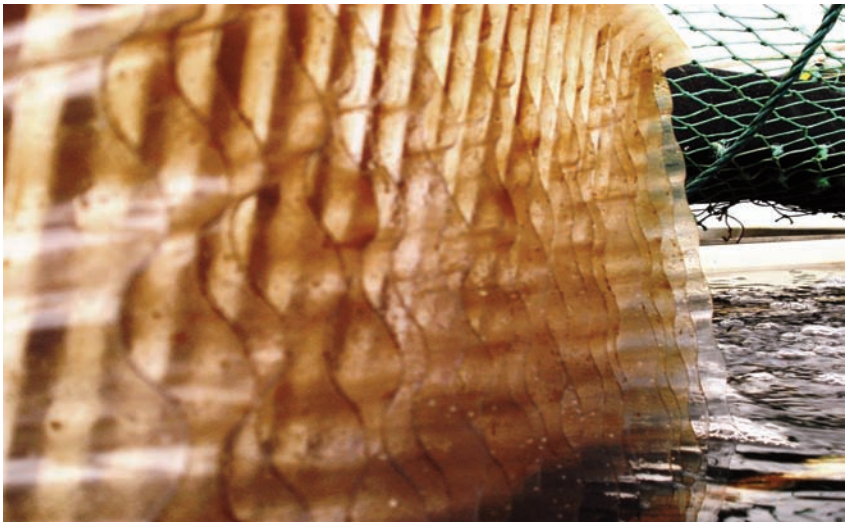


Plate 13: Close up view of plates in basket with recently settled juveniles visible at plate edges.

If there is diatom overgrazing or diatom collapse then the remaining or surviving juveniles will require supplemental diatom feed unless the spat can be quickly transferred to a weaning system and can be supplied with a suitable artificial or seaweed diet. If there is a heavy collapse it is best to siphon the bottom of the settlement tank

immediately or indeed if the juveniles are a few mm in length and the air temperature is not too high in the nursery unit then the tank should be drained and refilled quickly. Benthic diatom cultures can be used as an alternate food source at this stage and overcome the stress of transfer to a new system followed by the weaning onto a new diet.

There is a risk of heavy copepod infection in the tank if it is not cleaned immediately after diatom collapse.

Plate 14: A settlement area at Brandon Bay Seafoods. Note various shadings.

Plate 15: A settlement area at Mara Chleire Teoranta.

Chapter 6: Culture of benthic diatoms

6. 1. Introduction

Benthic diatoms can play a very important role in the abalone hatchery/nursery if used in conjunction with well managed naturally occurring biofilms on the settlement substrata. It is also very plausible that through correct diatom species selection abalone could be produced throughout the year, albeit on a smaller scale, without the need to use natural biofilms. As already mentioned some diatom species are an excellent source of food as is the extracellular mucous that some secrete. Other diatoms actively encourage settlement while being of little benefit as a food source. Over a period of a few years intensive culture of benthic diatoms was undertaken at the Shellfish Laboratory in Carna. The specific aim of this work was to mass produce benthic diatoms and assess their benefit on juvenile abalone growth and survival. Trials were also carried out to evaluate the nutritional benefit of different species. Procedures for the culture, maintenance and upscaling of these benthic cultures were established. These techniques may differ somewhat to those used in other hatcheries but they outline the basic principals of benthic diatom culture. Some of the methodologies used and results obtained during this work are included in this section.

6. 2. Establishing routine stock cultures

In any fish hatchery using microalgae it is essential to have a series of stock cultures which can regularly be used to start larger scale cultures and be in reserve in case other cultures collapse. Stock cultures must be hygienic and healthy and it is essential to avoid cross contamination with other species.

A batch culture technique was used throughout (Brown et al., 1975) where a constant volume of microalgae is cultured within a particular container and there is no replacement of the culture medium. The batch culture technique relies on utilization of the log (exponential) phase of growth by the algal cells. Holding or stock cultures of the various microalgal strains were maintained in Guillard's F/2 medium (Guillard, 1975) in 500ml flasks and were then used to produce the stock cultures as inoculants for further upscaling as required.

Routine practices were developed for maintenance/holding of stocks and cultivation to 5 litre flasks as start up for expanded levels of production. The basic procedure is as follows:-

A stock culture medium is prepared in 2 litre batches. This medium consists of seawater, which has been U.V. treated and filtered to 5 μm, to which 2ml of a nitrate, a phosphate, a trace metal and a sodium metasilicate solution are added. This solution mixture is referred to as Guillard's or F/2 medium.

This solution is then poured into the 500ml conical stock flasks. These are then sealed with a cotton wool bung and a piece of tinfoil. These stock flasks are then autoclaved at 15lbs per square inch (1.05kg/cm2) at a temperature of 121˚C for fifteen minutes. This autoclaving can be carried out in a pressure cooker. Once cold the stock flasks are inoculated with 50ml of a stock culture of the particular diatom species. These are then grown under continuous florescent illumination for 24 hours to initiate growth.

Thereafter they are maintained under normal illumination until required for inoculation of new stock culture flasks. Normally a minimum of two stock culture flasks of each diatom species are maintained at any time.

Nutrient constituents and chemicals

Nitrates		
Distilled water		2 litres
Sodium nitrate	$Na\ NO_3$	150g
Phosphorus		
Sodium di-hydrogen phosphate	$Na\ H_2\ PO_4$	10g

1 ml added per litre of seawater for 5 and 20 litre cultures

Trace Metals		
Distilled water		2 litres
Ferric chloride	$Fe\ Cl_3$	3.8g
EDTA Sodium salt	Na_2 EDTA	8.7g
Trace Metal Solution		1ml

1 ml added per litre of seawater for 5 and 20 litre cultures

Trace Metal Solution		
Sodium molybdate	Na MO	0.46g in 200ml water
Copper sulphate	$Cu\ SO_4$	0.98g in 200ml water
Manganese chloride	$Mn\ Cl_2$	18g in 200ml water
Cobaltous chloride	$Co\ Cl_2$	1g in 200ml water
Zinc sulphate	$Zn\ SO_4$	2.2g in 200ml water

1 ml added into Trace metals above

Vitamin Mix		
Distilled water		1 litre
Cyanocobalamin B12		0.0005g
Thiamine B1		0.1g
Biotin		0.0006g

0.5 ml added per litre of seawater for 5 and 20 litre cultures

Silicate		
Distilled water		2 litres
Sodium metasilicate	$Na_2\ SiO_3$	228g

1 ml added per litre of seawater for 5 and 20 litre cultures.
This is prepared separately from F/2 medium

Table 6. 1: Guillards F/2 Medium and Silicate solution for diatom culture .

6. 3. Upscaling benthic diatom cultures

During the initial trials it was estimated that abalone post larvae would require up to 1,000 litres of benthic microalgae at 5-7 day intervals to maintain an adequate biofilm during peak grazing periods in the standard 3m^3 Fasttank® nursey unit. However smaller quantities were sufficient to cater for lower post larval biomass during earlier development.

Initial microalgal upscaling was carried out according to established practices using 500ml stock cultures to inoculate 5 litre flasks. These 5 litre flasks were used as inocula for 20 litre flasks after 5-7 days while still in the exponential phase of growth. In turn the 20 litre flasks were used as inocula after 5-7 days to upscale to 1,000 litre levels in circular white 1m^3 polyethylene tanks. The entire process followed a series of standard practices outlined below:

- Stock cultures of each strain were held in 500ml flasks in Guillard's F/2 medium

- Sub-cultures were established by addition of 50ml of the stock to similarly prepared 500ml flasks which were grown for a week under continuous illumination and thereafter could be maintained for several weeks as inocula for 5 litre cultures. Culture room temperature was maintained at ca. 13-14˚C.

- Flasks with 5 litre of UV treated seawater, filtered to 5μm were autoclaved (other sterilization techniques can be used) and nutrient solutions of nitrates, trace metals and sodium metasilicate were added at 1ml per litre of seawater and a vitamin solution at 0.5ml per litre.

- Each flask was then inoculated with 500ml stock culture and grown for a week. Culture room temperature was maintained at ca. 13-14˚C.

- Twenty 20 litre flasks were likewise prepared and inoculated with a 5 litre culture grown for a week and these in turn were used as inocula for 1000 litre containers during the normal production cycle 1,000 litre bins were thoroughly cleaned and rinsed out with hot water from a power washer. They were then filled with 1μm filtered seawater and sterilized by adding 10ml of sodium hypochlorite which is neutralized after 24 hours with 10mg of di-sodium disulphite dissolved in 50ml of distilled water (for at least one hour).

- After an hour nutrients were added as 250ml of a nitrate/phosphate solution and 250ml of a sodium metasilicate mix and the tanks then inoculated with 20 litre cultures, these were then grown under continuous illumination (Mercury Vapour Lamps) and vigorously aerated to prevent cell adherence to the tank walls for five days before harvesting. The tank walls were also regularly brushed down. Tanks were then cleaned and prepared for the next weekly cycle.

6.4. Maintenance of biofilms

During the early stages of this work benthic diatom production was some 3,000 litres per week. This microalgae was produced in the algal mass culture room of the Shellfish Research Laboratory. Later this production had increased to 7,000 litres per week.

After inoculation of the 1,000 litre bins it was observed that in general they were harvestable after four days. However the concentration of cells was greater after five days and usually this was when they were harvested. It is essential when culturing diatoms to establish timing of peak cell concentration. Counts should be carried out using a haemocytometer on a regular basis.

A second algal production unit was established outside the mass culture room in an area directly adjacent to the settlement tanks. This consisted of five 1,000 litre bins on a raised platform with four Mercury vapour lamps suspended overhead. Identical procedures as those already described were followed.

However, this nursery building was a lean-to type shed and as such, had very little insulation and lacked the general hygiene of the algal mass culture unit. Little difficulty in microalgal production was encountered although it was noticeable that production rates slowed as ambient air and seawater temperatures dropped.

Production volumes were determined by the grazing rate of the post larvae on settlement plates and tank surfaces. This was assessed through both visual and microscopic examination of surfaces.

As the production system was modular, increased levels could be phased in response to requirements. During this research all five species of diatom were cultured to 5 litre levels but only two of these were routinely taken to 1,000 llitre stages. It was observed that *Navicula sp.* clumped less in the flasks and culture bins and also had a stronger cell concentration than the others. Similar qualities were observed for an isolated *Nitzschia sp.* When

selecting a diatom sp. for bulk culture it is important that in addition to being of high nutritional value, the particular strain is also adaptable to mass culture techniques. These diatom cultures were fed to the abalone nursery tanks using a submersible pump. The culture tanks need to be brushed during pumping to ensure that cells adhering to the bin surfaces were fed to the abalone.

The water level in the nursery tanks was dropped prior to feeding and the aeration was turned off overnight. This allowed easier attachment to the Fasttank surface and corrugated settlement plates. This process may slightly affect water quality but no resultant mortalities were observed.

A parallel feeding trial was also conducted to determine which strain of diatom or microalgae would promote best growth and survival. Seven microalgal species were compared during these trials. The trials were carried out in white 15 litre buckets and the abalone feed was not limiting.

Data obtained during this trial was statistically analysed and the two strains providing the best growth rates were a *Navicula* and *Nitzschia* species respectively. While they were not significantly better than all strains it is likely that they would have been if the trial had been conducted over a longer period of time or indeed if the trial had been initiated at a smaller shell length for the juveniles. It was also of interest to note during the course of this trial that *Tetraselmis suecica* and a species similar to related to *Ulvella lens* were both a poor food source for the juvenile abalone.

6. 5. *Ulvella lens*

Ulvella lens is a green macroalgae that is found naturally in many of the Southern Hemisphere abalone producing countries including Australia, South Africa and Chile among others. It is crustose and grows as prostrate rosettes forming a very tough macroalgal layer on settlement plate surfaces. Many hatcheries in the Southern Hemisphere will reuse their settlement plates on a constant basis in order to keep this *Ulvella lens* coating. When juvenile abalone are being deplated they are brushed off in salt water baths so as not to damage this macroalgae. Pregrazed films of *Ulvella lens* produced greater than 90% settlement and metamorphosis of *H. d. hannai* larvae while ungrazed *Ulvella films* induced 60-70% settlement and attachment (Takahashi and Koganezawa, 1984). In Ireland, while there have been recordings of similar species *Ulvella lens* itself does not appear to be present. Even if it is present it does not form the plate coatings as it does in the aforementioned southern hemisphere countries.

Ulvella lens is not a diatom and its life cycle is completely different. The adult macroalgal phase is referred to as being a sporophyte. To reproduce they release spores which are referred to as zoospores.

The zoospores are pear shaped with a red eyespot. They have four equal flagella which protrude from one end. After settlement they lose their flagella and after 24 hours they germinate and by the third day become a two-three cell germling. By the fourth day they become a 4-8 cell disc and by the eighth day this disc has an average diameter of 26.6μm. Initial zoospores can be recognized on these germlings by the time they have a 30μm disc diameter. By the nineteenth day when they have an average diameter of 76.6μm (60-107.5μm) they are capable of releasing zoospores.

There are two major factors that influence the release of Ulvella zoospores. They require a minimum temperature of 10°C while the ideal temperature range is between 15°C and 20°C. In this temperature range zoospore release is most intensive on the fourth to fifth day.

The closer the temperature approaches 20°C then the larger the zoospore release should be. They also need light to initiate a release and adult sporophytes can be prevented from releasing zoospores by keeping them in the dark.

6. 6. Culture of *Ulvella lens*

Ulvella lens has only been used in one Irish hatchery with a moderate degree of success so far. Attempts to culture it followed on from a sample being purchased from Hobart, Tasmania. A brief outline of the culture techniques used and also those used elsewhere will be outlined but as such it should be remembered that these techniques are only being developed and may be modified later.

Like diatoms or other microalgae there is a need to maintain stock cultures of *Ulvella*.

However, these stock cultures are not held in 500 ml conical flasks but on settlement plates or other surfaces within holding tanks. It may be necessary to build up these stocks over a period of time. They can be held in tanks that are shaded or indoors where light intensity is quite low. Water entering each tank should be filtered to 1μm to prevent diatoms and other organisms such as *Spirorbis* entering the culture. Nutrients can be added and a Guillard's F/2 medium with silicate has been used for this purpose (Daume and Ryan, 2004). A commercial plant food manufactured in New Zealand called Aquasol can also be added at 80mg/l (Krsinich et al., 2000).The water in these culture tanks should be changed weekly. Fresh plates should be added periodically to increase the percentage of *U. lens* cover within each tank.

To produce mass release of *U. lens* zoospores the plates are held in tanks with 70-100% shading for a period of two weeks. To further aid the mass release of zoospores the plates can be desiccated for

three minutes before being transferred into a static (no water flow) tank with clean plates and full daylight (no shading). The seed plates should be placed between clean plates throughout the tank. Zoospore release should commence within a few days and reach its maximum on the fourth and fifth day. Zoospore release tends to be greatest in hour proceeding first light. It can be observed as a yellow layer on the water surface if aeration is off but it is better on low to break up and disperse this spore aggregation.

Plate 16: Tank containing adult seed plates of *Ulvella lens* (Courtesy Boet Mor Seafoods).

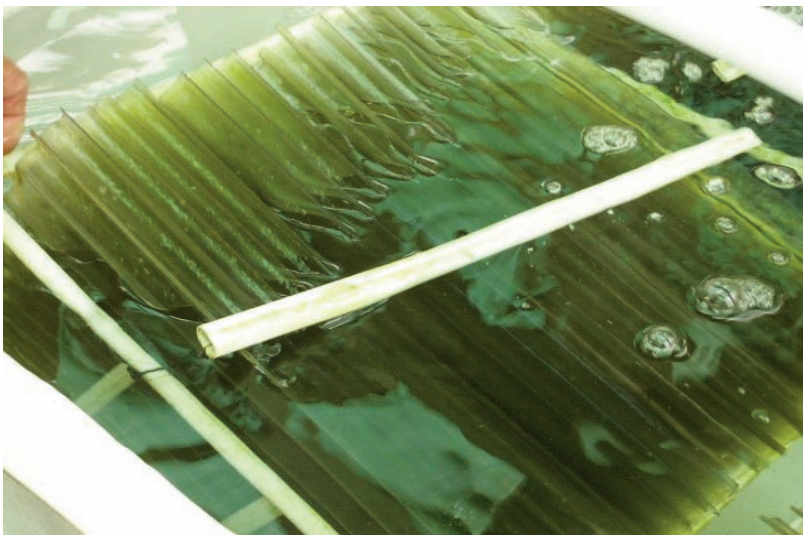


Plate 17: Basket of settlement plates heavily coated in *Ulvella lens* (Courtesy Boet Mor).

Nutrients should be added during this period and an F/2 and silicate mix is recommended at 40g/1,000 litres (Daume and Ryan, 2004). The seed plates should be removed after about eight days and returned to their holding tanks. This will help prevent introduction of competing algal species that may be growing on them. Water should be turned on after about ten days and larvae introduced ca. 1 week later.

6. 7. The role of benthic diatoms and *Ulvella lens* in abalone nursery culture

This project conducted at the S.R.L. was designed to improve juvenile survival during the nursery phase and also to establish a number of benthic cultures that could be of use to the industry. Survival was at most increased by a few percent. Most tanks would yield from approximately 3,000 - 6,000 juveniles which varies from 0.5% to 1-2% depending on the number of larvae introduced during settlement.

However, survivorship of 5% was achieved and this was attributed to addition of cultured diatoms after biofilm collapse. Traditionally at the S.R.L. 800,000 larvae were added per $3m^3$ (3,000 litre) settlement tank. In hindsight this was probably 4 times too many veligers.

While it was disappointing that survival levels were for the most part similar to those achieved in previous years, a number of positive and explanatory points were taken from this research. It appears that the mortality of the juvenile abalone occurred during the first three months after settlement and in particular this has been observed between 3 and 8 weeks (Leighton 1989, Roberts et al., 1999). These observed mortalities are thought to be primarily due to diet.

Kawamura et al. (1998) suggest that post-larval abalone cannot digest the diatom cell contents in their alimentary canal without first splitting open the cell wall with the radula. In that study they also observed that the size of the post larvae also affected their ability to digest particular diatom species. They observed that small post larvae (< 800μm) could not ingest *Cocconeis scutellum var. parva* because of this species high adhesive strength while at 1mm - 2mm they could simultaneously tear off and break open the cells with their radula. It has also been noted that the extracellular mucus of diatoms appears to be an important source of food for early post larvae abalone up to approximately 800μm (Kawamura and Takami, 1995; Kawamura et al.,1998).

The results of research at the S.R.L. showed that *Navicula* and *Nitzschia spp*. promoted best growth in the juvenile abalone. All of these diatoms had a maximum length of =10μm. Coincidently, it was also the case that these three strains of diatom were also best suited to bulk culture. While the three better performing microalgal strains of the trial were identified as being a *Navicula sp*. and two *Nitzschia spp*. and these findings are in agreement with other studies (Kawamura et al., 1998), this work did not address the most suitable species for early post larval juveniles. The initial size range of abalone used (ca. 5.5mm-7mm) was quite large and at that stage the juvenile abalone should be capable of removing all adhering diatoms from their surfaces, although the extent of cell

content digestion thereafter is speculative. However, Roberts et al. (1999) point out that as the mouth width of *Haliotis iris* is 30*µ*m two days after metamorphosis when the juvenile has a shell length of 350*µ*m the size range of diatom used in this study is potentially useful as a food source immediately post settlement.

Navicula britannica
Navicula ramosissima
Coccensis scutellum
Cylindrotheca closterium
Achnanthes brevipes
Achnanthes longipes
Amphora angusta
Nitzschia laevis
Nitzschia longissima
Nitzschia ovalis
Stauroneis constricta
Navicula ulvacea
Navicula cf. jeffreyi
Navicula incerta
Navicula agnita
Nitzschia divergens
Nitzschia thermalis var minor
Nitzschia pseudohybrida
Amphiprora paludosa var hyaline

Table 6. 2: Some of the principal cultured diatom species used in juvenile abalone production worldwide.

Over feeding and an excessive layer of biofilm can also lead to heavy mortality especially in the period of a few weeks after settlement. Some of the reasons suggested for this are 'smothering' (Tong and Moss, 1992), the obstruction of juvenile mobility, the harbouring of micro-predators (Ebert and Houk, 1984) and the creation of adverse water quality in the diffusive boundary layer (Searcy-Bernal, 1996). Considering some of the above factors, it seems crucial that at no stage, with the possible exception of the two days immediately after settlement, should the juvenile abalone be without food. During the first two days after settlement they are between lecithotrophic and microalgal grazers and energy is being supplied from yolk reserves. Thereafter a reliable food source is essential and any period without food will result in heavy mortalities. It would appear that there are two options which should increase current survival rates. Firstly, prior to introduction of competent larvae into settlement tanks, a natural diatom coating should be encouraged to bloom. However, this coating should not be too heavy to reduce the risk of 'smothering' It should be just a light 'browning' or 'dusting' of the plates. After settlement this

coating should be carefully monitored and a short period later, while this biofilm still exists, artificially cultured diatoms should be added. These species should be from the genera *Navicula* and *Nitzschia* with a maximum length of 10μm and should be suitable as a nutritional food source at this early stage. Thereafter careful monitoring of feeding surfaces will indicate the need to increase microalgal production as the abalone increase in size.

An alternative as suggested by Roberts et al. (2001) would be to induce settlement using an alternative to a diatom coating, these being GABA or mucus trails, and then after two days the introduction of artificially cultured diatoms. Roberts also suggests (pers.com) the addition of *Nitzschia ovalis* cultures for settlement induction and then *Nitzschia longissima* cultures thereafter as a food source. The removal of fine filtration, addition of nutrients and correct use of shading should then encourage growth of natural benthic diatoms.

Using *Ulvella lens* during the settlement phase would create a number of distinct advantages over the methods already described. This will be dependant on the establishment of reliable mass culture techniques for this macroalga. It has been demonstrated that *U. lens* encourages large scale settlement and metamorphosis of a number of abalone spp. including *H. d. hannai.* It can do this without the presence of diatoms, mucous or other settlement stimulants. It is less certain of its nutritional value to the newly settled juveniles at this early stage. It is a crustose green macroalga and as such it is likely to be too tough for the radula to scrape off the settlement surfaces. However a coating of *U. lens* does not preclude coatings of benthic diatoms. In fact, benthic diatoms can grow over the *Ulvella* layer without adverse affects to either. As a result, it is possible to establish a layer of natural or cultured benthic diatoms over *U. lens* just after settlement. It is thought that *U. lens* is quite a poor source of food until the juveniles are over 40 days old (Daume and Ryan, 2004). Thereafter it seems to be similar to most other diatom species. These same authors also suggest that using plates cultured for eight weeks results in heavier settlement as compared with plates coated for six weeks. It would seem advisable to have plates coating with *Ulvella* for as long as possible before settlement.

Another advantage of utilizing *U. lens* during settlement is its durability. It is crustose in nature and is not as susceptible to collapse within the tank as are diatom films. Poor water quality or heavy copepod infestation can lead to a complete diatom collapse with detrimental consequences for juveniles of a few mm shell length. *U. lens* is more resilient to periods of poor water quality and is too robust to be effected by copepods.

6. 8. Recommendations

1. Isolate or purchase a number of diatom cultures, in particular needle *Nitzschia's*, that are proven to be beneficial to juvenile abalone.

2. Conduct trials to establish which of these diatom species promote optimum growth at specific shell lengths.

3. Attempt to achieve higher microalgal culture densities than were attained at the S.R.L. This may be possible through light intensity manipulation, the addition of CO_2, using Jorgensen's culture medium instead of Guillards F/2 or various other factors.

4. Investigate the possibility of purchasing 'off the shelf' or proven diatom culture systems that should greatly simplify this process.

5. Aim to standardise the diatom culture procedure throughout the industry

6. Establish a methodology to mass culture *Ulvella lens*

7. Ensure this technology is incorporated into production of abalone juveniles in all hatcheries.

Chapter 7: Management of juvenile abalone during the early nursery phase

7. 1. Introduction

Management during the juvenile nursery stage is aimed at maximising survival during this most crucial of phases. This is the period after which perhaps 200,000-800,000 competent larvae have been transferred to this tank for settlement. It is possible to double or treble output by adopting correct management strategies. Such management techniques can be labour intensive and time consuming and depend very much on staff availability.

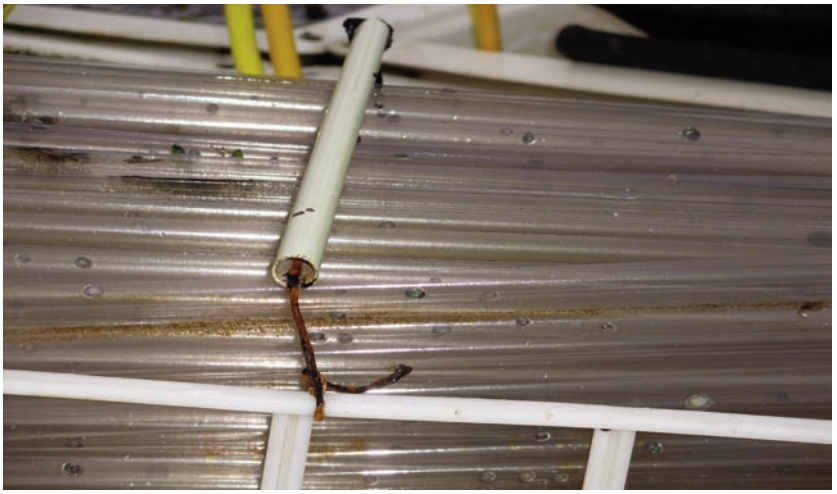


Plate 18: Juvenile abalone starting to overgraze plates (Courtesy Brandon Bay Seafoods).

However, while such a strategy is recommended it is by no means essential. Nursery tanks can almost be left untouched for long periods save occasional siphoning or draining to clean them. Generally, the tank will yield a low but steady supply of juveniles. It is very much up to the hatchery operator and the specific amount of spat required.

7. 2. Estimation of settlement success

About two weeks after settlement, carry out a guesstimate on the number of juveniles in each tank. Select a basket in the tank and assign it a number e.g. B7 (Basket 7). Choose 2 to 3 plates in this basket. Again, assign a number e.g. P1, P8, P19. Take out the plates, find a suitable angle with the light to see the juveniles. At this stage they are small (<1mm) and appear as no more than white flecks or specks. Using a hand counter, run your eye along each groove and click. Count one side of plate then turn it over and repeat. Repeat for another plate and a third if necessary. Do the same with a number of other baskets and get an average number of juveniles per plate and multiply by the total number of plates in the tank. In general 3-5 baskets should be sufficient to give an

accurate assessment of juvenile numbers in the tank. Replace the plates and baskets in the order and position that they were found in. This counting process can be repeated on a weekly or fortnightly basis and gives the grower an accurate idea of the amount of spat in a tank. It will also give an indication of mortalities should they occur and indicate time to transfer the abalone from the tank to another growing system.

7. 3. Monitoring of benthic diatom film

If the above procedure is being carried out then it will also enable the grower to assess the amount of diatom covering on the plates. Quite often it is possible to achieve a balance whereby the amount of grazing and the amount of diatom replenishment are the same. This is the desired and ideal situation. However, overgrazing can result in the diatom film being completely removed from the plates. If this happens the grower has a number of options which will, to an extent, be dictated by the shell length or size of the seed. If the seed are less than 3mm handling is quite difficult. Once the seed are 5mm or over they are quite robust.

If the diatom film is over-grazed and the juveniles are ca. 5mm then they are large enough to transfer out of the tank. They can be brushed off the plates into a weaning system or alternatively, they could be brushed into other settlement tanks with adequate diatom coating. If it is apparent that the diatom film was removed as a result of over-grazing then these animals should be split between two tanks. If the diatom film collapsed as a result of water quality issues or copepod infection then the priority should be to remove the decaying material from the bottom of the tank and also the plate surfaces. The causative factor must be addressed whether it is air or water supply etc. Again if the seed are over 5mm they can be moved to another growing system. If the seed in the tank are less than 5mm (e.g. 1.5mm - 4 mm) it is more difficult to transfer them. In such a situation it is preferable to add cultured diatoms to the tank until they are large enough to transfer. However, they can be brushed off if absolutely essential and transferred to another diatom coated settlement tank.

If seed are left unattended after diatom collapse then these will be inevitable mortalities. However, it is unlikely that all juveniles will die. After a period macroalgal species will start to colonise the tank walls and plate surfaces and many of the remaining juveniles will be able to utilise this as a source of food. Months later there should be a few thousand juveniles available for transfer to a weaning or growout system.

The hatchery manager should, through experience, be aware of the holding capacity of the particular settlement tank design in the nursery. Plate counts within weeks of settlement would indicate if the tank was correctly stocked. If overstocked then plates could be transferred to other tanks. Within a few weeks of settlement the

juveniles would be too small (ca. 1-2mm) for brushing off. To overcome that, a settlement tank without juvenile abalone but with plates and baskets, is bloomed up. Every second plate from each basket is then exchanged from the tank of juveniles to the empty tank. Plates with seed are replaced with fully coated plates from the empty tank. After a period the spat will start to distribute evenly throughout both tanks. This protocol should prevent over-grazing at a later stage. This will yield a certain amount of success. Alternatively, pump in cultured diatoms until they reach 3mm and move them to a weaning system on a powder diet. Similarly, if the seed are ca. 5mm and the tank is starting to be over-grazed and there are no weaning systems or growout systems available, then juveniles could be brushed from plates into a new fully coated tank. It is advisable for the hatchery operator to have a number of spare settlement tanks available at all times for such transfers, as described above.

If stocking densities, light intensity and water quality issues have been adequately managed, then there should be no need for spat transfer or supplemental diatom feeding during this period. The abalone can be left in the tanks for up to six months or more. This is dictated by weaning or growout space available. The juveniles can be removed at ca. 5mm - 6mm and transferred to weaning systems.

Alternatively, if they remain in the tank for a longer period then they may be transferable directly to growout systems. If the latter approach is being pursued then it is advisable to wean the spat in the tank onto seaweed for a period before removal. This is done by placing fronds of the brown kelp *Laminaria digitata* along the basket tops and edge of the tank on a weekly basis. If the tank contains *H. tuberculata* then dilisk or *Palmaria palmata* should be used instead. Generally the juveniles will crawl up from the plates and the tank bottom during the evening and night to feed.

When feeding these tanks with seaweed it is necessary to clean them more frequently. Normally with diatoms, very little cleaning is necessary. However, when the juveniles feed on seaweed there is a significant amount of faecal material produced which sinks to the tank bottom. This will decay, hydrogen sulphide (H_2S) will be formed and pockets within the tank could become anoxic. Depending on the amount of seaweed being fed, cleaning should be carried out between 2 weeks and a month. For cleaning it is a good idea to have a 25mm - 50 mm hose in the facility to provide pressure to remove the material and also to permit rapid refilling of the tank. The baskets can be removed completely or carefully stacked on each other in the tank to allow access to the tank bottom. If these settlement tanks are in a greenhouse, great care should be taken when cleaning on sunny days. The air temperature is likely to get quite high during the late morning and this can result in rapid desiccation of the juveniles.

Settlement tanks should be checked for air supply, water flow, temperature and 'crawl outs' on a daily basis. Crawl outs are those juveniles which are found a few centimetres above the water level of the tank. They should be brushed down in mornings and evenings.

Chapter 8: Protocol for conditioning, spawning, larval rearing and settlement

8. 1. Introduction

This chapter is intended to give a step-by-step guideline of the procedure in a moderate spawning using Tris and H_2O_2 that would yield between 1,000,000 and 5,000,000 larvae for settlement of either *Haliotis discus hannai* or *Haliotis tuberculata*. The number of larvae will be dependant on the quantity of gametes released and the larval survival rate thereafter. The theoretical spawning is being carried out on a Monday morning with settlement intended for Friday morning.

8. 2. Conditioning

- Condition 10-15 females and 4-6 males in the farms existing conditioning unit.
- Keep males and female separate.
- Hold at a maximum density of 4-5 per box.
- Select large (70mm-100mm) broodstock that are healthy and free of *Polydora* infection.
- Maintain the conditioner temperature at 17˚-19˚C.
- Feed and clean once or twice weekly.
- Provide them with a macroalgal mixture including *Palmaria palmata*, *Laminaria digitata* and/or *Ulva lactuca* and *Porphyra umbilicalis*.
- Leave in the conditioning unit for four to five months.
- Provide a 12L : 12D light regime.
- Monitor gonad development every three to four weeks.

8. 3. Spawning

- **Friday**
  Before spawning, thoroughly clean all equipment to be used. This includes larval rearing bins, airlines, airstones, buckets, siphons, plastic wash bottles, larval mixers, Sedgewick-Rafter slides, pipettes etc.
- Ensure reservoir and piping to and within hatchery are clean.
- Ensure the chemicals required are available and within date.

- **Monday a.m.**
- Prepare Tris and hydrogen peroxide (H_2O_2) solutions (See 3. 2.).
- Quickly rinse all equipment to be used in filtered seawater.
- Gently remove broodstock from conditioner.
- Ensure the UV unit is turned off.
- Place 3-4 females in each 10 litre bucket filled to the 8 litre mark.

- Divide the males between two buckets filled to the 8 litre mark. -
- Maintain water temperature close to conditioner temperare (17-19ºC).
- At ca. 8.15 a.m. add 52.8ml (8 x 6.6) of Tris to each bucket of females.
- At ca. 8.30 a.m. add 24ml (8 x 3) H_2O_2 to each bucket of females.
- At ca. 8.45 a.m. add 52.8ml (8 x 6.6) of Tris to each bucket of males.
- At ca. 9.00 a.m. add 24ml (8 x 3) H_2O_2 to each bucket of males.
- Turn off lights and leave hatchery.
- Observe abalone periodically using a low power torch
- at ca. 10.45 a.m. decant water and chemicals from buckets. Wash thoroughly and refill with filtered isothermal water.
- Monitor over the next one and a half hours for the initiation of spawning.
- Decant sperm to Pyrex beaker or similar container
- Siphon eggs through a pair of sieves (see 4. 2.), gently wash and transfer to buckets filled with clean isothermal seawater.
- Repeat this process until gamete shedding is complete
- Fertilise eggs in a number of buckets using high sperm density (20/ml - 30/ml).
- Decant off water, refill, allow eggs to settle and decant again.
- Briefly check fertilisation.
- Wash eggs gently on 75μm sieve.
- Complete this process inside 15 minutes, do not disturb developing embryos thereafter.
- After 45 min - 1 hour check fertilisation rate by the presence or absence of polar bodies or 1st cell division.
- Add freshly shed sperm solution if necessary.
- Leave the developing embryos undisturbed in the hatchery overnight with lights turned off.
- Clean and disinfect all equipment used and clean and disinfect surfaces in the hatchery.

- **Tuesday a.m.**
- Rinse all equipment to be used in filtered UV treated seawater.
- Start filling larval rearing bins, fit 'banjo' sieves, check for leaks etc.
- By 12 a.m. the trochophore hatch should be near completion.
- Start to siphon trochophores into buckets or sieves (90-150μm).
- Use plastic wash bottle to stop trochophores adhering to side of the bucket.
- Discard bottom 10-25% of bucket.

8. 4. Preparation of settlement tanks

- Initiated at least 2-3 weeks before larval settlement.
- Thoroughly clean plates, baskets and tank surfaces.
- Fill tanks with 10-20μm filtered seawater (allowing some diatom spp. through).
- Turn off water for a few hours daily.
- Add nutrient solution mix (see Table 6.1.) at beginning of this period.
- Adjust shading as diatom coating starts to develop.
- If green microalgae starts to coat plates then maximise shading.
- Rotate baskets in the tanks if necessary.
- If coating becomes too heavy then drain, wash down with seawater and refill.
- Before introduction of larvae ensure the temperature. is within a few degrees of the hatchery rearing temperature
- For most size settlement tanks introduction of a few hundred thousand competent veliger larvae is more than adequate.
- Turn off air and water for a number of hours or even until the following morning after larval introduction.
- Thereafter carry out plate counts to estimate settlement and monitor the benthic diatom coating regularly.

Chapter 9: Weaning stages

9. 1. Introduction

Initial abalone trials at the S.R.L., Carna did not incorporate weaning stages or weaning systems and indeed as already stated earlier, abalone juveniles can be transferred directly from settlement tanks to growout units, if they are close to or above 10mm shell length. However, commercial scale production does necessitate the use of weaning systems. Firstly, when such units are available to the grower, it means that the seed can be removed from the settlement tanks at ca. 5mm thereby freeing the tank for settlement of further spawnings. If spawnings were to be carried out between March and September then it should be possible to utilise each settlement tank twice over this period.

9. 2. Weaning tank design

Availability of commercial artificial diets has also played a major role in the development of weaning systems in abalone farms worldwide. While small abalone spat will wean on to softer weeds such as *Palmaria palmata* and *Ulva lactuca,* it is more difficult for them to do so on to the much tougher *Laminaria digitata*. However, they will readily wean onto crumb diets at this early stage. In fact many abalone species can be weaned onto powder diets at as little as 2mm - 3 mm shell length. This means that if a good weaning system design is used in conjunction with a powder or fine crumb diet the potential for increased spat production is enormous. Potentially the seed can be removed from the settlement tank in as little as three months or less. This means that the farm could potentially get three settlements annually per tank.

Weaning tanks are very varied in size and design in hatcheries throughout the world. There is as such no prototype weaning system and most farms will design their own. The design should facilitate ease of cleaning and observation of seed. Shelter for the juveniles must be provided where they can remain in relative darkness during the daytime. A feeding surface must be provided particularly if artificial diets are being used. There should be a good air supply and a water flow of a few litres per minute if possible. Most weaning trays tend to have a relatively small volume (50 litres -150 litres), are square or rectangular and usually little more than 9cm -10cm deep. Basically the water level will just cover the shelter provided for the juveniles. Many farms worldwide use black plastic corrugated plates as the shelter and feeding surface. The plates have perforations of a few cm on the surface that enables the juveniles to crawl through to access the food at dusk or night. Generally, they will have a series of vanes a few cm deep running perpendicular to the plate surface and parallel to the corrugation, which effectively keep the plate surface off the tank bottom and also provides attachment for the juvenile abalone.

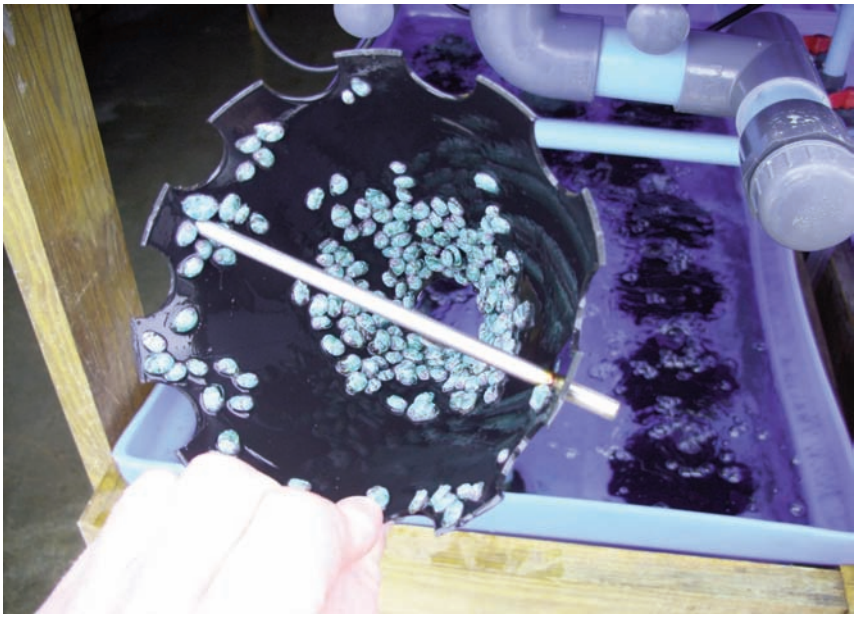


Plate 19: Weaning system in use at Mara Chleire Teoranta. In foreground is plastic weaning cone with juvenile *H. d. hannai.*

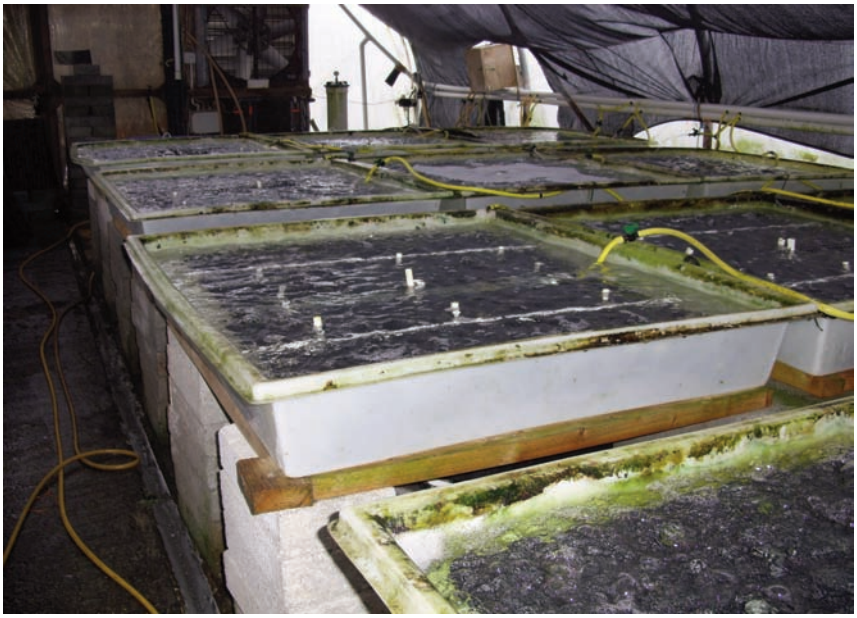


Plate 20: Weaning system of feeding plates and opaque polyethylene tray used at Brandon Bay Seafoods.

Normally weaning tanks are designed to hold between 2 - 5,000 spat at sizes between 5mm - 10mm.

The tanks are generally manufactured of polyethylene plastic or fibreglass with a white gel surface finish. If manufactured of white polyethylene plastic then it is possible to get a layer of benthic diatoms to coat on uncovered areas of the tank. This again has distinct advantages in weaning as these juveniles have just been removed from diatom covered plates. These diatoms can ease the

transition onto powder or crumb diets. The weaning trays can also be used to wean the juveniles onto seaweed at a later stage.

The length of time juveniles spend in the weaning system will very much depend on the size and density that they are placed into the tanks and the size that they are removed at. It is generally considered that all abalone stock on a farm should be graded three to four times annually and the juveniles in the weaning system should be treated in this way. One or more gradings should increase growth rates all round at this stage.

Weaning tanks generally need to be siphoned twice weekly, though one siphoning may be sufficient. Some tanks may have a bung placed centrally at the base of the tank and this may be used in the cleaning process. While most abalone farms will have their weaning tanks on a flow through system more frequently, they will be part of a recirculation system with recent advances in this technology. It is very important to ensure good water quality levels at this stage and more particularly if the weaning system is part of a recirculation unit. Overstocking, overfeeding, poor water circulation and build up of waste material will result in lower dissolved O_2 levels and reduced pH, particularily at the base of the tank. This will manifest itself as 'Shiny Shell Syndrome'. This is a silvering towards the whorl region of the shell. This is a result of the calcium from the outer layers of the shell dissolving. It does not adversely affect the abalone but if unchecked will result in fusion of the shell pores into slits. If it progresses further the slits will start to fuse and the shell structure will become extremely weak making handling almost impossible. If Shiny Shell Syndrome is observed then cleaning, aeration and flow rates should be increased. Stocking densities should also be reduced by grading and dividing the stock into other weaning units.

9. 3. Feeding artificial food

There are currently numerous commercial abalone diets available to the Irish grower. Many of these are French diets that are manufactured for *Haliotis tuberculata* but are also fine for *Haliotis discus hannai.* Companies including Adam and Amos, Abfeed and Le Gouessant are among the larger manufacturers of abalone food worldwide and their products have provided good results with all species tested. It is generally considered that high quality artificial diets, when correctly fed, will give a 1.2 - 1.3:1 Food Conversion Ratio (FCR). This compares very favourably with an FCR of ca. 20:1 for those fed on seaweeds (wet weight).

The principal constituents of an artificial abalone diet are proteins, lipids, carbohydrates, fibre and a binder. In general the protein source is from fish meal, soybean meal or casein. The amount of protein will vary with the diet type. Protein is utilised for growth and the amount used in the diet is generally ca. 30%. Too much protein in the diet can lead to water quality problems as the feed starts to breakdown. This

is particularly so if recirculation units are being used for growout or nursery stages. If fishmeal is being used as the protein source it is essential that the level is kept below 20%.

Carbohydrates generally make up 30%-60% of the abalone artificial diet. These are the main energy supply for the juveniles and the principal sources are wheat, soybean meal, maize and rice starch. It is considered that if the carbohydrate concentration is too high it may lead to poor protein utilisation.

Constituent	Range	Average
Protein	20-50%	30%
Carbohydrate	30-60%	47%
Lipid	1.5-5.3%	4%
Crude Fibre	0-3%	
Moisture		12%

Table 9. 1: Approximate composition of commercially available abalone artificial diets (Fleming et al., 1996).

Lipids have a very high energy value and are also a source of essential fatty acids and fat soluble vitamins. Abalone have a low lipid requirement as they are very efficient in its utilisation. If marine and vegetable oil are the principal sources they tend to be kept at 3%. *H. midae* has been observed to grow well with levels of 6% but in general lipid levels rarely exceed 5%. Abalone have a limited ability to digest fibre and quite often fibre is used as a binding agent in the food.

Binders are used to keep the food intact and the most common forms include starches, gluten or alginates.

The average stability of abalone feed is 2/3 days and they can lose up to 30% dry matter after 48 hours immersion. It is considered that diets supplemented with algal products tend not to be very stable. Diets containing a 1:3 agar/gelatine mix show the greatest stability, retaining ca. 70% of their dry weight after 24 hours immersion.

Feeding should be carried out as late in the day as possible. This reduces the amount of time that the food is in the water thereby reducing the amount of time for it to breakdown in the system.

Manufacturers will give specific feeding guidelines with their products. In general they will recommend feeding 1% - 2.5% of abalone bodyweight per day. They are also likely to recommend feeding every second or third day. When selecting a food it is better

to choose a smaller size pellet over a larger one as this reduces the energy the abalone expends in searching for it.

Food efficiency will depend on many factors such as diet type, temperature, species and the type of culture system being used. The juvenile abalone should be growing at a daily rate of 70-100μm. While there is much debate as to the advantages and disadvantages of feeding artificial feed versus kelp during the later culture stages, it does seem preferable to concentrate on artificial food during this period, while occasionally supplementing their diet with softer species of seaweeds. Plates 21 - 25 below show some of Adam and Amos range.

If a feeding plate is being used then it is quite easy to observe what is being consumed and the grower can then decide how much needs to be fed. It is advisable to remove uneaten food from the system every two to three days as at that stage it is breaking down, leaching and of little nutritional value.

It is probably best to leave juveniles in these systems until they are getting close to 15mm shell length. At this stage they are quite strong and robust and are able to withstand most handling. However, if this seed is being supplied to other ongrowing units then it is likely they will purchase the seed at a smaller size to reduce their outlay. It is unclear if this makes economic sense as mortalities will be higher in the earlier stages. It may depend on transport success and the type of system they are being moved too. At present spat of both species are sold for about 3.5 - 4 cent per mm or 35 - 40 cent for a 10mm juvenile.

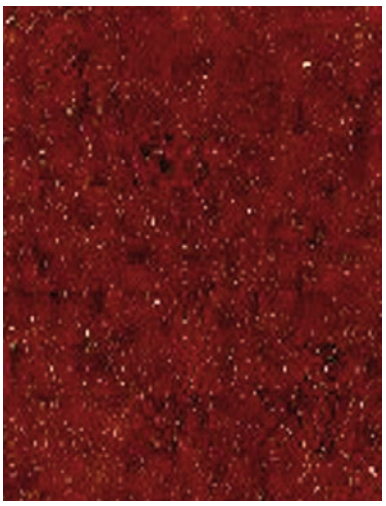


Plate 21: A plate powder.

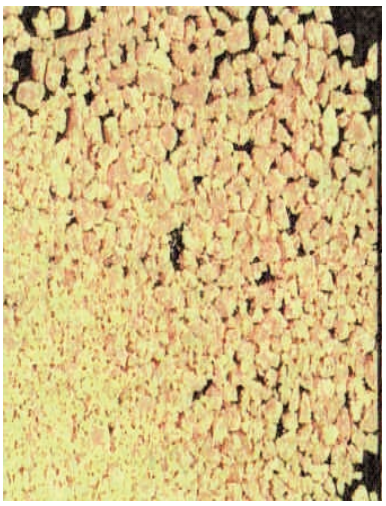


Plate 22: A coarse crumb powder diet.

Plate 23: A 5mm pellet diet.

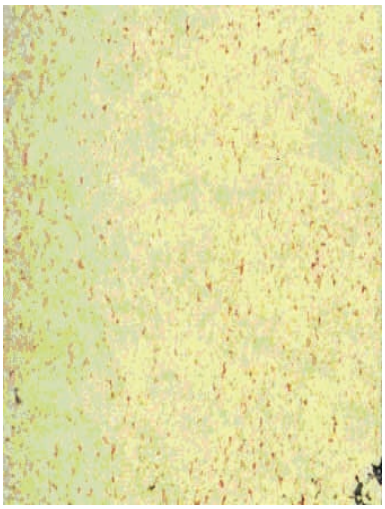


Plate 24: A fine crumb powder diet.

Plate 25: A broodstock diet.

Plate 26: Weaning plate at Boet Mor for artificial food with 2mm-4mm *H. tuberculata* on surface.

Plate 27: Black feeding plate for juveniles and adults (underside).

Plate 28: Top side of feeding plate.

9. 4. Handling of abalone spat

As a general rule the juveniles get stronger and more resilient to handling as they increase in size. When they are just a few mm in size it is best to avoid handling. If necessary, then a soft bristle paint brush should be used. It should be gently pushed along the grooves of the corrugated settlement plates.

When they reach a shell length of 3mm+ they can be removed by hand. They can in fact be removed by hand through most of their life cycle if they are removed soon after being taken from the water. Juveniles of 15mm+ can be more difficult to move particularly if they do not shift with the first pushing motion. Excessive pushing can result in the shell being torn away from the retractor muscle and the rest of the body. Even if this doesnt happen excessive pushing will stress the abalone for a long period. Then it may be necessary to use a blunt object such as a specially designed spatula or a butter knife.

It is best not to use excessive force with these as they may actually remove a slice of the foot and this is likely to lead to the abalone bleeding to death.

Several narcotising agents have been used. The abalone are immersed in these and after a period of time they release their grip and fall off their attachments.

9. 4. 1. Benzocaine

This is the shortened name for Ethyl-p-aminobenzoic acid ($NH_2C_6H_4COOC_2H_5$). A stock solution is made by dissolving 100g of Ethyl-p-aminobenzoic acid in 1 litre of 95% ethyl alcohol. A working solution can be made by adding 0.5ml to 1ml of the stock solution to 1 litre of seawater. A larger solution can be made by adding 1 litre of the stock solution to 1,000 litres of seawater in a large container. It is probably the most efficient narcotising agent particularly for *H .d. hannai* and is used in most countries. It does tend to cause irritation of the eyes if used for long periods, this may be problematic if large scale grading is being undertaken.

9. 4. 2. Magnesium sulphate

Magnesium sulphate salt is used very successfully in South African hatcheries to narcotise *Haliotis midae*. It is used at a ca. 4% ratio (30kg - 40kg /1,000 litres) and the abalone have excellent recovery rates. It has also been used successfully for grading spat of both species in Ireland. It is relatively cheap to purchase and very user friendly.

9. 4. 3. Carbon dioxide

Carbon dioxide has also been used to successfully narcotise both abalone spp. in this country. The water is supersaturated with the gas and abalone release their grip after a few minutes.

9. 4. 4. Other anaesthetics

Ethyl carbamic acid or urethane, chloral hydrate and diethylbarbituratic acid have also been used as narcotising agents but are not that successful and the juveniles show a slow recovery rate. Acetic acid (CH_3COOH) or common table vinegar can be used to remove juveniles from surfaces though its effect is less pronounced with larger abalone. Basically household vinegar is diluted to a 10% solution with water and put into a spray canister. It seems that it is more of an irritant than a narcotising agent but recovery rates are excellent if the juveniles are placed back into seawater quickly.

9. 5. Grading spat

Grading is a very important part of the rearing cycle on the abalone farm. It is recommended that it is carried every three to four months or three to four times a year.

The process is extremely labour intensive and particularly so if there is a large amount of stock on the farm. It is best to have a number of staff trained in the process. It is also likely that it will be carried out at off peak times during the farm cycle. Grading increases productivity on the farm. Abalone are graded into various size classes thereby reducing the competitive advantage that larger ones have over their smaller counterparts. While grading of larger stock on the ongrowing side of the farm is very important, grading at smaller sizes is also very beneficial to the performance of these size classes. Grading size class selection is very much up to the hatchery operator but in general when hand grading three size classes will be selected. If possible a grading should be carried out at or soon after de-plating. Sieves can be used in the grading process. The operator will know the size of abalone that will remain or pass through each sieve.

Grading is generally carried out by manually removing the abalone from their plates or shelters or by immersion in benzocaine or magnesium sulphate with them then falling off the plates. When manually removing them it is very unlikely that all come off in the first go. Larger abalone in particular can get a strong grip. Push them from the rear and left side of the shell never from the front and right side as this may damage the viscera. If a larger abalone resists then either use a specifically designed plastic spatula or a blunt knife to remove them. An alternative is to leave them on the upperside of their shelter or plate in the tank or basket and eventually they will move to the underside to avoid the light. While crawling they are easy to remove. The abalone should be returned to the water as soon as possible after grading. Small juveniles in particular are sensitive to desiccation and should be out of water for no more than twenty minutes. If an anaesthetic has been used the abalone should not be piled in clumps on top of each other as this will restrict oxygen flow to them at a very stressful time. Detailed accounts of length and weight measurements should be recorded and the new abalone baskets or tanks then labelled. Commercial graders for abalone are now available from a number of companies. They are expensive but may well be worth their price in terms of labour savings and also growth rates around the farm.

9. 6. Culling

This is an essential part of the production cycle in abalone culture just as it is in the production of other fish species. During the larval rearing process for bivalve shellfish, grading is carried out. This divides those with cohorts with different growth rates at an early stage. It is also possible to cull a certain percentage at this grade. However, grading

is not practical during the abalone larval cycle so larvae with very varied growth rates are settled on the plates together. A first grading is advisable after removal from the plates or early during the weaning phase. If the seed are hand graded into three size ranges it should be apparent that at least 5% - 15% of the lower grade is particularly small and the hatchery operator should consider their removal. This will free up space, reduce stocking density and feed quantity required. During all further gradings on the farm cycle this procedure should be considered. This culling seems more imperative for *H. d. hannai* than for *H. tuberculata*.

9. 7. Transporting juvenile abalone

This is usually associated with the shipment of juveniles from the hatchery to an ongrowing unit. In this country most journeys from hatchery to ongrowing unit can be made in eight hours maximum. Different companies will use their own packaging design, stocking densities etc., but basically it is necessary to have polystyrene boxes, plastic or mesh bags, industrial oxygen and possibly seaweed.

Many hatcheries will use a double layer of plastic bags. Into this are placed the seed intermingled with damp seaweed such as *Palmaria* or *Fucus spp.* The bags are filled with industrial grade O_2 and double sealed with elastic bands or cable ties. Three to five of these are then placed in a polystyrene box which is sealed with tape. If cooling blocks or ice are used then they should never come in direct contact with the seed. They should be placed outside the box on top of the oxygen filled bags. An alternative to using sealed plastic bags is to place a damp sponge layer on the bottom of the polystyrene box. A layer of weed is placed on this and then a layer of juvenile abalone would be placed on this. This would be repeated a number of times. A second layer of damp sponge is placed on top. If cooling blocks need to be used then they are placed on top of this sponge layer and under the lid. The box is then filled with O_2 and sealed with tape. During particularly warm spells of weather it may be advisable to transport the juveniles during early morning, late evening or at night time.

Chapter 10: General conclusions

At present the abalone industry in Ireland is going through an expansion phase with a number of new facilities being constructed. These facilities are, for the most part, ongrowing units that will depend on existing hatcheries to supply their seed demands.

These units, in addition to the existing units, require large quantities of seed to meet their ongrowing demands.

Current abalone production in Ireland is, at most, a few tonnes. Production figures anticipated from new ongrowing units vary from ca. 20 - 100 tonnes each annually. A 30 tonne facility would need to harvest ca. 0.5 million 60g abalone to achieve this. With the high mortality rate associated with *H. d. hannai* realistically the ongrower would need to have acquired ca. 750,000 of this species two years previously.

Combined Irish abalone hatchery production is up to 2 million seed per annum at present. This production figure is well short of the industry need. It is essential for the industry to address this issue. In the shorter term it seems that existing hatcheries need to dramatically increase spat production. This will require a high degree of forward planning. Each hatchery will need to have in place the infrastructure necessary to achieve their planned productivity.

This planning should start with having excess broodstock to provide the larvae required. The broodstock units should facilitate maximum conditioning capacity throughout the year. Realistically, there should be a minimum of a few hundred broodstock of both *H. d. hannai* and *H. tuberculata.* As current demand for spat is aimed to a larger extent at *H. d. hannai* then, realistically, the hatchery will require more of this species.

If broodstock are properly conditioned then spawning and larval rearing are quite straight-forward. For increased productivity it is necessary to have a surplus of settlement tanks. Output of 5mm - 15mm spat will vary between settlement tanks depending on size, design and food availability but realistically most will not exceed 35,000 with average figures being considerably lower. The hatchery operator must take normal survival figures per tank into account when deciding how many settlement units are required. Correct management of the diatom film will greatly enhance juvenile survival and decrease the period juveniles need to remain in the tank, thereby freeing it for further settlement.

Similarly, forward planning of the amount of weaning space required will facilitate higher growth rates after seed are removed from the settlement tanks. The hatchery operator should also consider having excess weaning space in order to prevent overstocking of seed in the

event of delays by the ongrower collecting the spat as this has a knock on effects right through the production cycle.

Weaning juveniles onto an artificial diet at 2mm - 3mm should also increase the farm's productivity and free up settlement tanks for further spawnings throughout the year. Sourcing of high quality and relatively inexpensive artificial food will be directly linked to this.

Likewise the incorporation of the green macroalgae *Ulvella lens* into the settlement/nursery phase should help increase survival of juveniles while on the settlement plates.

There is also an urgent need for the issue of *Haliotis discus hannai* broodstock to be addressed. As all current stocks in the country originated from a small number of broodstock, the genetic pool is very limited. It is well known in the industry that *H. d. hannai* are more susceptible to mortality right throughout their culture cycle than are *H. tuberculata*. If this issue is not addressed the future of *H. d. hannai* culture in this country is uncertain.

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