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An Illustrated Hatchery Guide for the Production of Clawed Lobster

Using a Green Water Technique

## An illustrated hatchery guide for the production of clawed lobsters. (Using a green water technique)

Ronan Browne, Gonzalo Pérez Benavente, Ingebrigt Uglem, and José Carlos Mariño Balsa.

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#### This guide was produced as part of an Aquareg project;



AquaReg an Interreg IIIC project – Involves co-operation between the regions of Galicia in Spain represented by the CETMAR Foundation, the Border, Midland and Western (BMW) in Ireland represented by The Marine Institute and Trøndelag in Norway represented by joint forces of the South Trøndelag and North Trøndelag counties. The overall objective of AquaReg is to provide opportunities and design strategies for sustainable development of peripheral coastal communities by promotion of interregional co-operation in aquaculture and fisheries.

Overexploitation and depletion of traditional fisheries, has lead to a reduction in fishing quotas and a re-structuring of the fleet all over Europe, which, in turn has caused a loss of traditional jobs and increased unemployment in many coastal communities. As a consequence, aquaculture has diversified significantly, and today aquaculture constitutes an important and flourishing industry with high expectations for the future.

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> Gnáthamh na hoibre an t-eolas Knowledge comes through practice



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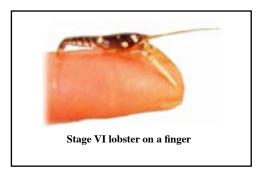
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Table 1. Regulations and management strategies

## An illustrated hatchery guide to producing European clawed lobster *Homarus gammarus*, using a green water technique

Ronan Browne, Gonzalo Perez Benavente, Ingebrigt Uglem and José Carlos Mariño Balsa



#### Summary

The objective of this project was to establish reliable and low cost methods for culturing European clawed lobsters (*Homarus gammarus*) under controlled conditions to a size suitable for their release into the wild. This illustrated guide is intended as a practical manual describing the production methodologies and the technical aquaculture considerations involved in the lobster larval rearing processes. These techniques are also applicable to other mariculture endeavors and may be used to demonstrate to students the considerations involved in marine larval culture. The fundamental strategies for producing postlarvae are that cleanliness and disease prevention are prioritized, and that the nutritional requirements for optimal larval survival are met.

Egg bearing female lobsters can be obtained from a variety of sources, for example lobster merchants, fishermen and even restaurants. Thereafter these lobsters can be held in individual containers within ponds which have a constant flow of seawater. During this period the lobsters are fed periodically. Subsequently, the broodstock lobsters whose eggs were close to hatching are not fed for several weeks to ensure that their waste products do not contaminate the freshly hatching larvae when they are transferred into the hatching vessels.

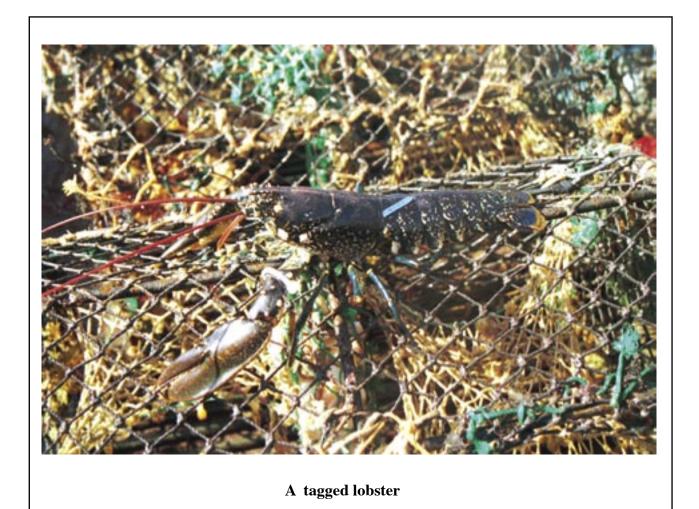
Prior to the introduction of lobsters into hatching vessels these lobsters with ripe eggs (about to hatch within 24 hours) are bathed in an iodine solution in an effort to remove external parasites and potential pathogens. The volume of the hatching vessels used was up to 500 liters; they were aerated and filled with  $1\mu$ m filtered and UV-irradiated seawater. Hatching vessels with broodstock were cleaned and refilled on a daily basis. The refilled vessels were allowed to acclimatise to room temperature and the same temperature as the vessels containing hatching females. This action reduced the possibility of temperature shock and prematurely hatched larvae.

At the time of hatching (night) the resultant larvae were collected using a hand net and weighed. To estimate larval numbers a linear relationship between the number of larvae and their weight was established. This was then used to estimate the number of freshly hatched Stage I larvae transferred into the larval culture hoppers.

The larval culture structure utilised can be any type of clean insulated building (with air temperature control) or even a mobile portacabin as was modified during this Aquareg study into a hatchery. The fundamental hatchery operation described in detail later in this report was based on a system of 90 litre (total volume) MDPE food grade hoppers, which were vigorously aerated from their base. Ideally two species of live phytoplankton (*Chatocerous muleri* and *Isochrysis galbana*) and *Artemia* with a known high level of PUFA's (Polyunsaturated fatty acids) were provided in each hopper. This combination provided adequate food, separation of lobster larvae and maintenance of water quality. Every second day larvae were transferred to freshly prepared hoppers. This was repeated until the lobsters reached Stage IV postlarvae (day 12 to 14 post hatch). This methodology provided a simple cost effective system for routine bulk production of these postlarval animals in their normal hatching season.

When approximately 70% of the larvae had reached the IV Stage, all of the larvae were scooped out of the hopper and transferred into a static seawater tank vessel (e.g. 500 litre circular vessel with a flat base) in the nursery area (outside of the hatchery). This acted as a separation device with the Stage IV swimming to the surface and Stage III lobsters sinking to the base of the vessel allowing the easy collection of the Stage IV's with a hand net.

Many of the techniques and structures described evolved over the period of this research. Observations on aspects of lobster biology pertinent for hatchery consideration are also presented in this report.



## Introduction

The demand for fisheries resources is increasing with the world population and the sustainability of all fisheries depends on the acceptance and application of appropriate management controls, which protect the breeding stock and maintains the environment they live in. Enlightened management measures are fundamental to any lobster fisheries self-sustainability and should always be the first choice when considering methods for increasing or preserving stock levels. The protection from landing, of sufficient, numbers of ovigerous or egg bearing female lobsters is a prerequisite for the protection and self sustainability of a fishery. There are various technical measures that can be introduced to promote stocks and enhance the economic returns from the fishery (see Table 1). Any management policy must also have a dynamic content, capable of adapting to new circumstances.

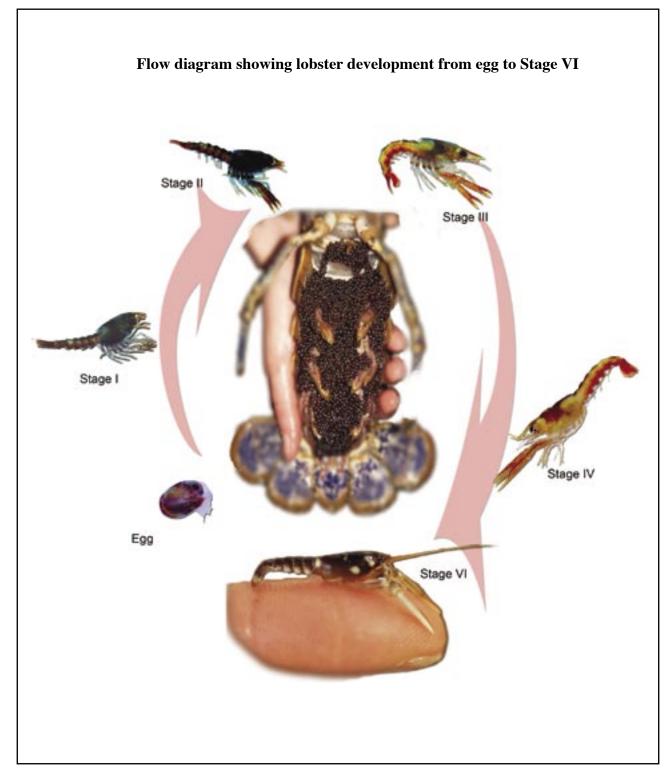
TABLE 1.   Regulations and management strategies.
Prohibition on the landing of berried females
Minimum size limit
Maximum size limit
V-notching
Licensing of fishers
Trap limitation (number)
Gear Regulation (type, size etc.)
Seasonal (or daily e.g. no fishing on Sunday) closure of fishery
Marine reserves
Escape vents
License fees used for lobster conservation

Historically there have been many recorded attempts with varying technical successes and failures to rear clawed lobsters since the 1880s on both sides of the Atlantic. These efforts have left many questions unresolved about the viability and efficacy of producing juvenile lobsters for release into the wild. The use of aquaculture techniques to produce juvenile lobster and their subsequent release into the wild also has the potential to create unrealistic expectations about possible returns. Therefore before embarking on such an exercise, an in depth evaluation of the potential for stock enhancement should be undertaken. This should include the establishment of a scientific monitoring programme, an understanding of the life history of the target species and an economic assessment of costs. Then, if after such an analysis it is decided that such a project has merit, a rigorous experimental plan incorporating all of the objectives, costs and the scientific considerations should be drawn up before work proceeds.

When writing about the biology, cultivation, stock enhancement and management of the European clawed lobster (*Homarus gammarus* L.), it is impossible not to refer to the findings for its American cousin (*Homarus americanus*, Milne Edwards) due to the similarity in the biology of the two species. The life histories of *H. gammarus* and *H. americanus* have been described and are subject to a number of well established terminological schemes. Despite the many similarities the behaviour of discreet life stages of *H. gammarus* in the wild is still less well understood than their American counterpart.

After hatching from eggs the lobster larvae develop through three free swimming or pelagic stages marked by molting (shedding of their shells). This process takes 10 to 14 days in a hatchery environment (temperature dependent) while it is thought to be completed in approximately one month in the wild. These three free swimming larval stages use expodites and their thoracic appendages or pleopods (swimmerets found under their tails) for locomotion. The larvae of both *Homar*-*us* species up to Stage IV *postlarvae* are regularly found in the plankton. The seabed populations

of European and American lobsters are replenished by Stage IV postlarvae which undertake diving forays to the benthos, to explore the seabed and where they then decide either to settle or to swim to a more suitable location for settlement. This pelagic to benthic transition and early benthic life stage of lobsters has received significant scientific attention. In North America these early benthic or settled lobsters have been located. However to date, no new recruits or shelter restricted juveniles lobsters with a carapace length (CL) less than 20 mm, have been recorded in European waters despite extensive scientific sampling.



An objective of this Aquareg project was to establish reliable, low cost methods of culturing lobsters (*H. gammarus*) under controlled conditions to a size suitable for release into the wild. To achieve this, cultivation systems had to be established for broodstock care and conditioning; larval development; nursery

nursery and the ongrowing of post larvae. The design of the physical systems used and the operational procedures used to produce post-larval lobsters are described in this guide. For more detailed information about the ongrowing of later stage postlarvae the reader should consult Uglem et al. 2006.

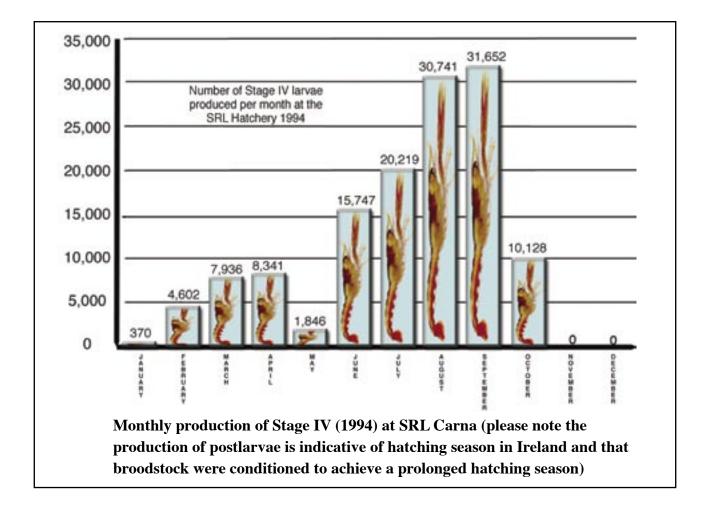
Many of the techniques described here in this manual have evolved over many years of trial and error and the hatchery principle is based on the concept of using aerated larval rearing cones developed in Maine USA by Beal et al. 1993. There are also differences in the overall approaches taken to cultivate the European clawed lobsters and these are mentioned in this report. Also, described in this report are aspects of clawed lobster biology pertinent to their cultivation. In brief, this manual describes the practical techniques involved in the culture of Stage (IV) lobsters and their transfer into nursery and ongrowing units.

#### Hatchery design and operation

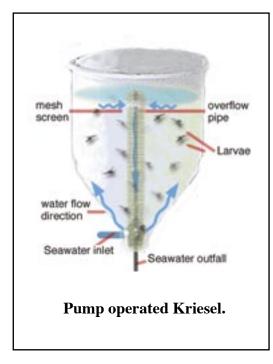
Most aquaculture shellfish and marine finfish hatcheries are based on broadly similar biological principles, though the equipment used and procedures followed may vary. The hatchery practices described here are an amalgam of techniques and ideas adapted to rear juvenile lobsters. Many ideas are borrowed and have been modified to suit the operating requirements of this investigation. This report covers work undertaken in Ireland during the 1990's and in the course of this study. A key to the long-term successful operation of a larval hatchery is that the unit is isolated from potential contaminants and that the room used is easy to keep clean.

In successful hatchery operations strict guidelines (cleanliness and hygiene) should be adhered to, but with so many variables the production of lobsters can in many ways be an art rather than a precise science. An operator may require good judgement, enthusiasm, experience, and dedication to doing the mundane tasks such as cleaning. These qualities are not easily taught and are usually acquired through experience.

Experience has shown that it is most efficient to rear larval lobsters during the natural reproduction season for clawed lobsters as survival is higher during this period and production costs lower. The added advantage of operating a hatchery on a seasonal basis is that other marine species (mollusks or finfish) can be grown within the hatchery when lobster larval production is not ongoing. As can be seen from the 1994 Shellfish Research Laboratory (Galway, Ireland) production graph (overleaf), June to August was the most productive months when the greatest production of lobster occurred. Unlike Beal et al. 1993 who released postlarvae directly into the sea after being cultured in the aerated culture vessels, each of the Stage IV lobsters produced had to be separated from other more juvenile stages and then placed into individual compartments before being counted. Out of the 131,500 Stage IV lobsters produced in the year 1994, some 98,000 or 74% were cultured during these months (June to August). The extended nature of the 1994 production season reported was achieved by conditioning the lobster broodstock. Early hatchings during the months of January to May were obtained by elevating the temperature of the water the broodstock were held in, while later hatchings were obtained to a lesser extent in October by chilling the water.



When planning a hatchery operation a major limiting factor in reaching production targets for lobster releases is the availability of suitable ovigerous females at all times during the production season. The successful production of postlarval lobsters is strongly linked with the conditions that broodstock lobsters are held under. For the work undertaken in Ireland we were fortunate in obtaining the majority of our broodstock lobsters from a lobster merchant who held them under semi natural conditions in a lobster pond.



#### Alternative hatchery/ larval culture design

Pump operated Kreisel (larval rearing vessels) systems have traditionally been used to rear lobster larvae for stock enhancement purposes and are still favoured by many operators. In the author's experience of making and using this type of system with inert foods the average survival could be low (approximately 10% or less). However, this should not deter the reader from investigating the use of such systems as there are many accounts of these systems that have been successfully operated to produce large numbers of postlarvae.

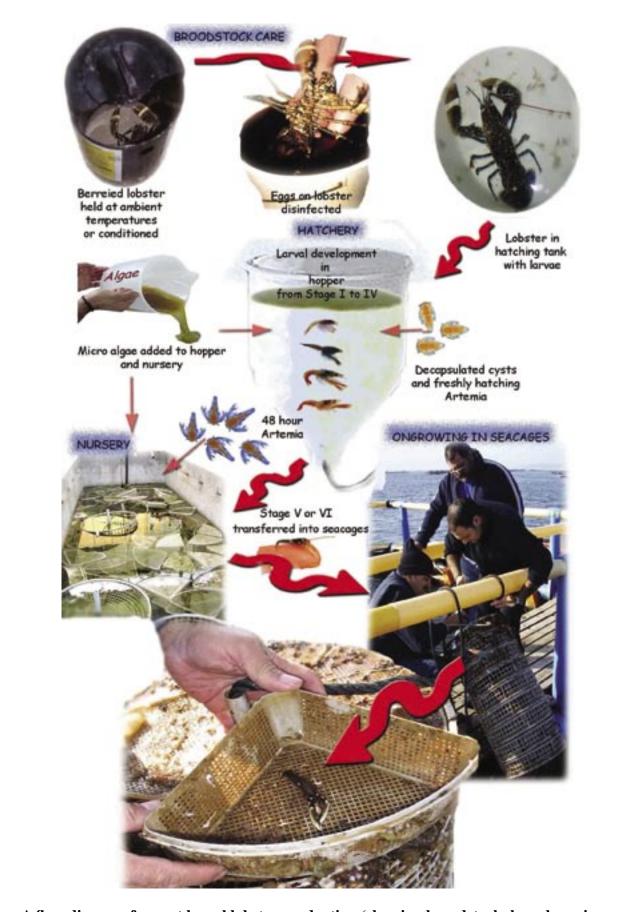
#### Green water technique for rearing clawed lobster larvae

At the Irish American Technical Exchange on Aquaculture in Galway, Beal et al. (1993) reported on a larval culture system that had been developed in Cutler, Maine, USA. It involved the use of two species of phytoplankton to enrich *Artemia*, which were then fed to lobster larvae in conical 500 l vessels. Routine survival rates of 30 - 50% were reported. It was decided to carry out trials using this type of system and after a visit by Beal in 1993 aspects of the methodology used in Maine were adopted and modified in Ireland to suit requirements of the European lobster *H. gammarus*. When the hatchery was established it was found that larvae after 11 -14 days in the hatchery, had an average survival rate of approximately 40% during the normal hatching season from Stage I to Stage IV (Browne and Mercer 1998). However the reader should be aware that there were times at when survival was somewhat variable.

#### Postlarval lobster production

The production cycle of lobsters for stock enhancement purposes consists of broodstock holding, hatching vessels, larval rearing hoppers, algal culture and nursery systems (with air temperature control). These phases are similar to the operation of a shellfish bivalve hatchery and it is essential for successful hatchery operations that the seawater is of good biological quality. Another prerequisite is that the equipment used is readily available and that it is cost effective to operate. Expensive equipment with the exception of a UV system, an air blower and seawater supply pumps were avoided in this project. An illustration of the production process for culturing juvenile clawed lobster using green water and Artemia is presented on the next page (this includes the nursery phase of ongrowing lobsters which is not discussed in this document). For further information about the nursery care of lobsters the reader should consult Uglem et al. 2006.





A flow diagram for post larval lobster production (showing broodstock, larval rearing, nursery and postlarval culture in sea cages suspended from a raft).



# Ovigerous/berried female lobsters for larval production.

Early lobster hatcheries at the end of the 19th century relied on eggs taken directly from berried females and hatched separately in water agitation devices. However the number of eggs that hatched was low and it is now known that a female lobster is a more effective incubator than any man made device.

For successful lobster production the selection of broodstock, their packing procedure and transportation, the holding facility and management, conditioning and hatchery methodology are all important to the number and viability of the larvae obtained.

When operating a hatchery it is important that each berried female is carefully inspected before being introduced into a hatchery environment, if there is a choice in the broodstock available then the lobsters chosen should be:

- (1) Large females (CL >90mm) in preference to smaller ones because of superior fecundity and egg quality;
- (2) Strong, active and intact animals (the lobsters ideally should not be missing claws or any of their pereiopods legs);
- (3) Only lobsters with large amounts of healthy eggs (Animals with off-coloured e.g. orange opaque eggs should not be taken, as these are dead).



# Collection transportation and care of broodstock

Once broodstock were selected, they were weighed (for record purposes) and carefully packed in polystyrene fish boxes with their tails tucked underneath them to protect their eggs. They were covered with damp sacks or sponge and a polystyrene lid placed on to the box. It is important that they are kept cool (<  $16^{\circ}$ C), moist and still during transportation and that the journey time is as short as possible. Otherwise the eggs will become unviable.

On arrival, lobsters were sorted according to their degree of egg development and held individually in black 20 litre perforated drums. Each drum had large ventilation holes for good water exchange and a concrete base which acted as a weight and prevented the lobster from turning the drum on its side and escaping. As lobsters are solitary crevice dwelling organisms these shelters provided ideal low light conditions and it was found that egg loss and mortalities were significantly reduced when compared to lobsters that were held communally. The drums holding the lobsters were arranged in sequence according to the degree of egg maturity in raceways. A continual flow of ambient unfiltered seawater was passed through each raceway with vigorous aeration at several points. Periodically the broodstock were examined for egg development and remained in the ponds until transfer to conditioning systems, or to the hatchery. Monitoring egg maturity allowed continuity of larval supply to the hatchery.

Broodstock were fed on a variety of foods if they were to be held for a prolonged period of time (months - over wintering). Their diet typically consisted of *Mytilus edulis, Carcinus maenas,* and/ or fish, depending on availability. For at least two weeks before the eggs were due to hatch these lobsters were not fed. It appeared that long term storage of broodstock was detrimental to survival and growth of larvae in the hatchery and it was preferable to obtain freshly landed lobsters with eggs close to hatching and not to feed the broodstock. The raceways were cleaned regularly without disturbing the broodstock which were transferred directly into an adjacent clean raceway (exposure to air can be detrimental to egg quality).

Fecundity of ovigerous lobsters is an important consideration in postlarval production planning. Variable egg loss is known to occur amongst captive female lobsters. The management philosophy described was designed to result in well fed, unstressed animals that are less likely to fight each other or remove eggs from their brood.

#### Conditioning

By using temperature manipulation of the water that the broodstock are held in it is possible to accelerate or reduce the development of lobster embryos, and to provide larvae for most of the year for hatchery purposes. Despite this ability it was the experience that best survival of larvae occurred during the natural hatching seasons of the lobsters and it was more economical to produce postlarval lobster during this period.

#### **Hatchery operation**

One of the main criteria for producing Stage IV lobsters is the rigorous application of hygienic techniques for the culture of larvae and the greatest enemy to the production process was complacency in operational procedures. All operators working within the hatchery undertake fundamental (but essential) hygiene practices. For example at the entrance to the hatchery disinfectant footbaths were used for cleaning footwear (ideally the boots should be only used within the hatchery confines). Before starting work the operators cleaned their hands and thereafter wore rubber gloves. The gloves were also washed in between each job of work within the hatchery. All clothing worn within the hatchery must be clean (preferably overalls or laboratory coats should be worn). It is also recommended that operators should begin working in the hatchery and finish all of their work within the hatchery before working with animals outside the hatchery or with raw seawater (unfiltered and non UV treated). Finally, and most importantly the hatchery should be isolated from potential contaminants.

#### Cleaning broodstock and transfer into hatching vessels



Berried lobster being bathed in iodine solution

When the eggs of the broodstock were sufficiently ripe for hatching in the conditioning unit or holding ponds, the broodstock were brought into the hatchery (isolated from the larval rearing and algal culture areas) and bathed in a 1% solution of Betadine<sup>(1)</sup> (100ml Betadine<sup>1</sup> in a ten litre container with filtered (1µm) seawater that had been U.V. treated). Most of the body (including claws and walking legs, which are cleaned by hand to remove bacterial film) except for the mouth parts and gills of the animals (as the iodine is toxic to lobsters) were submerged in the solution for up to seven minutes. It has been observed that eggs that are very close to hatching should not be submerged for the same length of time (less than seven minutes) as this may result in some mortality of the larvae.



Berried lobster in 500 litre tank with shelter and recently hatched larvae

After being bathed in Betadine solution the broodstock were rinsed in a water bath of 1 $\mu$ m filtered U.V. treated seawater and placed into the broodstock bins. Which were filled with seawater (UV & 1 micron filtered) at 18°C to 20°C. Shelters made of PVC pipe were provided to reduce stress. A white 500 l bin was fitted inside a black bin, thus permitting the broodstock to be kept in darkness during the day by placing a black lid over the top of the bin and also allowed any larvae that had been released to be easily seen against the white background by the person who is collecting them.

Each broodstock hatching vessel was moderately aerated and the broodstock lobsters were transferred into a freshly prepared vessel every day or even twice per day as required. Starvation of broodstock for several weeks prior to introduction to hatchery vessels was desirable. Unfortunately this was not always the case as ripe lobsters were on occasion brought directly from lobster merchants or fishermen into the unit. During the first few days, these lobsters sometimes excreted waste, necessitating more regular cleaning. Broodstock were not fed in the hatchery and were generally kept under these conditions for four to eight days, dependent on the quantity of eggs they were carrying and the demand for Stage I larvae in the hatchery.

(1 Standardised Betadine - Antiseptic Solution: Aqueous Providone Iodine solution for microbicidal preparation of operative sites.) After the broodstock have released all, or most of their larvae, they were removed from the hatching vessels, fed and then returned to the lobster merchant.

#### Incubation and egg loss

Egg masses are carried beneath the lobsters' abdomen where they are kept aerated by pleopod movement and routinely cleaned by the animal. To survive the incubation period, an embryo must be securely attached to the pleopods, and remain free from disease and predation. Premature egg loss is attributed to adverse conditions and stresses encountered during the incubation period.



#### **Embryonic Development**

During attachment to the pleopods the eggs develop and increase in size. The eggs also undergo a transition of colour change. The colour of the eggs goes from a dark olive green at the time of extrusion onto the pleopods (or swimmerets) of the lobster. The colour then changes to black, then red and finally a translucent blue or green prior to hatching. The incubation period of the eggs is variable, but as a guide it is approximately nine to twelve months.

Embryonic development is temperature dependent. The approximate time to hatch may be predicted at known temperatures by observing the Embryonic Eye Index (Perkins, 1972, Beard, et al. 1985). This is done by periodically measuring the increase in size of the oval embryonic eye in a small sample of eggs.

Embryonic Eye Index = (length + width of eye pigment) 2

When the eyes first appear, they consist of darkly pigmented crescents surrounded by a halo of lighter tissue. The eye index is first measurable for *H. gammarus* at about 50  $\mu$ m (Beard et al. 1985). An eye condition index of 600 to 620  $\mu$ m represents complete embryonic development for *H. gammarus* (Beard et al. 1985). This is greater than that recorded for *H. americanus* of 560 $\mu$ m -580 $\mu$ m (Talbot and Helluy, 1995). However with experience it is relatively easy to visually estimate the approximate time to hatching of mature lobster eggs without the aid of a microscope.

The lobster larvae pass through the nauplius stage in the egg; further development occurs accompanied by embryonic moults in the egg resulting in the post nauplius or metanauplius.

#### Hatching

At night a female lobster typically adopts a characteristic pose just prior to releasing a batch of larvae. Balancing on the tip of her pereiopods (legs), she slightly inclines her cephalothorax (head) downwards and extends her abdomen upwards at a 45<sup>o</sup> angle. She then rapidly fans her pleopods, expelling the newly hatched larvae and may repeat this several times. The liberation of larvae takes less than a minute and occurs once a night. In Ireland it has been noted that the hatching times at night are generally earlier in the evening during the months April/ May and progressively become later at night in August (e.g. 11.00 pm to 12.30 am) in synch with the day/ night photoperiod. Successive batches of larvae are released from the same lobster at nearly the same time each night until hatching has been completed. It has also been observed that some lobsters held under continuous light often initiate hatching shortly after the light is turned off.

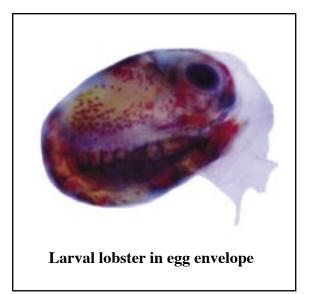
The hatching period for an individual female lobster can last from three to fourteen days dependent on the quantities of eggs being carried, with larger females producing greater numbers of larvae and taking longer to release all of their larvae. Once hatching begins, the number of larvae released generally reaches a peak between day 2 and 6 in the hatching vessels and is usually completed by day 8 to 14.

Daylight hatching of larvae was noted occasionally during this study, generally shortly after the daily water change of the 500 l hatching tank. This was thought to be due to temperature differences (circa.  $1-2^{\circ}C$ ) of the fresh seawater and stress when transferring broodstock to a new hatching tank. Examination of the resultant larvae revealed no apparent abnormalities. On some occasions however small premature larvae resulted. Daylight hatching was also noted during winter months (January / February), when photoperiod was reversed for day-night in the hatchery for two to three weeks per individual lobster.

At hatching, internal pressure bursts the outer egg membrane, which is sloughed off and the inner membrane then splits. The larvae are swept away by the current produced by the beating pleopods. The stage that is released from the egg envelope at eclosion is the prelarva (or prezoea). Immediately after hatching, the prelarva undergoes a moult resulting in the first larval stage (Stage I). The larvae assume a planktonic existence and swim to the water surface. Hatching of lobster eggs around Ireland held at ambient temperatures normally occur between April and the end of September.

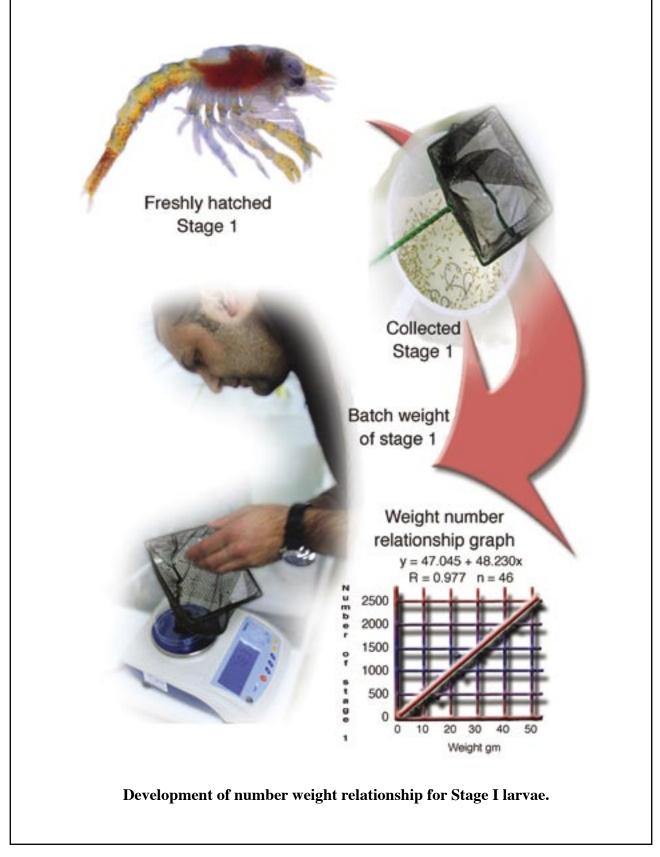
For the successful operation of a hatchery operators need to know the stocking density of their larval rearing vessels (hoppers). For this reason a number (larvae) weight relationship was established for stage I (*H.gammarus*) larvae as described by Beal et al. 1993 for stage I *H. americanus* lavae. This involved the collection of freshly hatched (ten minutes to one hour post hatch) larvae in an aquarium net. They were then dab dried, weighed and counted.

The linear relationship established between the number of Stage I larvae and their weight is illustrated on the next page in a graph. It shows that approximately 1,000 freshly hatched Stage I larvae weighing approximately 20 g.





A lobster that has been V notched

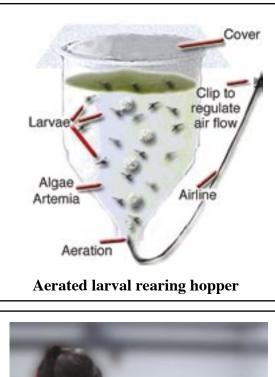


#### Larval rearing

Ideally a lobster larval rearing system must maintain both larvae and prey in suspension. It should maintain good water quality over a two-week period. Inadequate nutrition and poor water quality may cause high mortality of larvae and fluctuations in pH, salinity, temperature, toxins. Also, disease may be reduced or even avoided by the adoption of good husbandry practices.

It is important that the hatchery used for producing lobsters is well insulated and isolated from potential disease reservoirs. The structure used should be able to maintain stable air temperatures for the growing of the larvae during winter and spring. Within the hatchery white ninety litre (total volume - larger vessels were tried as had been used in Maine but the fecundity of the European lobsters was too low outside the main hatching season to operate these vessels efficiently) polyethylene hoppers were mounted on tables, with aeration tubes inserted through the base of each hopper providing large air bubbles (no air stone is used). The 5 to 8 mm airlines are simply pushed through a drilled hole of slightly less diameter than the airline (pressure fit) and connected to an air distribution pipe situated above the hoppers. It is important that the main air distribution pipe is significantly above the hoppers to prevent backflow of larvae and algae down the airline in the event of the air blower failing. Clips attached to the airline were used to regulate airflow to the hoppers. A transparent sheet of unexpanded polystyrene covering each vessel prevented or reduced aerosol cross-contamination between the hoppers. These transparent covers also permitted light for the larvae and algae. At all times no more than one active hopper was uncovered.

Each hopper can be easily lifted out of their table mounts to ease the task of cleaning. The hoppers are





Cleaning a larval rearin hopper

first rinsed with hot fresh water, then cleaned with a soft sponge and chlorine disinfectant solution. They are then thoroughly rinsed with freshwater before being refilled. Throughout the cleaning process the airline is left open to prevent contaminated water settling or disinfectant becoming trapped in the airline.

Access to the hatchery should strictly be limited to personnel only working on the production of postlarval Stage IV lobsters. Chlorine footbaths were used when entering the hatchery and all equipment (nets, buckets, etc.) was thoroughly disinfected (chlorine bath for plastics and iodine baths for permeable materials such as nets and brushes) and cleaned in hot freshwater before and after use. No equipment was brought into the hatchery, without being cleaned and disinfected.

Freshly hatched Stage I larvae were swiftly collected with a disinfected aquarium net from the hatching vessel, dab dried on tissue paper and then weighed (to estimate numbers using number/ weight relationship previously established). The larvae were then immediately transferred into a ninety litre aerated conical shaped hopper, containing two species of phytoplankton (*Chaetoceros and Isochrysis*) which had been cultured aseptically and *Artemia* cysts that had been decapsulated using a chloros (chlorox) solution and incubated for a period of approximately 12 hours. There was generally a mix of freshly hatched, hatching nauplii and decapsulated cysts. Periodically the density of *Artemia* was inspected visually by dipping a glass beaker into a hopper and looking at the quantity of *Artemia* present. If the numbers had significantly reduced then more *Artemia* were added to the hopper. Rubber gloves were used by the operators when dipping the glass beaker into a hopper, thus permitting easy disinfection of the gloves in an iodine solution which was then rinsed away with hot fresh water. The glass beaker was also disinfected in a similar fashion between uses.



Transferring lobster between hoppers with an aquarium net

Approximately 600 - 1000 Stage I larvae were added to each hopper depending on the number that hatched (weighing approximately 18 g). Larvae from no more than two consecutive days hatch were combined in any single hopper (preferably only one nights hatch). At high stocking densities, lobster larvae from hatch to Stage IV demonstrate a high level of cannibalistic behaviour. These antagonistic encounters are reduced by vigorous aeration. It is important that the aeration comes from the base. The air supply line should not be in the water column as this results in significantly reduced survival. In the hoppers, Artemia were held in high concentrations to reduce the search periods of the lobster larvae, and fresh Artemia were introduced into the culture tank as required. These concentrations of predator and prey made the maintenance of good water quality essential and necessitated total water changes every second day (48 hours). This negated the use of a sophisticated filter system for larval culture, but does require more manual labour. The methodology also had the advantage that each hopper was an isolated or quarantined unit reducing the possibility of spread of an infectious agent through all of the culture vessels in the hatchery. The hoppers were labeled with masking tape that could easily be removed and transferred onto the freshly prepared hopper every second day.

Larvae were transferred into fresh hoppers of algae and *Artemia* every second day using a large aquarium net. In the hatchery air temperature ranged from 20 to  $24^{\circ}$ C and the temperatures of the algae that the larvae were cultured in ranged from 18 to  $21^{\circ}$ C. When the hoppers were emptied of larvae, the algae and Artemia were siphoned out. The Artemia were collected on a 90 µm sieve and used as a food for post larvae in the nursery systems which are described later.

#### Nutrition in the hatchery

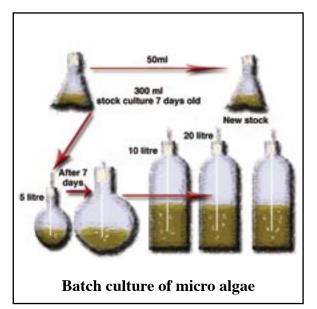
McDowell (1989) reports that at larval hatching of the American (*Homarus americanus*) clawed lobster and the initiation of larval feeding, there is a shift from endogenous to exogenous energy reserves. Larval lobster Stages I to III have similar energy requirements and possess high activities for protease, lipase and amylase. Lipid is of prime importance as an energy substrate and the turnover rate for lipid in lobsters can be rapid and weight specific metabolism increases with successive larval stages. In postlarvae however, the dependency on lipid as an energy substrate is diminished. Changes in physiology correlate with morphological changes in the mid-gut gland. By two moults after metamorphosis, lobsters have energy storage and metabolic patterns similar to adult lobster. From the authors experience with the European lobster and McDowell's findings it is important in a hatchery environment that larval lobsters receive a food high in Polyunsaturated Fatty Acids (PUFA's) and with adequate quantities of essential fatty acids (EFA's) for their development and growth.

#### Microalgae

In the lobster hatchery the supply of dependable, nutritionally complete and cost effective food is critical. Microalgae have been extensively used in mariculture as a food for all growth stages of bivalves, as a larval food for some species of crustacean, as well as for rearing zooplankton (e.g. rotifers and *Artemia*). Microalgae have also been used to overcome the nutritional variation of *Artemia* by enrichment and to consume inorganic nutrients and absorb various contaminants in the culture system helping to maintain water quality.

Green-water culture or the addition of cultured micro algae to larval rearing systems is now a standard method of culture for prawns and fish such as sea bass, cod and turbot. Carefully controlled low density green water can also be successfully utilised for culturing lobster larvae, but high algal densities may be detrimental to the lobster larvae.

Phytoplankton or micro-algae during these studies were grown using "batch" culture. The basis of the batch culture system is that the algae are maintained in pure culture. Part of these cultures are used to inoculate successively larger vessels over a two week period before being added to the hoppers or fed to Artemia. Sterile techniques are essential in the algal culture process to avoid collapses in algal growth and cross contamination of the species that are being cultivated.



The culture conditions of micro algae and their growth phase are known to affect their lipid and fatty acids contents. Many microalgal species have proven to be an excellent food; some however have been less successful. Microalgae also show wide variation in both content of protein and carbohydrate. Other factors that should be taken into account are differences in size and digestibility of the algae cells. Because some microalgae may be deficient in one or more key nutrients, many mariculture larvae are reared on a combination of two or more algal species (mixed algal diets). This can result in improved growth rates probably due to the better balance of nutrients than monoculture. The density of algae used in the larval rearing hoppers was approximately 150 cells per micro liter.

In summary the nutritional value of microalgae depends on its biochemical composition and the nutritional requirements of the feeding animal/predator. For cultivating larval lobsters in Spain *Iso-chrysis galbana* "Tahitian" also known as *T. iso* was successfully used on its own, but more typically a combination of low concentration *T. iso* and *Chaetoceros muelleri* were used. For postlarvae a combination of 48 hour post hatch *Artemia* (that are collected from the larval rearing hoppers) and *Tetraselmis suecica* may be used in the nursery system.

#### Artemia feed

A broad range of organisms have been used to feed hatchery produced clawed lobsters in various projects. Attempts have also been made to formulate artificial diets that meet lobsters feeding and nutritional requirements; these have achieved varying degrees of success. To date the lack of cost effective reliable artificial rations in the hatchery has been an impediment to the development of mass lobster culture. Lobster hatcheries still rely on natural food products (frozen mysids, krill, adult *Artemia* or mussel meat) and during this investigation live *Artemia* (brine shrimp) were the main food organism used in the hatchery and nursery operations. In our studies the nutritional strategies involved feeding *Artemia* nauplii with a known high PUFA content. While Beal et al. 1993 used

micro algae to enrich Artemia that were several days old.

*Artemia* are an optimal prey size for many decapod larvae, as they may be harvested at progressively larger sizes, permitting juvenile stages or beyond to develop. Unlike rotifers, copepods, amphipods and other live food organisms that have to be maintained in continuous culture, *Artemia* nauplii can be produced when required (incubation period of <24 hours) using live cysts. The cysts may be stored for long periods at 4°C and are commercially available.

When referring to *Artemia* it is important to identify the origin or brand of the cysts used as variations of the nutritional value of nauplii occurs. Watanabe et al. (1978, 1983) analysing different sources of cysts and batches from the same source determined their fatty acid profile and demonstrated differences in essential fatty acid (EFA) content; Navarro et al. (1993) agreed with this finding. Watanabe et al. came to the conclusion that the EFA content mainly determines the dietary value of a foodstuff for larvae. EFA are necessary for physiological functions and like vitamins cannot be synthesised. Subsequently it was demonstrated that the amount of essential fatty acids, such as eicosapentaenoic (EPA) acid 20:5µ3 determine the nutritional quality of *Artemia* for crustacean larvae

The feeding regime employed with *Artemia* also plays an important role in successful culture of an organism. For example Morris (1956) found that fish larvae fed older *Artemia* nauplii did not grow well, despite their gut being filled. He attributed this to reduced yolk reserves in the *Artemia*. Dye and Omando (1977) recommended the use of freshly hatched nauplii containing a rich yolk reserve.

The larval feeding strategy involved the addition of decapsulated *Artemia* cysts and recently hatched nauplii with a known high PUFA's to the hoppers at densities of approximately 15 to 25 per ml. Developing larvae were transferred every second day into freshly prepared hoppers with freshly hatching *Artemia* until Stage IV was reached.

#### Hatching Artemia

The hatching process of *Artemia* is critical to lobster larval and postlarval production. *Artemia* cysts are arrested gastrula, encapsulated with a hard lipoproteinaceous shell. These shells if not removed may be harmful when eaten by larvae as they cannot be digested and may block the gut of the larval animal. The cysts are also another substrate on which bacteria and other fouling organisms may grow.

For several reasons, the use of decapsulated (shell removed) cysts is preferred. Decapsulation was first described by Sorgeloos in 1979 and subsequently improved by Bruggerman et al. (1980). It involves the hydration of dry cysts with tap or seawater. The procedure that was proposed involved the use of 2.6% hypochlorite solution to oxidise the chorion from the encapsulated embryo. All that remains is the *Artemia* embryo surrounded by the embroynic cuticle and the outer cuticular membrane. The viability of the organism is not affected when the process is carried out correctly (Sorgeloos et al 1980). The decapsulated cysts are then collected on a 60 to 90 micron sieve, and then rinsed immediately with tapwater or seawater to remove the hypochlorite. An additional strategy that is highly recommended is that the hypochlorite is neutralised with sodium metabilsulphite. The treated cysts thereafter may be used directly or stored in a saturated brine solution or damp at 4°C. The reader should note that great care should be practiced when undertaking decapsulation and all safety procedures recommended for handling the chemicals must be undertaken (ideally the procedure should be undertaken in a fume cupboard and the reader should consult people

with decapsulation experience before attempting the procedure).

The advantages of decapsulated cysts are:

(1) Separation of hatching debris is unnecessary, as the thin embryonic cuticle is the only membrane remaining after hatching.

(2) Untreated cysts are covered with microbial material. Once treated the exterior of the cysts are rendered sterile, reducing the risk of bacterial contamination.

(3) The non-buoyancy of the cyst is an advantage as the unhatched cysts do not tend to aggregate on the sides of the hatching vessel or hoppers above water level. They also remain in suspension with moderate aeration.

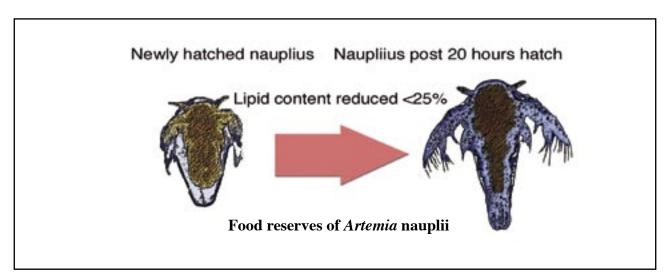
(4) The hatching of some strains is improved.

(5) The decapsulated cysts may be offered as a direct food source to the larval lobsters and may therefore be used as an emergency feed in the event of an unexpected hatch of lobster larvae.

(6) The cysts have 30% to 50% higher energy content than the nauplii (Vanhaecke and Sorgeloos 1983).

(7) It is possible that non viable cysts for hatching may be used as a direct feed, improving the commercial value of *Artemia* that have a low hatch rate.

It is important to note that *Artemia* undergo changes in biochemical composition with their life cycle (Benijts 1985). Instar I nauplii are unable to feed and live solely on their energy reserve (Dye 1980). Benijts et. al. (1976) demonstrated that from instar I to instar III, the calorific value decreased by 27% and dry weight by 20%. Correct harvest time is therefore essential if *Artemia* are not to be enriched prior to being fed to larval lobsters.



#### Feeding Artemia to lobsters

The feeding regime plays an important role in the cultivation of lobster. Under-feeding can result in cannibalism, while overfeeding may bring about fouling and stress. The concentration of feed affects rate of feeding, growth as a product of energy uptake and survival.

#### Strategy of feeding H. gammarus larvae adopted in this study

For this project it was decided to purchase *Artemia* cysts that were known to have a high PUFA content and feed the lobster larvae on freshly hatched nauplii or decapsulated cysts. Using decapsulated 12 hour incubated (at approx 20 °C) *Artemia* cysts of known high food value, it was possible: to establish a

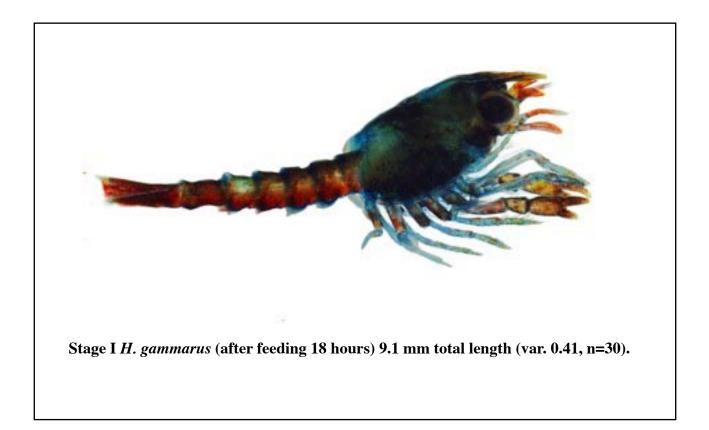
consistent feeding regime, to reduce the number of operations involved in preparing feed, to keep the volumes of algae low, and to reduce opportunities for contamination. Approximately 6 to 8g of *Artemia* cysts are required for each lobster culture vessel of 70 to 80 liters dependent on how heavily stocked it is with lobster larvae. The initial density of *Artemia*/hatching nauplii typically ranges between 15- 25 per ml in each culture vessel.

#### Larval development of lobsters

Lobster larvae have three free swimming stages, which are thought to be completed in approximately one month in the wild. The raptorial feeding of lobster larvae and the early benthic stages on small plankton (<  $500\mu$ m) is well established. The successive larval stages are marked by molting or ecdysis. The three larval stages, sometimes called mysid larvae, are regarded as equivalent to the zoeal stages of other decapods because they use thoracic appendages for locomotion (Williamson 1982). The stages are readily distinguished by the degree of pleopod development they display. It is believed that most lobster mortality occurs during their free-swimming stages. At Stage IV, or the postlarval stage they start to explore the seabed for suitable substrate in which to settle.

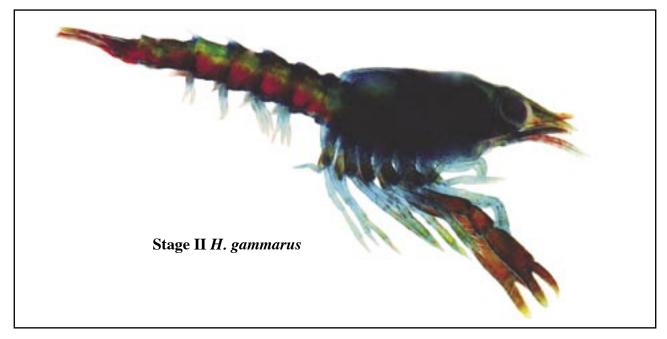
#### Stage I

Stage I larvae are pelagic in nature and display a positive response to light. Immediately on hatching they rise to the surface of the water in the hatching tank. There is considerable variation in their colour up to 18 hours after hatch. Red and yellow pigmentation is produced by chromatophores that are distributed over defined areas of the body. First stage larvae are segmented, their cuticle is transparent and their internal organs visible. Approximately 24 hours post hatch the cuticle darkens in colour (blue / black) and becomes opaque.



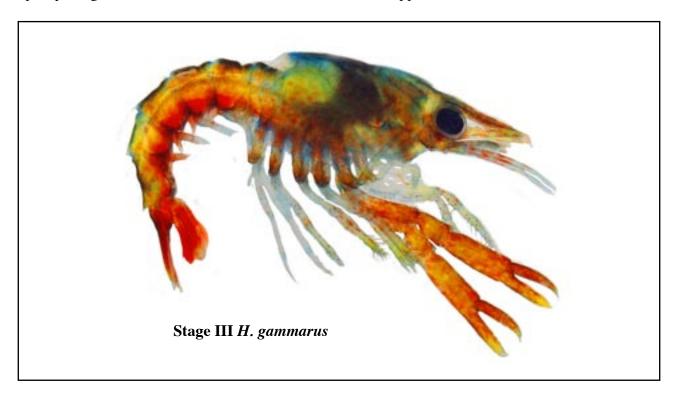
#### Stage II

Stage II larvae closely resemble stage I in behaviour and colouration. In this stage the pleopods project as bifid processes, lacking a fringe of plumose setae, characteristic of later stages. The swimmerets on their tail (uropods) are not free.



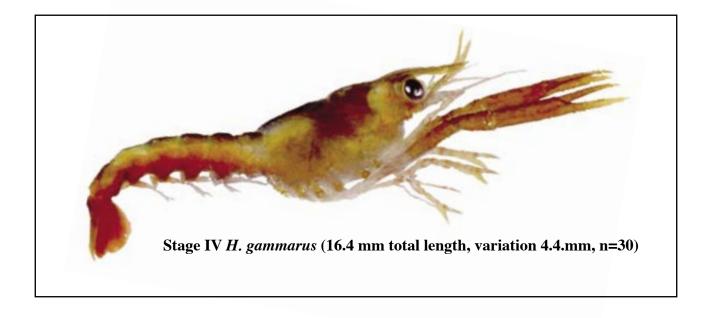
#### **Stage III**

Stage III larvae maintains their planktonic existence by means of their expodites. The claws are relatively larger than in the proceeding stage. Pleopods are also larger and consist of two paddle shaped processes, which are constricted at the stem. The branches of the distal margins are fringed with hairs. Stage III in the absence of strong upwelling currents tends to sink to the base of their culture vessel. Mortalities occur due to aggression and  $O_2$  depletion if they are left in this state. It is usually at this stage if the intermolt period becomes protracted mortalities are seen to occur and attributed to a build up of pathogenic vibrio bacteria within the larval culture hopper.



#### Stage IV postlarvae

Stage IV is referred to as postlarvae (Cobb 1988) or megalops larvae. They differ markedly from Stage III larvae and resemble adult lobsters rather than zoea. They are strong directional swimmers (Cobb et. al. 1989, pers. obs.) using their pleopods, while pereiopods are used for walking. Stage IV are strongly planktonic for 4 to 5 days after their metamorphosis, but they gradually become benthic over several days.



Survival and number of Stage IV postlarvae produced per hopper over a two year period appears quite variable. Increased survival appears to be related to the hatching season (Ireland) i.e. lower survival in the early period of the year January to March, with increased survivorship coinciding with the natural hatching period of *H. gammarus* around Ireland.

The survival figures of approximately 40% (when the hatchery in this study was operating well) for larval survival to postlarvae in the hopper system compare favourably with others published for *H*. *gammarus* cultured in Kreisel's of around 10% (Beard et al. 1985, Beard and Wickins 1992, Burton 1992, Cook 1992, Uglem et al. 1998). The survivorship in this examination are comparable to those quoted by O'C Lee and Wickins of 20 to 40% (1992). Nevertheless the mean survivorship of approximately 40% is somewhat below the percentage survival that is regularly quoted by authors for H. americanus. This ranges from a low of 25% to regular reports of 70% (Hughes et al. 1974, Schur et al. 1976, Cobb 1976, Van Olst et al. 1980, O'C Lee and Wickins 1992, Chang and Conklin 1993, Waddy 1998). Reasons as to such differences between survivals may be related to vessel/ system design, nutrition and species.

#### **Postlarval lobster collection**

Generally during periods of peak production all Stage IV postlarvae and Stage III larvae present in a hopper are harvested using an aquarium net when the majority are adjudged to have reached Stage IV. These animals are placed into 500 l vessels with seawater. Stage III larvae settle to the bottom of the tank and the Stage IV postlarvae which are active swimmers are scooped out with an aquarium hand net into a bucket and placed into individual compartments using a plastic tea strainer. It has been observed that Stage IV's tend to lose their chelae (claws) if left in the larval rearing hoppers for several days after metamorphosis. For this reason it is important that they are removed promptly after they have moulted into postlarvae It has also been seen that some of Stage III will moult through to the IV stage in the separation vessel over a 24 hour period if *Artemia* and algae are added and the temperature of the water is kept to approximately 20°C. It is also important that there is enough aeration to keep the stage III larvae in suspension. Alternatively if space is limited it is suggested that the remaining Stage III's are released into the wild in deep water.

#### Feeding postlarvae and ongrowing Artemia

Once the hoppers are emptied of lobster larvae, the remaining *Artemia* were siphoned out and collected on a 90  $\mu$ m sieve submerged in water. *Artemia* recovered from the hoppers after two days of culture were either fed directly to postlarvae in the nursery systems or ongrown. In periods of peak postlarval production and at times when there was a reduction in the availability of *Artemia* from the hatchery, cysts with low HUFA content were decapsulated and enriched on algae (*Tetraselmis suecica*) before being fed to postlarvae.

#### Nursery

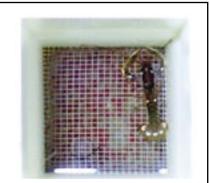
When ongrowing postlarval lobsters the highest survival rates are generally obtained by placing them into individual compartments. These compartments prevent antagonistic encounters and cannibalism amongst the postlarvae. However, the capital, running and maintenance costs of such systems are generally very high.

Various diets have been used to feed postlarval lobsters in compartments. These have typically included mysids and chopped mussel meat. Unfortunately, these diets also significantly contribute to the operating costs of growing postlarval lobsters. The expenses incurred with such systems include the purchase of the feed, the labour costs for feeding the lobsters or the capital costs required to provide an automated feeding system. Such inert diets also make it obligatory that the lobsters are checked frequently. The systems that utilize these diets require a regular supply of pumped seawater and the compartments that house the lobsters must be cleaned (usually by hand) to remove any uneaten food or moribund lobsters. All of these cost combined can make it prohibitively expensive to rear lobsters for any cost effective stock enhancement trials.

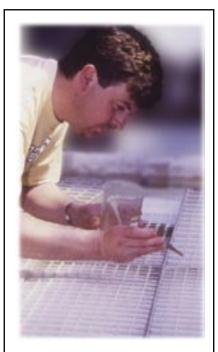
In an effort to reduce the labour input and associated feeding costs for postlarval lobsters this project has focused on developing novel cost effective methodologies for ongrowing post larvae to a size suitable for release into the wild. One of the techniques focused on is culturing the lobsters in sea cages,



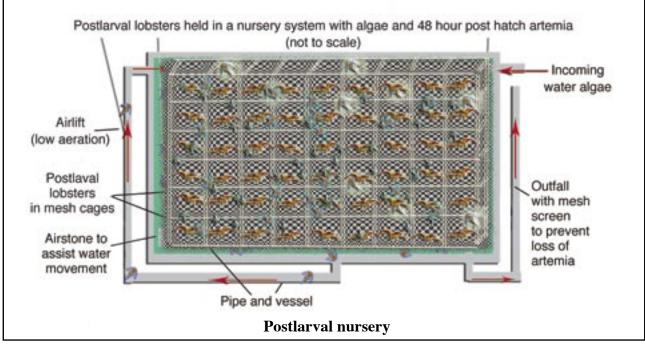
Seperating Stage IV from Stage III



Stage VII+ in a compartment Nursery



Postlarval lobsters in compartments being fed by hand



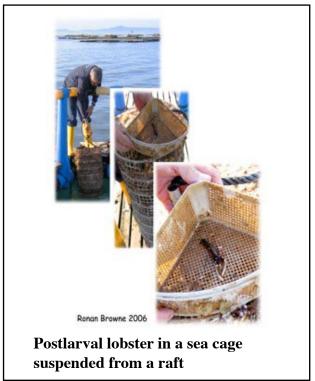
within which the lobster postlarvae feed on plankton and the naturally occurring fouling organisms that settle on the walls of their seacage.

Before the lobsters can be transferred from the hatchery to sea they are first reared to Stage V or VI in a nursery unit. At these stages they are robust enough to survive the rigors of the sea. To culture them to this size, the postlarvae (Stage IV) are transferred from larval hoppers into individual compartments for ongrowing. These compartments or sea cages have a mesh size large enough to permit the free movement of *Artemia* and are placed into an down welling/ nursery unit. Forty eight hour post decapsulated *Artemia* from the hatchery are added to the nursery vessel and the postlarval lobsters quite happily and effectively remove/filter these out of the water column. By maintaining a density of at least 1 Artemia per ml in the nursery vessels a mean survival of 80% has been achieved. The reader should note that care must be practiced in ensuring that there is sufficient water movement and food supply for the postlarval animals. This type of system is similar to that described by D'Abramo and Conklin (1985) and O'C Lee and Wickins (1992). A supply of algae (*T. suecica*) and fresh unfiltered seawater should also be added to this system. Gentle agitation and aeration were provided at the base of the task has means of the task to this system.

the base of the tank by means of an airlift and airstones.

#### Ongrowing

When the postlarvae in the nursery have molted at least once to the V or twice to the VI Stage or even larger they are generally ready for transfer from the nursery unit to the sea in their compartments. For more information on the performance of the lobsters at sea the reader should read the report by Uglem et al. 2006. An alternative method for growing postlarval lobsters is the use of communal rearing systems where the lobsters are simply added to a seasoned (fouled with naturally occurring organisms) substrate that provides shelter for the animals. Postlarval lobsters may also be grown in individual containers and fed by operators on a daily basis.



# Conclusion

The culture of *H. gammarus & H. americanus* throughout their initial pelagic larval stages has resulted in many publications describing various methods to achieve maximum growth and survival. The choice of system adopted is related to the availability of components, food source for lobsters, the budget of the project, the people available and the personal preference of the operators. When deciding on the most appropriate system for producing postlarval animals, each system must be evaluated with these considerations in mind.

Larval survival can be very variable in a hatchery environment, especially when culturing out of the normal reproduction period. The production of larval and juvenile lobsters can be fraught with many crises and problems if not properly managed. The number and severity of these events can make the difference between failing and successfully reaching production targets. However by applying strict operating guidelines and undertaking adequate hygiene practices this green water technique is very successful in producing very high levels of larval survival.

While stock enhancement using hatchery produced lobsters offers the enticing possibility of boosting recruitment to a fishery. The process can be costly and the return of lobsters released highly variable. Therefore, the key to successful self sustaining lobster fisheries is an understanding of the biology of the animals and the enlightened application of management regulations to ensure that the optimal return from the fishery is achieved.

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#### Step by step checklist for producing postlarval lobsters

Safety within the workplace must be the major priority before any work begins. All people working within a hatchery should be aware of all the health and safety issues involved in what they are doing and have adequate training and safety equipment before starting work.

#### Hatchery

Filters are to be cleaned and put into a freshwater chloros bath each afternoon (gloves, face mask etc... should be used).

After filters are put back into their cartridges housings the seawater should be allowed to flow for half an hour or more (until all traces of chloros have been removed) through the UV unit (turned on) and all of the pipe work in use after the filters.

Ensure that 1 micron ( $\mu$ m) filter is clean and that the UV system is turned on before turning on seawater to rinse cartridge filters.

The floor of the hatchery should be cleaned and mopped each evening.

Nets should be rinsed with hot freshwater, and then placed into a freshwater iodine bath. Before use they should be rinsed with freshwater.

Covers of hoppers should be kept in place to stop aerosol cross contamination between hoppers. These lids should be cleaned in a bath of chloros/ freshwater and then well rinsed with freshwater. Make sure that after the seawater supply to the hatchery has been turned off that the UV is then turned off.

#### Broodstock

• Broodstock should not be fed several weeks before their introduction into the hatching tanks.

• Disinfection- Use betadine, povodine iodine solution. Approx 100 ml in a ten litre bucket of UV filtered seawater. The duration of the treatment will depend on the development stage of the eggs. Broodstock are only disinfected prior to bringing them into hatching tanks. It may be useful to disinfect them on a daily basis if it is found that it can be done without adversely affecting egg viability.

• The broodstock should be transferred on a daily basis into a freshly prepared hatching tank containing UV filtered seawater. Make sure that the temperature of the water is similar to the tank that they are being held in. Temperature shock and stress will result in the premature hatching of larvae.

• Temperature shock will cause premature hatching of larvae as will prolonged exposure to betadine.

• Collection of larvae, larvae should ideally be collected at night using a disinfected aquarium net.

The larvae should then be weighed (a number weight relationship should be established. Typically up to 18 gm of larvae may be held in a hopper. 10 to 15g of Stage I larvae will be adequate for stocking a hopper.

• Larvae from a particular female will generally hatch at the same time each night.

• Make sure that the lights are turned off at the same time each evening. You may want to try photoperiod manipulation- that is fool the lobsters into thinking that night is day.

• No more that two days hatch should be held in a hopper, and preferably only the hatch from one night should be cultured in a hopper.

• Counting of larvae. Larvae are dab dried (twice) in a net and then weighed. Thereafter they are counted and the results used to establish a length weight relationship (this procedure should be repeated periodically over the hatching season). The length weight relationship once fully established can then be used to estimate the number of larvae in a hatch (ensure that this procedure is carried out quickly).

• Make sure that air is turned on before adding broodstock to hatching tanks, however too much air may result in the displacement of larvae from the abdomen of the broodstock.

#### Larval rearing

• Cleaning of hoppers. Larvae are to be transferred every second day into a freshly prepared hopper.

• Larvae are collected using an aquarium net and transferred directly into a freshly prepared hopper. The hopper is then siphoned empty; *Artemia* should be collected in a 90 micron + mesh and may be fed / added to communal rearing / sea cage lobsters. The hopper is then to be cleaned with hot freshwater and chloros.

• New Artemia (6 to 8g decapsulated and then incubated 12-14 hours before being introduced into a fresh hopper), fresh algae and UV filtered seawater at the correct temperature. Larvae that are to be transferred are to be caught using an aquarium net and moved directly into the freshly prepared hopper. After larvae have been removed from a hopper, the air to the hopper is to be turned off and the remaining contents are to be siphoned through a sieve 120 micron onto the floor. This is done to collect any uneaten Artemia, which may then be used as a feed for postlarvae. The hopper is then to be washed with chloros and rinsed with hot freshwater. When there is water and chloros in the hopper the air is to be turned on. This is to prevent any water in the airline from contaminating the freshly prepared hopper. The lids are also to be washed as described.

• Preparation of hopper. At least one hopper should always be available for the transfer of lobster larvae.

• During the course of the lobsters cultivation the level (number) of *Artemia* should be visually checked using a glass beaker, this also allows the operator to study the development and wellbeing of the lobster larvae. This beaker should be washed and cleaned before being used in another hopper.

• Transfer of larvae every second day, using a disinfected and rinsed aquarium net. The process should be carried out as quickly as possible

• Hygiene, when the operator comes into the hatchery the footbath should be used (with disinfectant), the operator should then wash their hands before starting work, watches and jewellery should ideally be removed. The operators clothes should only be used in the hatchery and ideally a clean laboratory coat or overall should be worn. It's best that the operator wears rubber gloves for several reasons. Firstly it allows disinfection between operations, the operator will also be able to comfortably withstand higher hot water temperatures, and also as chloros is corrosive the gloves offer protection.

• Always ensure that there will be one freshly cleaned and prepared hopper available for transferring larvae into.

#### **Post-larvae**

• Collection of Stage IV larvae, when it is adjudged that the majority of the larvae have reached the fourth stage they are to be collected with an aquarium net and placed into a tank with an air stone (optional), the air may then be turned off. The stage IV larvae will swim to the surface and can be collected with an aquarium net. It is important that the vessel is large. i.e. 500 litre so that the larvae are not too crowded and that they do not suffer from oxygen depletion.

• Transfer to storage/ongrowing trays. The use of a plastic tea strainer is essential for moving lobsters into individual containers.

• Unused/ uneaten live Artemia from the hatchery may be fed to postlarvae. Hygiene is not as critical with postlarvae as it is with larval culture.





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