Project no.: 286903

Project acronym: NEPHROPS

Project title:

"Development of new techniques in hatchery rearing, fishery enhancement and aquaculture for *Nephrops*"

Research for the benefit of SMEs

Deliverable 4.1 Hatchery handbook

Due date of deliverable: Month 35

Actual submission date: Month 35

Start date of project: 01.02.2012 **Duration:** 36 months

Organisation name of lead contractor for this deliverable: Swansea University, UK

Report status: Final

Project co-funded by the European Commission within the Seventh Framework programme (2007-2013)		
Dissemination level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Report no.
Deliverable 4.1 - Hatchery handbook

Project Title
NEPHROPS - "Development of new techniques in hatchery rearing, fishery enhancement and aquaculture for *Nephrops*"

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Please cite as:

Powell A, Cowing DM, Scolding JWS, Shepherd-Waring T, Eriksson SP, Lupatsch I, Johnson ML, Shields RJ, Gowland D. (2015). *Nephrops norvegicus:* Hatchery Handbook. Deliverable 4.1., *NEPHROPS* project, EU Framework 7 Project no. 286903 [URL and date accessed].

Note from author, January 2020:

EU Framework 7 funding permitted public dissemination 5 years after the end of project funding. *Nephrops* was a 36 month project finishing end of January, 2015. This manual is the deliverable for WP4, itself based on two technical reports based on research over three seasons (2012-2014).

***NEPHROPS* project**

Nephrops norvegicus: Hatchery handbook

Centre for Sustainable Aquatic Research
Swansea University, 2015

1. Introduction

1.1. Overview

Nephrops norvegicus ("*Nephrops"*) has a number of common names: Norway Lobster, Langoustine, Dublin Bay Prawn and Scampi. In the UK alone, *Shellfish News* regularly estimates that the species commands approximately 40% value (*ca.* £100 million/annum) of total shellfish landings. *Nephrops* also contributes significantly to the capture fishery sector across the NE Atlantic Ocean and Mediterranean Sea. The value of the fishery has partly driven an historic research effort, which has included reproductive biology and fecundity, and larval recruitment and settlement of *Nephrops*. This has increased in recent years, particularly with rising awareness of the potential effects of ocean acidification, and a desire to promote sustainable fishing practices across the industry.

Safeguarding the resource has been a key driver to seek and validate new technologies, and to inform and assist their uptake across the fishing industry. This has included the development of scalable hatchery facilities, equipment and know-how, promoting survival of captive larvae and allowing onward release of significant numbers of post larvae ("PL") at appropriate locations.

Release of hatchery reared lobsters for ranching, restoration or remediation inevitably draws parallels with *Homarus sp.* culture, an established practice that has led to a number of hatcheries that operate, or have operated, across NW Europe and the Eastern seaboard of the USA since at least the early 1900's. A detailed overview, including the historical background, techniques, equipment and other useful information, is provided by Burton (2003) and Nicosia and Lavalli (1999) and forms essential reading.

Whilst the ecology and reproductive biology of both *Homarus sp.* and *Nephrops* is broadly similar, there are key differences that demand modifications to rearing larvae and PL successfully. Indeed, efforts to rear *Nephrops* started in the early 1970's to our knowledge and have continued at small scale with varied success (see review by Powell and Eriksson, 2013). This handbook is written using information gleaned from these reviews, contemporary lab scale know-how from University of Gothenburg, Sweden, and the results of pilot commercial trials conducted at the Centre for Sustainable Aquatic Research (CSAR), Swansea (supported by North Bay Shellfish Ltd, Orkney). The general approach has been to use *Homarus sp.* techniques as a "stepping off point", with gradual optimisation over the course of 3 seasons (2012-14).

There is currently scant accepted best practice regarding large scale culture of *Nephrops.* The handbook does not make claims regarding economic viability of initiating a hatchery, and as research continues elements of the handbook will probably be superseded by advances in the field and juvenile culture. However, our approach demonstrates much improved survival of larvae at pilot commercial scale. We hope this handbook provides a solid foundation to produce copious early stage juvenile *Nephrops* for future commercial and R&D operations, and transferable techniques for other aquatic invertebrate species that may be similarly investigated to support research agendas such as pure science or applied research (safeguarding the fishery, food security, ecological restoration, and the effects of climate change).

1.2 Reproductive biology

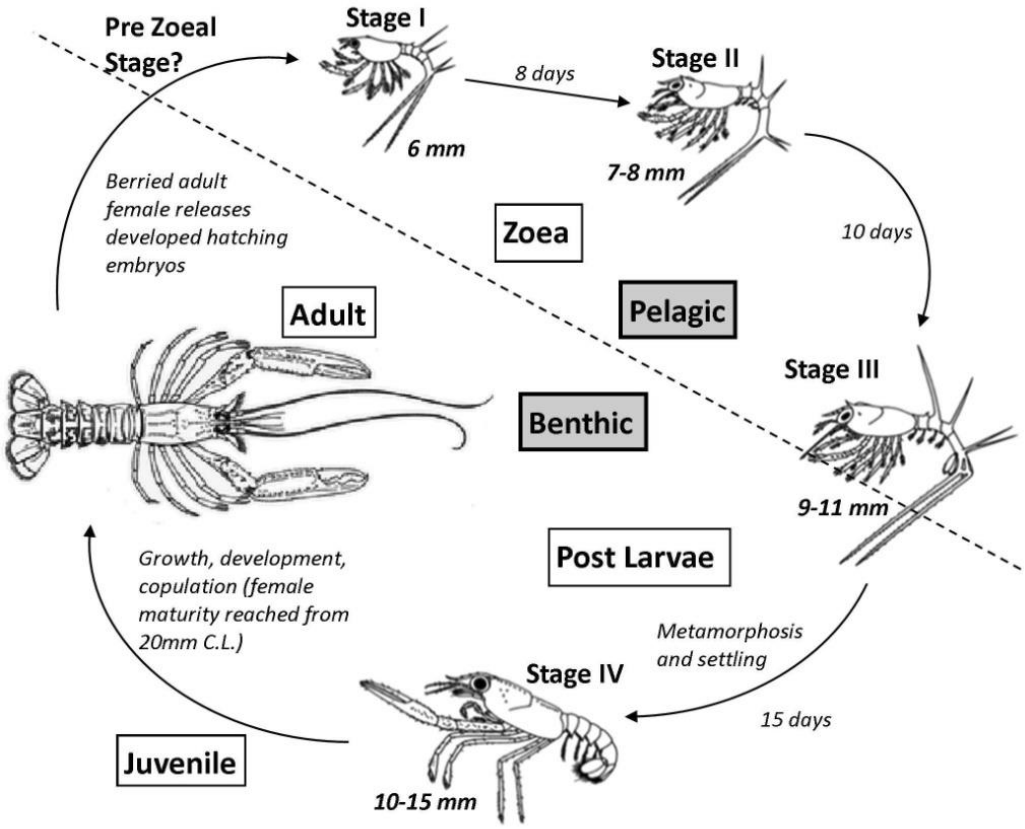
This short summary of reproductive biology, ecology and aquaculture of *Nephrops* is provided to give the reader a background to the life stages, to a level required to follow the handbook. Further reading is recommended, particularly Powell and Erikson (2013), Smith (1987), and Farmer (1974).

Adults (female broodstock). Lobsters are dioecious or gonochoristic (have separate male and female sexes), with the onset of maturity in either males or females variable across populations. Maturity can occur in quite small females from *ca.* 2cm carapace length (CL). It is generally believed that females have an annual reproductive cycle, with the developmental stage of the ovary determined according to ovary colour and size/volume. Fertilisation of female gametes (ova, or eggs) is likely to occur internally, after copulation and subsequent transfer of a spermatophore, which contains male gametes (sperm).

The timing of key events across the reproductive cycle is dependent on seasonal temperature (i.e. latitude, ocean currents) and typically occurs earlier across their southern and western most range, certainly with comparison between S. European, UK and Scandinavian populations. In late summer in the North Sea, fertile eggs are extruded from the ovary. These reside externally underneath the abdomen as a clutch (lobsters are termed “berried”). Eggs remain attached via the swimming legs, and develop slowly over the winter. From April to June eggs develop and change colour from black to green and finally orange/pink. Advanced embryos eventually hatch and are released directly into the water column, and for the remainder of the summer the non-berried females moult, mate and feed. Similarly, latitude also affects the incubation period for the eggs, according to a degree day effect, with between *ca.* 120 to over 300 days required for Southern Mediterranean or North Atlantic populations respectively.

The fecundity of females also varies, with between 900-6000 eggs/female recorded, depending on abdomen width (animal size), geographic location or fishing ground, and sediment. Adults may reside at hundreds of metres depth depending on population, and seem to favour muddy sand allowing them to construct simple burrows. The species appears to suffer from egg loss, which alongside natural stressors may be increased via anthropogenic stressors such as fishing pressure. Trawled lobsters appear to lose more eggs than creel caught specimens.

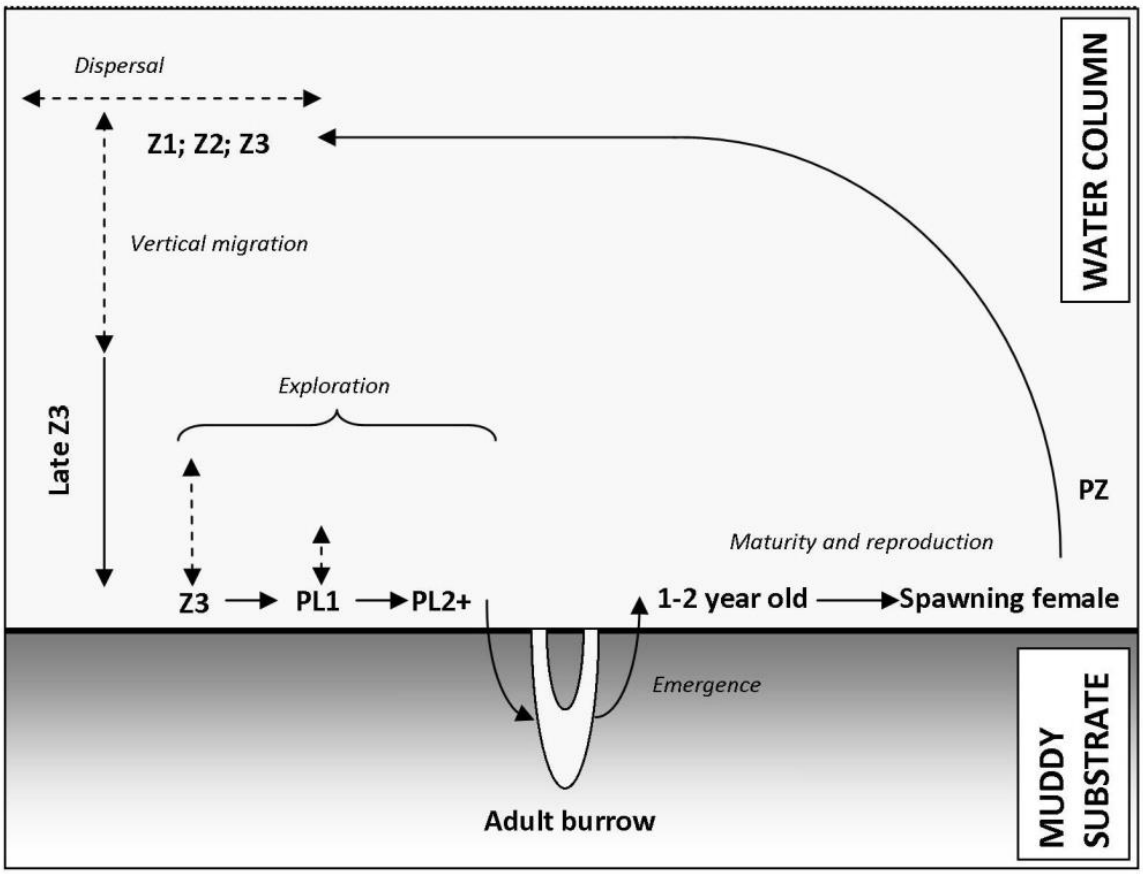
Larvae. Larvae are released into the water column, over approximately one week from an individual female, typically at dusk. Larvae are motile and move toward the surface (they are initially positively phototactic, i.e. are attracted to light). They then enter the plankton assemblage in the top 20-40m and undergo DVM (Diel Vertical Migration). DVM is a daily, rhythmic vertical movement where organisms move up in the water column during darkness, and reside deeper during daylight hours, to optimise foraging success and reduce the threat of visual predation. Larvae are likely to eat small zooplankton during spring plankton blooms, such as *Acartia sp.* and *Calanus sp.* copepods (smaller crustaceans up to 2mm total length). Although larvae are motile, currents distribute larvae laterally during pelagic phase development (i.e. whilst residing in the water column).



***Nephrops norvegicus* life cycle (Powell and Eriksson, 2013, after Santucci, 1926)**

There is a general agreement that three successive larval stages exist: Zoea (Z) 1, Z2 and Z3. Intermoult duration increases slightly between successive stages and depends primarily on temperature, and at 15°C this may last from 8-12 days between stages. Larvae are distributed during this period, which could last several weeks in cool, spring temperatures in the NE Atlantic. Total larval length increases from *ca.* 6 to 12mm over this period. In appearance, larvae don't resemble adults and possess spines that emanate from the abdomen and tail, which probably have an anti-predatory function and may assist buoyancy. Late Z3 larvae become increasingly benthic, or "semi-benthic" (they reside ever closer to the sea floor rather than the higher water column).

PL (Post larvae) and juveniles. Metamorphosis to the initial PL stage IV is illustrated by a distinct change in body shape, with obvious chelae, the development of a fan shaped tail and a reduction in spines. In appearance they now represent a small adult. PL stage IV can still swim, but generally reside and explore the substrate. They are capable of digging small burrows in appropriate substrate, and experimentally have been shown to favour muddy sand. Juveniles also thought to share larger burrows commensally with adult *Nephrops*. At 15°C, it requires *ca.* 20 days for a PL stage IV to moult to stage V, and then successively longer intermoult periods to subsequent stages. It probably requires a few years for juveniles to attain sexual maturity, from 15mm total length at PL stage IV to a 20mm CL (minimum) as a young, sexually mature adult.



***Nephrops norvegicus* life cycle (Powell and Eriksson, 2013, after Smith, 1987)**

1.3 Overview of hatchery effort

To our knowledge, R&D for *Nephrops* larviculture began in the 1970's in Portugal, with infrequent research published in the scientific literature to the present date. A dozen or so research papers have investigated broodstock care, larval and young juvenile rearing at laboratory scale across Portugal, Scotland, Isle of Man, Ireland, Germany and Sweden (see Powell and Erikkson, 2013 for further information).

In brief, larvae have generally been collected from captive female broodstock (fished using static pots or creels), and allowed to hatch naturally in aquaria. Some larvae have been taken from plankton trawls or from incubated eggs removed from gravid females (albeit with limited success). Larvae have been typically raised individually or sometimes communally in small containers (usually less than a few litres in size), such as beakers or perforated vessels, with frequent water changes and aeration. Most commonly, Brine Shrimp, *Artemia sp.* have been used as a live feed, although green water systems have also been investigated at small scale, i.e. vessels of a few litres. Some studies have fed *Crangon crangon* eggs, small pieces of mussel and shrimp, wild plankton and microencapsulated feed, usually provided in excess. Water flow was relatively high/exchanged regularly in static systems, otherwise water quality declined and hygiene and survival compromised.

Overall, larval survival from Z1 to PL stage IV has been limited to approximately 5%, with very few PL recruited and high juvenile mortality after settling on benthos. There are few common threads amongst the research to suggest how success could be improved, or upscaled, with one exception: Temperature appears to be key factor, with many studies demonstrating that an optimum of *ca.* 15-16°C expedites larval development and improves survival between Zoeal stages. In addition, research efforts at the University of Gothenburg, Sweden since the late 1990's have shown that *Nephrops* can be reared communally in large cylindro-conical upwelling tanks, in a similar manner to *Homarus sp.* A modest number of Zoea 3 and PL stage IV have been reared to enable robust experiments to be performed, although much remains to be optimised.

The table below provides a concise overview outlining the differences between *Homarus* and *Nephrops* which demands refinements of the typical *Homarus* protocol.

	Homarus sp.	***Nephrops norvegicus***
Broodstock robustness	Hardy. Can occupy shallow water/lower intertidal zone; physically larger animals. Appear to be less stressed after transport or introduction to hatchery.	Occupy deeper water, offshore, in burrows, with less incident light and more stable water physico-chemical parameters.
Fecundity	Fecundity may regularly exceed 10,000 eggs/animal; the species is physically large at maturity.	Fecundity typically 1-2,000 eggs/animal, often less. Shorter hatching season (late Spring to mid-Summer in UK)?
Egg loss	Minimal; alongside high fecundity, this suggests far fewer broodstock required to provide significant larvae.	Can be high, depending on quality of husbandry and reduction of other stressors.
Larval robustness	Larger, few spines, generalist appetite, can withstand high energy/aerated systems/short periods of emersion.	More delicate, smaller, with more complex exoskeletal architecture. Cannot be moved in nets.
Larval development	Rapid, appear to be eurythermal and can metamorphose within 10 days at temperatures up to 20°C (i.e. perhaps half the duration of *Nephrops*).	Limited to a ~17°C maximum, mortality occurs between Zoeal stages; low energy system sub-optimal for semi-benthic stages.
Cannibalistic tendancies	Larval density/survival probably limited by highly cannibalistic nature. Physical separation required for PL.	Comparatively less during larval stages; can be reduced after settling/adult stages by rearing at low density.
PL care	Appears satisfactory out of sediment; Aquahive technology established for mass culture of Z3 onwards to young juveniles.	Juvenile survival not studied widely but appears challenging; little knowledge of optimal diet/growth.
Release protocol	A range of approaches attempted and used including scuba, underwater flumes, release at low tide.	Never attempted or considered to our knowledge; best practices not established.

2. Broodstock

Procurement of berried adult females, of sufficient quantity and quality, is essential to produce significant numbers of larvae and PL stage IV. Adult females are, of course, procured from wild stocks and during capture, transport and after arrival in the hatchery, optimal husbandry is required to ensure that both the individual females and the developing eggs are exposed to as few stressors as possible. It is however, advisable to check any national regulations or regional byelaws regarding landing of berried females, and minimum landing sizes.

2.1 Procurement and hatchery location

Location. It is beneficial to source broodstock ("brood") and eggs as close to the hatchery location as possible. There are several reasons for this:

- Reduced emersion period – local procurement (i.e. within a few hours between removal from creels to introduction to hatchery water) will decrease stress, and the logistics required to move brood. Stressors will include dehydration, temperature fluctuations, noise/vibrations and incident light intensity, wavelengths and photoperiod. Since the animals are aquatic, emersion will reduce gas transfer and promote build up of waste products, causing physiological changes to the respiratory, excretory and circulatory systems.

- Improved water quality – It is likely that the water used in a marine hatchery will be sourced locally, for example a flow through system or to top up recirculating systems (RAS). This will likely reduce stress of removal and introduction to different water bodies. Better still, procurement of water from depth and adjacent to *Nephrops* grounds would ensure a water body with identical physico-chemical characteristics would be used to rear the brood, and for onward culture/release of larvae and juveniles.

- Improved biosecurity – Movement and release of aquatic animals is under increased scrutiny at both national and European levels (e.g. Council Directive 2006/88/EC, "*on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals*"). *Nephrops norvegicus* is a host for the parasitic dinoflagellate *Hematodinium perezi*, a pathogen that has shown ability to infect several decapod species, causing "Bitter Crab Disease". Therefore a number of statutory bodies may demand formal biosecurity measures, licencing, record keeping, inspections, etc. to reduce the risk of geographic spread of disease.

- Local stakeholder support – Responsible sourcing, and introductions of genetically different stock may worry national or regional stakeholders. Although the species has a prolonged larval period with a likely extensive distribution, release of juveniles with local provenance may appease such concerns. It would certainly assist collaboration with local fishermen who could sell, if not "loan" berried females, but expect to see tenable results prioritised at or adjacent to their fishing grounds; indeed, this relationship should be one of the key drivers behind initiating hatchery or R&D operations.

Brood supply. Provenance of brood is key to maintaining medium term survival of the brood population within the hatchery. *Nephrops* are distributed throughout the continental shelf of the North Eastern Atlantic and Mediterranean, at particular concentrations (at fishing grounds) that make commercial fishing viable. The majority of the fishery employs offshore trawling, which is generally not suitable to source brood. This is a high energy, prolonged capture method using a large towed net, with the catch stored on ice for several days before landing.

For the purpose of maintaining healthy, active berried females as broodstock in the hatchery for months after capture, it is vital that adults are sourced from vessels that use static gear such as pots or creels. *Nephrops* creels are similar to crab or lobster pots; adult and juvenile *Nephrops* are attracted into the baited pots, which are lifted regularly, inspected, re-baited and returned. Adults are retained and individually placed vertically, head and chelae uppermost, in a matrix of individual rectangular compartments (tube trays) capped at both ends with a large perforated plastic tray. This ensures that each adult is kept separated from each other and maintains some humidity, thus reducing stress, and preventing egg loss by reducing tail-flicking. Alternatively, the brood may be held communally in baskets. This requires the chelae to be "banded" (with small elastic bands) in order to reduce fighting and damage at high density.

Suggested procurement approaches are outlined below. Seasonality needs to be considered, i.e. it may be best to receive berried, ripening females in the months just before probable larval release, and during the typical hatching season. The onset and duration depends on geographic location, and it is worth preparing industry colleagues some months beforehand and request updates on the numbers of females in the catch. This is relatively straightforward as berried females are obvious, with a colour change of the eggs which suggest imminent release of larvae.

- ***Direct procurement.*** To allow control of broodstock care from moment of capture to introduction to the hatchery, it may be possible to accompany a suitable vessel (e.g. collaboration with fishermen during normal operations, or a specifically contracted private or research vessel). Although outside the expertise of this handbook, perhaps this could be taken a stage further, e.g. creels thrown from the shore into deep water, or even hand collection via SCUBA in shallower water in appropriate habitats.

- ***Commercial Creel Fisherman.*** If it isn't possible to directly accompany/collaborate with a fishing vessel, it may be possible to organise collection soon after the catch is landed. This would preferably require that as the creels are hauled, berried females are separated into a tube tray to reduce additional sorting and handling stress at a later time.

- ***Shellfish merchants/transporters.*** Since individual creel-fishing operations can be relatively small in scale, co-operatives or private shellfish merchants exist which allow landings to be collated, transported and sold more effectively via an economy of scale. It may be possible to intercept brood

at stages of the supply chain (selected from holding tanks; from vivier HGV at the roadside during transport). The high turnover and multiple sources suggest a better likelihood of obtaining berried brood on a regular basis. However, there is a possible disadvantage of reduced knowledge regarding the provenance of the animals during capture, higher handling frequency, etc.

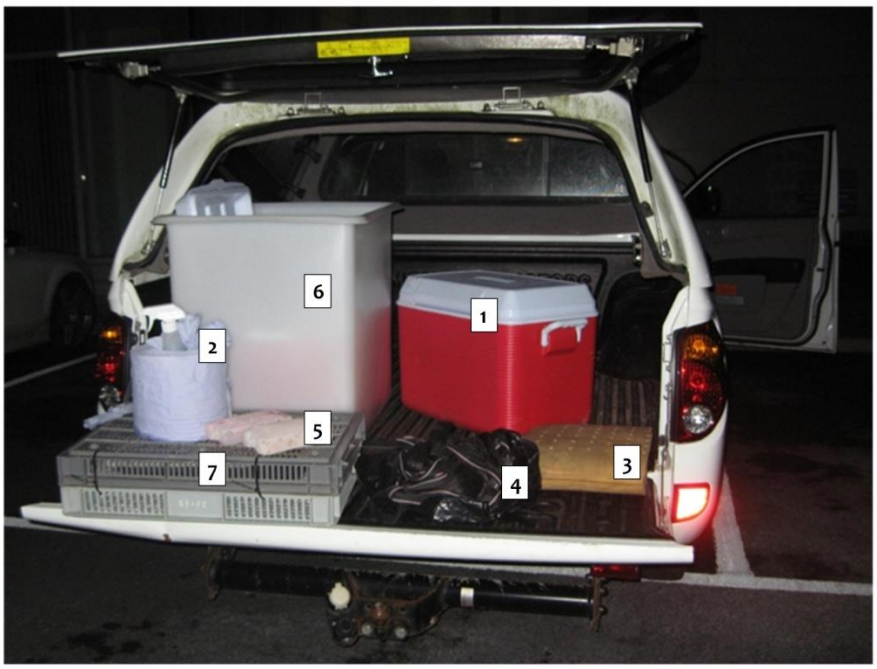
Transport route and method. Whilst international transportation is technically possible, it requires much logistical planning and documentation from the exporter and importer. The expense and time required to transport brood in this manner, in addition to the benefits for local procurement outlined above, suggests this is not a straightforward approach. The transportation method used will likely depend on the transport duration, as well as the holding facilities at the hatchery; there is a wealth of research on decapod transportation which would be useful as additional reading. The following is based on our practical work, designed to optimise survival but also minimise logistics.

- ***Damp transportation.*** Transportation out of water is possible but is only recommended for relatively short distances with this species. A journey time of approximately 2-3 hours did not seem to significantly reduce survival of brood for 2 weeks after introduction to the hatchery, but it did increase egg loss. Very short journey times would likely require less preparation and would be preferable. Damp transportation for long durations (4 - 10h) inevitably seemed to cause high mortality of the brood within days to weeks after introduction to the hatchery.

  The brood need to be kept moist, cool and in darkness throughout the journey. The tube tray should be placed inside a large insulated container, opened very slightly to assist air circulation whilst delaying temperature change. Surrounding the tube trays with damp tissue paper or seaweed, regular spraying with a fine mist of chilled seawater, and addition of chiller blocks (not in direct contact with the brood) is also worthwhile. Finally, it is also important to tightly secure the container (e.g. the boot of a car), and to reduce vibrations by sitting the container on top of cushioning.

- ***Wet transportation and vivier systems.*** Vivier systems are typically used by commercial shellfish merchants since there is a premium for live lobsters at point of sale. The best way to transport live adult *Nephrops* in tube trays is via lightly aerated vivier systems, which provide the animals with conditions which, best mimic those in their natural environment. This is the only viable method when transporting broodstock over long distances for many hours, in order to prevent mortality of females and deterioration of egg quality.

Vivier transportation allows regulation of a relatively constant temperature. Ideally, a constant temperature regime should be maintained for the *Nephrops* throughout the supply chain (from inhabited water, vivier transportation and hatchery holding facility). Regulation of oxygen levels and salinity in vivier transportation also helps to reduce respiratory, osmotic and other physiological stress of the brood and eggs, and thus assist viability and development.



Recommended materials for brief transport of* Nephrops *in tube trays (encased in grey plastic trays). *A large container (white box) is used to prevent spillage and reduce incident light in the pick-up vehicle. The insulated boxes (red) and/or cool blocks may be used to maintain temperature. Tissue or paper roll, dampened by cold seawater, will maintain humidity to a degree. Finally, a robust, ratcheted luggage tie will anchor the entire ensemble to attachment points, taking care to sit the containers on a large cushion.*

Equipment for transport – coolbox, leave lid ajar ***(1)****; atomiser containing seawater; roll to wet with seawater* ***(2)****; cushioning* ***(3)****; straps to prevent movement* ***(4)****; cool blocks* ***(5)****; large container to prevent spillage* ***(6)****; tube tray between plastic trays, containing lobsters* ***(7)****.*

2.2. Broodstock holding and husbandry

On arrival at the hatchery. The brood should be transferred to the holding tanks as soon as possible to allow re-immersion of animals that have been transported out of water; for brood that have been transported wet, or over very short distances, there is probably a reduced urgency. For brood transported in damp conditions, it is very tempting to immerse the tube tray immediately or indeed upend the tray gently into the water. To do so would promote tail flicking, and any non-banded lobsters will immediately grab each other via the chelae. This creates a mass of lobsters which is very difficult to separate, and will cause stress and in some instances, physical damage or autotomy. Brood need to be individually removed from the tube-trays or container, and placed in the tank tail-first to ensure air escapes from the gill cavities (underneath the cephalothorax) to avoid creating an air pocket. This is also a good opportunity to quickly check egg colour, and separate the brood into populations that are approaching hatching.

Tail dipping is often practiced on incoming broodstock in *Homarus sp.* Hatcheries: Briefly, the abdomen and eggs of large female brood are held in disinfectant (usually an iodophore) for several minutes to remove parasites, fungi and bacteria before they are introduced to the hatchery system water. The cephalothorax of the individual female is held above the disinfectant to prevent gill damage, and egg and larval survival and quality are apparently unaffected. This process is easier to achieve with a few females arriving per day, although more challenging with a large influx of smaller *Nephrops* brood; these may be procured in greater numbers and require rapid re-immersion. A quarantine tank may be one option to hold animals and allow them to recover, allowing some to be disinfected and moved to a main system at a later date.

However, to our knowledge *Nephrops* broodstock have not been tail dipped and therefore the effects of the disinfectant on developing embryos are not known. From the hatchery literature and recent operations, there have been few reported problems from larval diseases caused by parasites or pathogens. Experimental application of dissolved ozone to females for one week (420mV, *ca.* 20ppb total residual ozone) did not cause obvious mortality or developmental problems with survival of adults or eggs, but did reduce bacterial load. This could be an alternative way to maintain hygiene in larger groups of physically smaller brood, likely in a *Nephrops* hatchery (Appendix 1).

Holding tank design. All varieties of flow-through holding tank are suitable for maintaining brood, but by far the most convenient is a shallow raceway-type tank at waist height, which allows easy access to the animals for husbandry and observation. It is possible to accommodate brood communally in larger, deeper tanks during the early stages of embryonic development and then move them into shallower raceways when eggs are close to hatching. The following approaches have been used:

- *Deep communal holding tanks* – brood are held communally in *ca.* 1-2000L tanks. The chelae are banded, and short sections of 50mm PVC pipe and/or 150mm half pipe refuges have been provided. The disadvantage of keeping the broodstock in deep tanks is that it is very hard to check egg quality and development without increasing the amount of handling, since they need to be manually checked with a net. Larvae are also difficult to collect, and it is recommended to use deep tanks for short periods only prior to hatching.

- *Shallow raceway* - is much preferred as it provides much more control for lobster husbandry. Tanks can be cleaned easily, and it is possible to get a better idea of the general condition of the females and whether they are dropping eggs and eating food. Water depth must be altered so that the brood lie in several centimetres of water. Brood however need to be banded, with refuges, with a density of no more than 10/m^2, and it is difficult to track individual egg development in communal tanks.

- *Shallow holding tanks with perforated cells* - as above, but brood are kept individually. It is possible to track individual egg development over time, allowing management and prediction of larval production. The brood tend to be less stressed as they do not come into direct contact with one another, and banding is not necessary. The dividing trays are placed on two lengths of pipe to keep the trays off the bottom of the tank, and with the cell perforations this allows adequate water exchange, whilst faeces and detritus can fall through and collect on the bottom of the tank. Larvae can also leave the cells relatively easily during hatching. However, it is still necessary to clean the cells individually and build-up of waste does occur underneath the cells, and some experimentation is required with the height of the cells and the water depth: The lobsters must be immersed but the cell height needs to be well clear of the water level, so the brood can't climb into adjacent cells. A heavy sheet of plastic could also be placed over the top of the cells to prevent escape, if necessary, and will also help reduce incident light.

  A variant on this theme is to make pens rather than cells – i.e. there is no raised floor, with the perforated plastic acting only as walls, which will assist cleaning. The walls need to be heavy enough so that they cannot be moved by the brood, and well constructed so that gaps don't form at the base. An additional plastic sheet may also be required as a false floor, if the vessel base is distorted.

- *Bespoke tanks with larval collection system* – North Bay Shellfish Ltd devised a more sophisticated system that solves most of the problems affecting hygiene, stress and larval collection. Adult *Homarus gammarus* are maintained individually in 30L tanks supplied with a low flow and minimal food. Larvae are released into the tank outflow, which leads to a gutter that collects larvae from

several brood. Under gravity, larvae are deposited into a collection vessel. This method could be adapted for smaller sized brood, and commissioned for long term hatchery operations.

Clockwise from top left. *Communal raceway, note dropped eggs ad aggression; and raceway containing a cell matrix (3 x 10 cells, ca. 15 cm x 7 cm). Note water input and aeration is at opposite end of raceway to the outflow banjo filter. The brood and larval collection system at North Bay Shellfish Ltd* ***(bottom).***

The table below provides an overview of the positives and negatives of brood culture systems:

	Communal raceway	***Cell or pen matrix raceway***	***Larval collection system (Homarus sp.)***
Aggression	Not reduced, brood able to interact freely.	Not reduced, but brood unable to interact. Moulted females/eggs not cannibalised.	Seemingly reduced; individual water inflow likely to reduce pheromone transfer.
Banding	Strongly advised – appetite reduced so less effect on feed intake/manipulation (?)	Not required.	Not required.
Egg loss	Some, dependent on banding.	Reduced.	Reduced.
Limb loss	Some, dependent on banding.	Eliminated.	Eliminated.
Escape	N/A or None.	Very occasional; possible to gain purchase in cell matrix apertures if water level high.	Eliminated.
Physical space	Brood allowed greater movement. Unknown impact on welfare or physiology.	Reduced, unknown impact on welfare or physiology. Sufficient space required to allow animals to tail flip to release eggs.	Could be increased compared to cells, but may become space limiting and reduce absolute numbers of brood in hatchery.
Cleaning	Straightforward, able to perform daily cleaning throughout raceway via siphon.	Challenging to clean with siphon underneath cells, due to matrix floor, but flow rate seems satisfactory to maintain water quality. Potential to redesign matrix?	Requires occasional cleaning of tanks.
Density	At high densities, aggression is considerable. Density appeared to be optimal at 10-20 brood/m^2	Greater capacity to keep more broodstock: between 16-32 brood/raceway, *ca.* 30-40/m^2	Limited to number of individual tanks
Egg checking	Challenging as broodstock are mobile – potential to use spare tank (?)	Straightforward with mirror/using a pipe, and a proforma.	Would need a mirror, or perhaps only populated with hens with advanced eggs from a holding tank.
Larval collection	Little hindrance to collecting area. However, no barrier between communal adults and larvae (could create a barrier adjacent to outflow?) Adults seen to eat larvae on one occasion.	Apparently satisfactory. Adults separated from larvae, although some larvae probably lost underneath or between cell matrixes (potential to redesign matrix/use larger apertures?).	Guttering system allows automatic collection of larvae into pre-fed rearing vessel. No manual collection required in the evenings.
Construction requirements	Minimal, could be used for other projects out of season.	Minimal, could be used for other projects out of season. Cell matrix requires some preparation.	Substantial and would need a trial period to optimise. Relatively permanent structure for many anticipated future seasons. Original concept for larger *Homarus* spp.

Husbandry and water parameters. Optimal water parameters and brood husbandry within the brood tanks are important to reduce brood stress, assist collection of larvae and maintain larval condition for onward larval rearing.

- *Tank outflows* - All holding tanks, other than those with a collection system, should have a mesh filter covering the outflows in order to prevent hatched larvae escaping. A "banjo" filter is appropriate, as it can be angled fairly easily to dictate water depth. This filter can be easily constructed using a cross section of large pipe and mesh. Mesh size is 1-2 mm, allowing small particles to escape whilst ensuring the larvae do not. A smaller mesh size could clog rapidly, which increases the risk of the tank overflowing.

- *Tank inflows* - It is recommended to situate the tank inflow at opposite ends to the outflow, to allow for maximum water exchange. It also serves the purpose of allowing any hatched larvae to be gently washed towards the outflow, where they congregate and can be easily collected. The inflow should be angled away from the nearest cell to reduce stress on the lobster inside.

- *Water flow* - It is important to establish appropriate water flow. If the flow rate is too low, the dissolved oxygen concentration of the seawater may decrease, particularly if there is high biomass in the tank. The flow rate of the tanks is also important for maintaining the temperature of the water in the holding tanks (if air temperature is greatly different). Too high a flow rate may force any hatched larvae to become pinned against the outflow mesh. Whilst this may require some experimentation, we used an inflow rate of *ca.* 1L/minute for raceways of 0.5-1m^2.

- *Aeration* - Adding aeration to the tanks assists water circulation within the tanks as well as maintaining a suitable dissolved oxygen concentration of the sea water. Aeration can be provided by placing air stones at various positions within the tanks, but care must be taken to prevent bubbles from buffeting a cell containing a lobster which cannot move away. For the same reason, air flow in to the tanks should be relatively low. Aeration near the inflow will allow well aerated water to perfuse downstream across the broodstock, and away from the outflow where larvae may collect.

- *Temperature and salinity* - should be as close as possible to that used in transportation, considering that from the wild/holding tanks, temperature may have increased or decreased slightly during transit. Temperature may be changed slowly, with the rate of change probably not exceeding 0.5°C per day, in order to manage (i.e. retard or expedite) egg development. Auxilliary chilling may also be used to keep some raceways cooler than ambient to assist new arrivals and manage hatching rates according to likely degree days (Appendix 2).

- *Light intensity and wavelength* - In their natural habitat, *Nephrops* can live at up to several hundred metres depth, typically in burrows. Light intensity and wavelength is likely to be very different to that experienced by other decapods that inhabit shallow water or the intertidal zone. Lighting could be manipulated by organising low ambient lighting (we used *ca.* 0.3 Einsteins) and also using opaque tanks/lagging with black plastic, and using black covers to reduce intensity further. Brood should only

be uncovered for short periods whilst husbandry duties are being undertaken. It may be possible to use a red filter, but the colour of larvae and live feeds (dark translucent orange) makes manipulating larvae more difficult.

- *Photoperiod* - Photoperiod is likely to affect the on-set of hatching and therefore should reflect the photoperiod of the natural environment at that specific time of year. As larvae tend to hatch around dusk and into darkness, it may be an option to hasten the onset of dusk so that it is possible for hatchery staff to undertake larval collection earlier in the evening.

Siphon cleaning of raceways, and feeding adult broodstock.

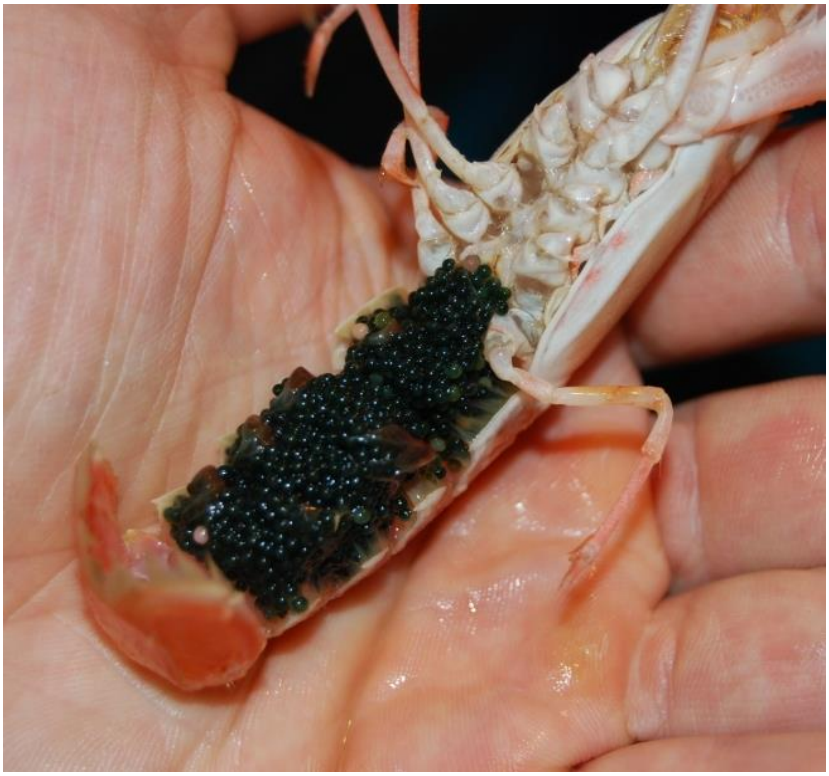
Feeding – similarly to *Homarus sp.*, *Nephrops* broodstock can be maintained on a range of frozen seafood such as raw frozen as well as cooked frozen prawns, mussels and squid. It was best to defrost any food before feeding, and worth varying the diet to promote a broad range of micronutrients and trace elements. Fish muscle tended to fragment and leach rapidly into the tank; formulated feed (e.g. penaeid shrimp maturation pellets) wasn't tested but may be appropriate.

Female appetite is apparently reduced whilst carrying eggs, and in any case it is important to not over-feed since excess food and faeces can increase bacteria loading and reduce water quality. *Ca.* 2 grams of food (say, one prawn, one squid ring, half a mussel, etc) should be fed to each individual 2-3 times a week; our experience showed a high appetite if fed once a week, whilst feeding every day was wasteful. However, this ration can be monitored and reduced if it appears that the brood are leaving large quantities of food uneaten.

Cleaning & hygiene **-** In order to maintain low bacterial levels in the water of the holding tanks regular cleaning must be undertaken. This can be done by using a siphon to remove any uneaten food, faeces and shed eggs which tend to build up on the bottom of the tank. This can become tricky if the holding cells are suspended on pipes as detritus builds up underneath them. In this case, it is best to try and access this area using a long siphon which fits underneath the cells. It is normally good practice to clean the tanks the day after feeding as there tends to be more faeces present and any uneaten food can be removed before it starts to decompose. To reduce weekend workload, perhaps feed on Monday and Thursday, and clean the day afterwards.

Outflow filters were cleaned frequently for two main reasons. Firstly, to prevent blockage, leading to the tank overflowing and the potential loss of larvae. Secondly, larvae tend to aggregate at the filter after hatching; it would be detrimental to the larvae to come into contact with faeces, detritus and associated bacteria which have become stuck to the mesh of the outflow filter.

Handling – In comparison to *Homarus sp.,* berried *Nephrops* are less robust, have a lower tolerance to being handled frequently and will drop eggs. They are also smaller and more fragile, and care must be taken when handling and moving them, to prevent egg and limb loss. When lifting *Nephrops* out of the water, both hands must be used: One to gently but firmly grab the back of the carapace, and the other to support and cup the abdomen to prevent flicking. Another method was to use a short length of 50mm PVC pipe to surround a lobster from behind, thus lifting the *Nephrops* out of water in a reservoir, particularly useful when transporting lobsters quickly between tanks.

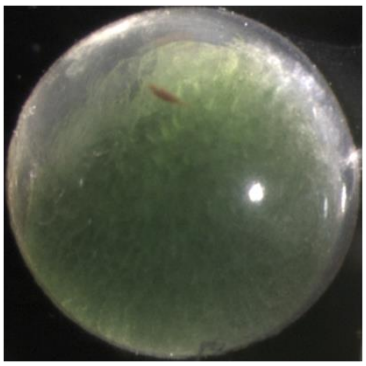


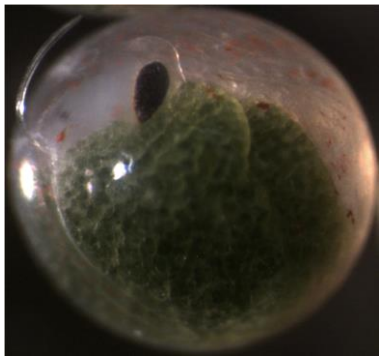
Top Left: *Using a pipe to gently remove an adult to inspect eggs;* ***Top Right:*** *use of dental mirror as a less invasive way of checking eggs.* ***Bottom Left:*** *Black-green eggs of unripe female (up to stage 6), and* ***Bottom Right:*** *Orange-pink eggs of female with mature eggs likely to hatch (stage 8). Stage 7 exhibits an olive grey colour.*

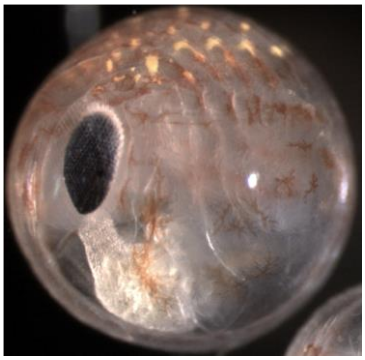
Egg checking – Regular of checking of egg development, perhaps once a week (maximum), was useful to determine which females are likely to initiate hatching. Females can be lifted out individually, and although this likely increases stress, the eggs can be examined much more thoroughly. Alternatively, the brood can remain in the water and a mirror (such as a dental mirror) can be employed to examine the eggs under

individual females. Due to movement of the animal and distortion caused by the water, it is only possible to get a rough idea of egg development according to colour – however, often this is often sufficient and it is certainly less invasive.

Egg stages – for the purposes of a hatchery**,** egg colour alone may be sufficient to assist stock management during hatching. However, it is worth providing more background on precise egg characteristics to allow understanding of the ripening process, and to anticipate the onset of hatching earlier in the year. The egg mass colour is by far the easiest indication of the egg stage of development and can be seen at a glance. For a more thorough indication of egg development, a small quantity of eggs need to be removed from the egg mass and examined. This can be done by carefully teasing away a few eggs using a pair of forceps, placing them on a Petri dish and observing under a low power dissecting microscope.







Left to right: *Green eggs (up to stage 6) with little or no eyespot developed; Stage 7 with green yolk, eyespot and some carotenoid colouration; Mature eggs likely to hatch (stage 8) with obvious eyespot, no green yolk, and near developed limbs (Courtesy of Susanne Eriksson, UGOT). These would approximately correspond to black-green, olive or orange clutches respectively with the naked eye.*

Hatching and larval collection – Larvae hatch from females from the onset of dusk and later into the evening. Work at CSAR over two seasons suggest there may be a vague circa-semilunar influence, although this was not been proved. For a particular female, larvae tend to hatch over about a week, with absolute numbers released following a bell-shaped curve with respect to time.

It was relatively unusual to observe hatching, with larvae usually already present near or at the filter upon inspection. The process was similar to *Homarus sp.* although with slightly less active shaking of the abdomen. The abdomen extended slowly, with the swimming legs showing rapid movement. This broke the egg capsule (eclosion) enabling mature embryos to be released. Occasionally several minutes were required for the larvae to unfurl (i.e. extend the abdomen fully) from embryonic posture.
Best practice suggests larval collection soon after hatching, reducing the likelihood of being eaten by brood, buffeting against the filter, and allows first feeding within hours of hatching (presumably this would likely happen in wild populations). Unless a sophisticated larval collection system has been considered, collection must be done manually, perhaps within 2-3 hours following dusk. A head torch should be worn to help identify larvae, as the hatchery should be in darkness at this stage. Due to the water flow within the brood tank, larvae will tend to accumulate near the filter on the tank outflow. Depending on flow and how soon

after dusk larvae are collected, larvae may also be distributed throughout the tank. When a particular female has started the hatching process, then there may be a high number of larvae in the occupied cell and adjacent cells.

It is also worthwhile checking again early in the morning to assess further overnight hatching, i.e. larvae that have accumulated following collection the previous evening. These larvae are probably still viable. A large number suggests that collection should happen later in the evening, which may be assisted by artificially advancing dusk by altering the photoperiod.

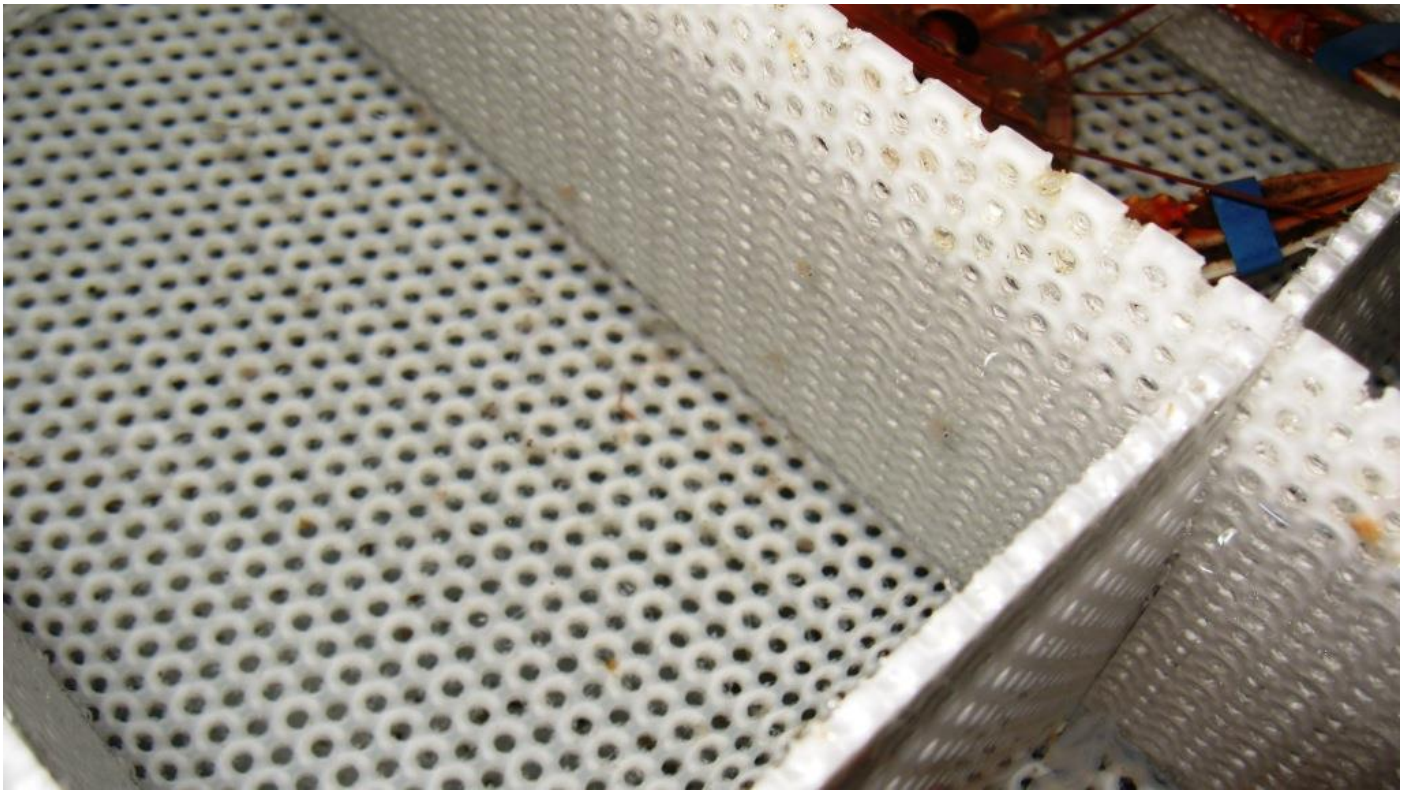
Nephrops larvae are smaller and more fragile than those of *Homarus gammarus* and need to be handled with great care. This is mainly due to their long spines which can be easily broken or tangle if handled incorrectly. Water to water transfer is required, as using a net will tangle larvae in the mesh. The best practice for collecting newly hatched larvae is to always keep them submerged and prevent them from coming into contact with any surface. Using a "gravy baster", effectively a large pipette (with the aperture widened slightly by cutting off a few centimetres at the tip) proved very effective for larval collection. Individuals can easily be counted by placing a fingertip over the aperture, and temporarily collected in a beaker or jug. A known quantity of larvae can then be transferred to a rearing tank, containing food items.

Record sheets – It can be very useful to keep record sheets for:

a) the number and survival of brood being held, and larvae hatching numbers (daily)

b) egg development (perhaps weekly-fortnightly).

Recording egg development over time will allow for predicting hatching times and number of larvae. Keeping records of tank cleaning and broodstock feeding will help ensure the animals are kept in good condition. Examples of record sheets are found in Appendix 3.



Above, *larvae recently spawned in a cell adjacent to female;* ***below,*** *larvae gently washed onto banjo filter ready for collection via basters or large pipettes.*

3. Larvae

Until recently, the majority of *Nephrops* larvaculture had only taken place on a small scale, using small vessels such as polystyrene cups or beakers to rear individual larvae. However, this has now been improved and *Nephrops* larvae could be reared on a scale more comparable that of *Homarus sp.* hatcheries.

3.1. Vessel design and operation

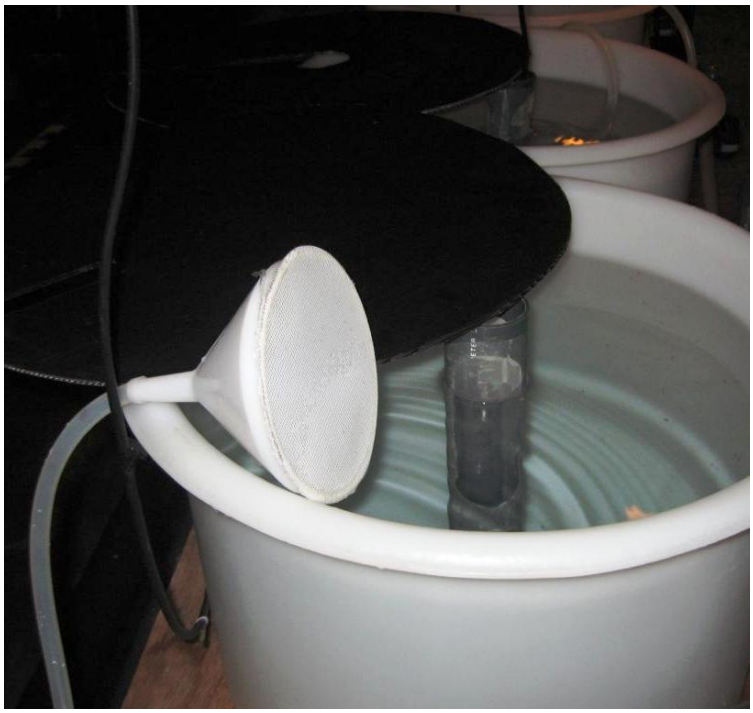
As with the established culture of *Homarus sp.*, the vessels used for the large-scale culture of *Nephrops* larvae are *ca.* 90L cylindro-conical vessels, also known as Incubators or Paxton "hoppers". *Nephrops* larvae require a defined upwelling current from the bottom of the hopper, which keeps the larvae in suspension. High aeration cannot be used to agitate the larvae as they are too delicate, and therefore a relatively sophisticated baseplate is required to maintain upwelling water flow.

Since the open sea has no corners, the shape of the hopper and baseplate has a limited number of edges which could reduce "dead spots" of circulation. This prevents, but does not abolish, build up of detritus in corners. It also maintains suspension of larvae in the water column away from the dead spots. Perhaps this could be improved with a bowl/goblet shaped hopper without corners, but to our knowledge this vessel is not available at the numbers or volume required.

Water inflow and movement. Water input into the hoppers is supplied at the bottom of the vessel to create an upwelling current, rather than a simple gravity feed into the hopper from above. This current is produced by means of a dispersal base-plate which sits snugly in the bottom of the larval rearing vessel, with a large central hole to accommodate a standpipe (see Appendix 4 and 6 for design, fitting and suppliers; note, this is the author's method for vessel construction, other approaches may be equally valid).

A number of small holes, *ca.* 2mm diameter, were drilled into the baseplate, radiating from the centre to the margin. The holes allow a relatively laminar flow of water to be forced into the vessel. A number of designs were trialled, including 8, 16 point stars (both hand drilled by eye, and precision drilled with a lathe) and finally a precision drilled multi-holed design. The latter seemed to work best, as this reduced dead spaces between holes where larvae could reside; it also created a more uniform flow. However, increasing the number of apertures also reduces the speed of the upwelling water, and if the available water pressure is limited then a trade off may be required at the design stage, depending on system specifications.

The base-plate is bespoke for the hopper, by using a lathe to chamfer the edges to an angle which matches that of the lower, conical part of the tank. The plate is then securely fitted to the bottom of the tank and non-toxic silicone placed around the edge to ensure a good seal. This is vital, since at high pressures water would otherwise escape from the base-plate margins, and also prevents breaking the baseplate away from the hopper when removing the standpipe.



Above, *hopper baseplate and air curtain around standpipe in action (for full details on design see appendix).* ***Top right,*** *detail of filter siphon.* ***Right,*** *Artemia removal in progress. Note covers have been removed, and tape on diaphragm valve marking inflow.*

Water outflow. Water exits the hopper via a central standpipe which emanates from the centre of tank. The height of the standpipe controls the water level in the rearing vessel and should be placed snugly in the centre of the baseplate prior to filling the hopper: Water enters the hopper until it overflows through the standpipe and returns to the system via a drain underneath the tank. The central stand is wide enough to allow the water to escape at the same rate as the water is entering the tank, i.e. maintain water turnover which could become relatively high. If the pipe is not wide enough, the water level in the tank will slowly rise and eventually overflow.

Typically, a *ca.* 90L hopper is maintained at about 75L, making use of the majority of the hopper volume. The waterline is thus 5-10 cm below the lip of the vessel, providing the operator with a few minutes to correct any errors in inflow rate (prior to overflowing).

Outflow filtration. The standpipe is surrounded by a mesh filter, so that larvae and feed remain in the hopper but water can freely exit. The filter is made from a larger diameter pipe with mesh windows cut into the side; this slides down over the standpipe. The filter is slightly longer than the standpipe (approximately the same height as the hopper) so that it sits well above the waterline. This allows any difference in water level, or splashing, to still be filtered appropriately. For *Artemia sp.* ("*Artemia"*) live feed, the upper limit of mesh diameter is 125µm. It is also good practice to have a small section of larger mesh diameter (say 500 µm) above the face of the main filter and above the waterline, to allow water to escape if the smaller mesh becomes clogged leading to a rise in water level which could lead to overflowing.

Aeration. Since *Nephrops* larvae showed little cannibalism (with appropriate feed rations), and hopper water turnover rates are relatively high with a low biomass, high aeration is not required to separate larvae or to ensure oxygen saturation. This is fortunate since high energy aeration (as applied to *Homarus sp.* to reduce cannibalism) damages *Nephrops* larvae within hours and leads to high mortality.

Aeration is better applied as a gentle air curtain, preventing the build-up of *Artemia,* as well as detritus, on the outflow mesh. Due to the high water turnover, it is usual for *Artemia* to accumulate against the mesh, eventually stripping feed from the water column and potentially starving the larvae. This can be prevented by securing an air source at the very bottom of the outflow filter, below and adjacent to the mesh window. A slow trickle of bubbles then rises up along the length of the filter, allowing much of the *Artemia* to be re-suspended in the water column.

A T-bar is sufficient to introduce an air curtain against one face of the filter, maintained in position by an elastic band. A collar of pipe with several air holes was investigated, although this likely requires precise engineering to allow air to evenly escape from all apertures evenly and simultaneously.

Hopper operation. As with any larval rearing system, it is important to control the rate of inflowing water into the tanks. This is especially the case for rearing *Nephrops* larvae as they are extremely fragile and can be damaged by vigorous water movements if the flow is too high, and by contact with the bottom of the tank (and each other) if the flow is too low. Therefore fine-tuning the inflow rates daily is recommended in order to get the best rearing conditions. This fine-tuning is made a lot easier by using a diaphragm valve for each of the larval rearing vessels, which provides greater control.

The precise design and construction of the hopper is likely to vary between units, and due to the slightly variable hydrodynamics, inflow rates will need to be monitored and optimised on a hopper by hopper basis. As a general guide, when the larval tanks are initially stocked with newly hatched and active Zoea 1 larvae, the water inflow rate should be around 4 – 5 L/min (maximum). As the larvae develop and moult (late Z2 and Z3), the inflow rate will have to be increased gradually to around 10 L/min in order to keep the larvae in suspension and away from the bottom of the tank.

Using absolute L/min figures is of some use, but perhaps the best way to define a correct inflow is to watch the larvae for a few moments. A high number near the baseplate likely requires a slight increase in flow.

Artemia and larvae pinned against the mesh suggests that flow should be reduced. For individual hoppers, it also helps to mark optimal flow on the dial of the diaphragm valve for future reference.

Water parameters and lighting.

- ***Temperature.*** Researchers have raised *Nephrops* larvae in small vessels over a range of temperatures (from 7°C to 21°C), with several papers showing that the optimal temperature for culturing *Nephrops* larvae is about 15 – 16°C. At lower temperatures, larvae develop much more slowly, moulting is less successful and mortality increases. Increasingly above 17°C, the larvae become close to their thermal tolerance and this also reduces survival. A temperature of 15 – 16°C allows relatively rapid larval development, which also promotes good survival and assists recruitment of PL. It also allows a 3-4°C buffer in case there are any accidental increases in the temperature of the system water. Lastly, if brood are also present in the same water system, it isn't too challenging to add auxiliary chilling to maintain a fraction of the water at a lower temperature to control egg development/accommodate new arrivals at a suitable temperature.

- ***Salinity.*** Larvae appear less sensitive to salinity changes, and have been grown successfully from 28ppt to full oceanic salinity. Perhaps this tolerance is an adaptation to the extended larval period which may disseminate larvae into inshore and offshore locations, or during DVM along a halocline. Low salinity (below 20ppt) does however appear to reduce survival.

- ***Lighting.*** As with the brood tanks, which may be adjacent to the hoppers, room lighting is reduced (we used *ca.* 0.3 Einsteins) and black plastic covers are used to reduce incident light further. To help cover the hopper, the filter should be approximately the same height as the hopper. Reduction of light is also important since *Artemia* are phototactic, and may collect in a specific part of the hopper if it is more illuminated than other areas. Photoperiod was ca. 12h:12h.

- ***Other water parameters.*** High water quality is required, whether due to a flow through system or excellent filtration and hygiene in RAS. The high water turnover maintains dissolved oxygen in the hoppers, and was generally above 80% saturation, bearing in mind the live feed also have an oxygen demand.

  Dissolved nitrogen was maintained at satisfactory concentrations (ammonium and nitrite, 0.02mg/L and nitrate, 1mg/L) and alkalinity 200 ppm $CaCO_3$ equivalent.

  When the hoppers are constructed and glued from new, it is also worth considering running them without animals and discarding the water, soaking them for a period, or using potassium permanganate flowed to waste, to remove any residues from glue, plasticware, etc.

3.2 Feeding and nutrition

Feed choice. *Artemia* is a ubiquitous live feed for many hatchery operations, and lobster culture has been no exception. A transition to more convenient sterilised plankton preparations has been successful at commercial scale, in addition to a wide variety of other feeds investigated at R&D institutions. In the past, a wide range of different types of food have been investigated for *Nephrops* larvaculture. These include dry feeds, preserved *Calanus sp*. in various size grades, algae, wild plankton, and live feed such as *Artemia* nauplii, copepod *Acartia tonsa*, mysids, amphipods and isopods.

Many factors need to be taken into account when deciding on a diet for commercial scale use, such as expense, ease of procurement, resources to prepare or store, batch quality, and how it behaves once in the rearing vessel (i.e. whether it stays in suspension and is available to the larvae).

Some *Homarus sp.* hatcheries use sterilised plankton feed, unfortunately this led to fouling on the long larval spines and rapid mortality. Small dry feed sank/leached into the water, especially during the first few days with reduced upwelling speed, and seemed to reduce water quality (or were not available to the larvae). At the time of writing, the best food for *Nephrops* larvae culture remains live *Artemia* nauplii in our hoppers. *Artemia* are relatively easy to culture and enrich at scale. Once added to the tank, they are robust enough to remain in suspension for long periods of time and therefore the larvae need to be fed less frequently. However, the types and brands of feed used in this study were not exhaustive.

Artemia nauplii culture. *Artemia* survive in an encysted form for long periods, and can be readily bought in bulk from many aquarist suppliers and refrigerated until needed. Cyst decapsulation and the hatching process are relatively simple (full details in Appendix 5). *Artemia* fed to *Nephrops* are 24h post hatch, at Instar II stage. This stage is slightly bigger and has begun filter feeding, allowing more enrichment to be imbibed. A wide range of *Artemia* enrichments are available on the market, and most of them supply a range of HUFAs, vitamins, probiotics, etc. No product preference is implied here, although long term enrichment (up to an additional 16-22h after Instar II) was favoured, since only one daily feed was performed and the enriched *Artemia* had a long residence time in the hopper.

Artemia density. It is important that feed is constantly available to the *Nephrops* larvae within the hopper, so that it is not a limiting factor for growth and survival. Adding sufficient *Artemia* nauplii to the culture water, to achieve of 5 *Artemia* ml^{-1}, is a workable candidate density. A higher density of *Artemia* can lead to clogging of the outflow filter, additional fouling of the tank, and is wasteful. A lower density may be suboptimal, but this depends on the efficiency of the design and operation of the upwelling and aeration technique. We didn't find the need to supplement feed density later in the day, although enriched *Artemia* was maintained on ice until use, to reduce metabolism and thus potential evacuation of enrichment.

Left, *removing enriched, cold* Artemia *from an aerated bucket;* ***Right,*** *gentle introduction of calculated volumes into a hopper.*

Nutritional optimisation and feed removal. After hatching and enrichment, harvested *Artemia* were counted and fed to larvae as quickly as possible. This can be challenging to achieve in a timely manner during hatchery operations, and if larvae are hatching it is useful to keep some *Artemia* for the first feed in the evening. Since the *Artemia* can survive harsh conditions, ice can be used to reduce their metabolic rate. This lengthens the period of time which the *Artemia* retain the enrichment, by reducing digestion and excretion. The enriched *Artemia* stock can be simply placed in an aerated bucket, itself placed in a polystyrene box full of crushed ice.

Depending on the hatchery staffing system, larvae should be fed early in the morning once the enriched *Artemia* has been harvested. Before this can happen, the previous day's *Artemia* needs to be removed from the hopper. After 24 hours in the hopper at ambient temperature, the *Artemia* has very likely reduced the enrichment and thus will be of little nutritional value to the larvae. The old *Artemia* are removed by means of a filter siphon, i.e. 1-2cm width flexible tubing with a 1mm mesh-covered funnel attached to the end. The siphon is set up in the tank with the mesh-covered funnel below the water-line and with the waste water containing the feed is drained to waste. The water in the hopper is replenished as usual by the inflow; typically, the water level decreases below the standpipe during this period. This process takes about 45 minutes, depending on the efficiency of the filter. Once very few *Artemia* nauplii remain in the larval tank, the filter can be carefully removed and the larvae can be fed with the newly enriched *Artemia*.

Surprisingly, very few larvae seem to become fouled on the filter, although this probably depends on the power of the siphon (i.e. the width and head of the water) and larval density. To reduce the likelihood of

fouling, a cover can be placed over the filter in an effort to draw the phototactic larvae away. This will also do similar to the *Artemia*, but doesn't appear to overly reduce the removal efficiency. If a larva is trapped, the filter should be moved slowly in the hopper water or gently perfused with water using the gravy baster, until it is freed.

Alternative filtration techniques could be used. For example, a second mesh filter could be constructed incorporating a 1mm mesh. With care, particularly at high densities, this could temporarily replace the 125 µm filter until the *Artemia* are removed, and then swapped back.

3.3. Larval handling technique

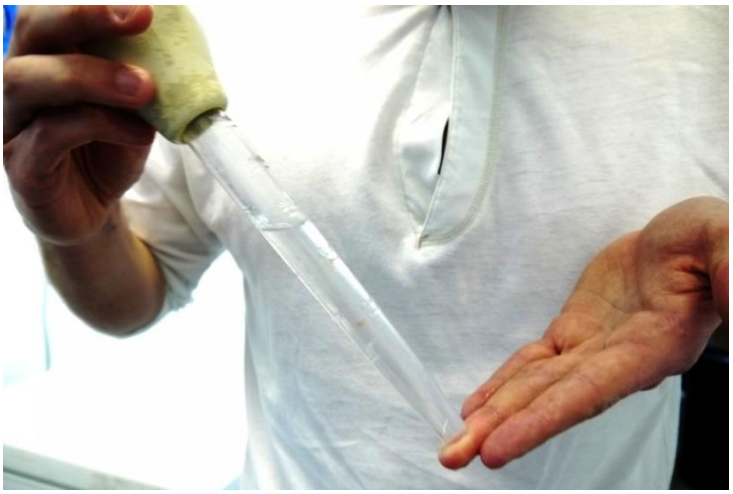
The larval stages of *Nephrops* are fragile and great care must be taken when handling them. The most risky periods are during collection from the female; when removing them for periodic cleaning/inspection; and when preparing Z3 larvae for metamorphosis.

- ***Beakers and jugs*.** For *Homarus sp.* larvaculture, sieves, nets and tea strainers are used to move larvae between hoppers. This cannot be done with *Nephrops* larvae due to the fragility of their spines. In fact, it is best practice to keep the larvae submerged at all times. Therefore, when moving larvae, a beaker or jug can be used to scoop the larvae and seawater out of the tank together, resulting in little damage being done to the animal. The problem with this method is that a lot of water will be moved as well as the larvae, but this can prove useful when collecting hatching larvae from brood.

- ***Buckets.*** As above, buckets are useful to collect larvae, particularly when temporarily emptying a hopper to allow cleaning. Provided this process is rapid and aeration is supplied, a relatively high concentration of larvae can be maintained in this way for short periods. Zoea 3, which are semi-benthic and will quickly collect at the base of buckets, need particular care.

- ***Gravy or Turkey basters.*** Effectively a large Pasteur pipette, a baster and head-torch are essential for manipulation, inspection and transport of individual/small numbers of larvae. The baster must be transparent, and the first 1-3 cm of the tip should be removed and smoothed (with sandpaper) so the aperture is large enough to accommodate the larvae – an additional increase in diameter will be required at the Zoea 3 stage, as the size and spine length expands further.

  This method is also useful for the close examination of individual larvae. Once the larva has been sucked up into the pipette, and whilst the end is still submerged, a finger can be placed over the end to prevent the water and larvae from escaping. The pipette can then be removed from the water and lifted up to the light to allow detailed viewing.



Clockwise from left: *Using a gravy baster to move and study individual larvae; temporary holding of larvae for counting/cleaning hopper; careful replacement of larvae into a hopper using a jug.*

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Husbandry of individual stages. Recently hatched larvae, Z1, Z2 and Z3 stages require slightly different husbandry requirements. The following case study of a hopper describes the typical approach over *ca.* 20-30 days at 15-16°C.

- ***Accruing hatched larvae.*** The number of larvae which hatch in a single night will be related to the number of ripe females with mature egg stages, the size of the females (and hence clutch size), and as the hatching season progresses the quality of ongoing broodstock husbandry. Larvae hatch from dusk and should be collected with a baster, jug/beaker, and a head-torch from brood tanks. Larvae should be counted accurately and transferred to a hopper pre-fed with *Artemia* (preferably maintained on ice until the evening).

  During larval collection, you may notice that some larvae are precocious and very active, others may require more time to become active, and some appear moribund. Different levels of larval activity have been recorded since the early 1970's and accruing larvae over 3-4 days in a particular upwelling hopper will give them the best chance to develop fully.

- ***Starting a hopper.*** Larvae from consecutive nights can be accrued in the same hopper until the density of larvae in the tank reaches 300-350 (*ca.* 4 larvae/L). This is the maximum number recommended for the hopper design and operation described above. Larvae should not be accrued for more than 4 nights in a row, and ideally over less time, to reduce the "age gap" between oldest and youngest larvae. This may reduce cannibalism, but presuming development rate is relatively constant, this will concentrate recruitment of PL stage IV over a few days. A larval stocking density of over 350/hopper is possible, however this may lead to a lower overall survival, so there is a trade off between low density (high survival), and high density (lower survival). However, it is likely that the hatching rate, absolute numbers of larvae and available resources will dictate final hopper density.

  To remove any moribund or dead larvae that did not thrive within a few days after hatching, the accrued larvae must be counted again into a cleaned hopper. Counting can be performed into an aerated bucket and then the larvae returned, or into an adjacent empty hopper. Counting can be accurate (e.g. for R&D purposes or to monitor hatchery success) or a good estimate, to confirm satisfactory initial larval density. This number is referred to as "Day 1", although one must consider a proportion of the larvae will be a few days post-hatch. The absolute number of larvae on day 1 is the total (100%) against which subsequent percentage survival at later time points can be calculated.

- ***Zoea 1*** (1400-1800 µm CL)***.*** For the first few days, larvae will reside in the top half of the tank with minimal upwelling. As the Z1 become older, they will rely more on the upwelling circulation and spend less time near the surface. The frequency of counting and cleaning is dependent on primary function of the hatchery (commercial or R&D) and how clean the hopper appears, but removal and counting every 6 days did not appear to cause any obvious stress or mortality.

  Larvae originating from brood that had been in captivity for 2 months suffered higher mortality in RAS. It is therefore worth considering a steady supply of brood throughout the season, rather than recruiting the majority of broodstock near the start of the season.

- ***Zoea 2*** (2100-2600 µm CL)***.*** The Z1 larvae will start to moult into Z2 larvae around day 5-8 (day 8-11 post hatch at 15^{o}C). To the naked eye it is quite difficult to notice this change, although this may improve with experience. Z2 larvae look very similar to Z1 larvae, just slightly bigger. In addition to timing, one of the best indications that the larvae are going through a moulting period is to spot tiny translucent moulted exoskeletons floating on the water surface or on outflow filters.

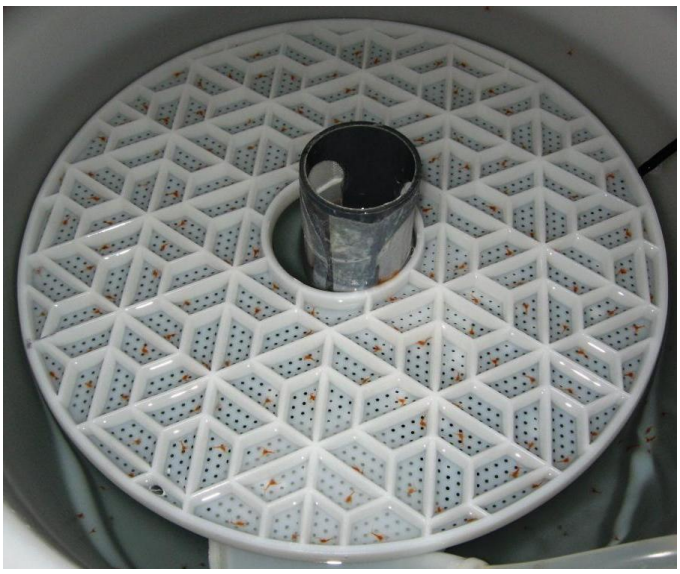
  Some larvae will not moult successfully, and die. In an optimised hopper with excellent husbandry, survival may be 70-75% at day 6.
- ***Zoea 3*** (2800-3400 µm CL)***.*** Z2 larvae will moult to Z3 at around day 10-13 (day 13-16 post hatch). Z3 have a substantially larger body and tail spines, and possess 2 small paddle shaped structures (developing fantail) either side of the spines. Larvae will spend more time near the baseplate and will become very reliant on the upwelling and circulation, which will need to be increased.

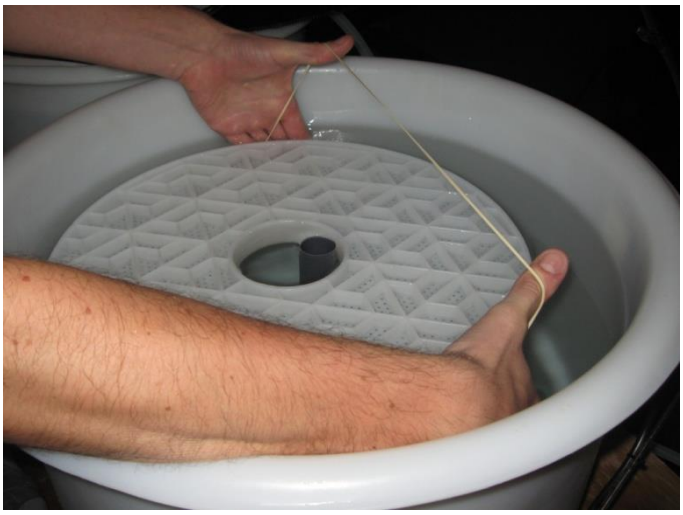
Again, some larvae will not moult successfully, and die. In an optimised hopper with excellent husbandry, survival may be 60-65% at day 12.

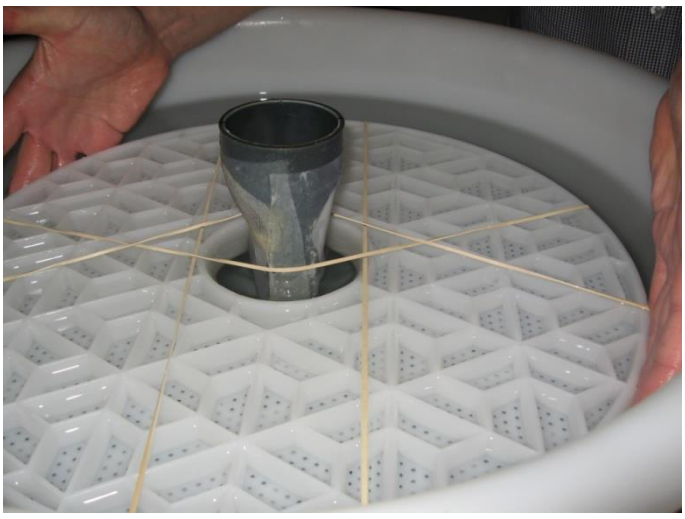
- ***Late Z3 and metamorphosis.*** At around day 15 (up to day 18 post hatch), the majority of the larvae will have moulted to Z3. At this stage, there may be up to 200 large larvae in the hopper, under rapid upwelling flow. Z3 tend to become snagged on the mesh at such high densities, (although this may be reduced in a lower density hopper). If the larvae remain in the hopper, mortality will increase. By placing the larvae in Aquahive trays, this will safeguard the larvae, allow daily inspections, reduce injury during metamorphosis, and will improve PL recruitment. This is relatively common practice in *Homarus sp.* Hatcheries that have access to Aquahive equipment.

  An Aquahive plate is set up in an adjacent rearing vessel so that it is floating on the surface. Inflow and aeration are turned off. Larvae are transferred into individual plate cells one at a time, using a large-aperture baster. A second aquahive plate is then placed securely on top, taking care to line up two holes at the margins of the plate. It is worth keeping the outer cells empty at this stage to reduce any escapes. The entire ensemble is then secured by a number of elastic bands (4 minimum) and is then submerged *ca.* 10cm under the surface, and is held in place by the elastic bands secured to the filter. The banding and submerging/retrieval procedure requires practice and a steady hand, not least a hopper that is wide enough to provide a margin permitting manipulation.

  The tank is fed normal rations of *Artemia* which can now pass through the cells via the small holes in the bottom of the Aquahive plates. Water upwelling need only be very low inflow, aeration is increased to circulate feed but placed above the plates (to reduce the risk of air pockets forming in the cells). Cleaning doesn't demand filter siphoning as the larvae are safe in the Aquahive plate. The plates are temporarily removed from the standpipe and allowed to float, whilst the filter is simply removed for *ca.* 30 mins.







Clockwise from left: *Copious Z3 in a hopper; Z3 removed to an Aquahive plate; second plate placed on top and secured with wide elastic band; minimum of 4 bands secured, with two wrapped around filter; plates submerged and aerated above the plates to ensure distribution of live feed and reduction of fouling at filter.*

3.4. Cleaning and hygiene.

As with any intensive aquaculture practice, it is important to maintain hygiene to prevent proliferation of bacteria, fungi and other pathogens; excellent water quality and quarantining of brood should assist this. Other equipment which is frequently used, such as beakers and basting pipettes, should be cleaned and disinfected thoroughly after use and stored in a clean, dry environment. However, one must be very careful not to transfer any disinfectant residues into the larvae tanks as this could cause mortality. Allowing equipment to dry before use is also an important step because it will dehydrate/UV will reduce viability of adherent bacteria.

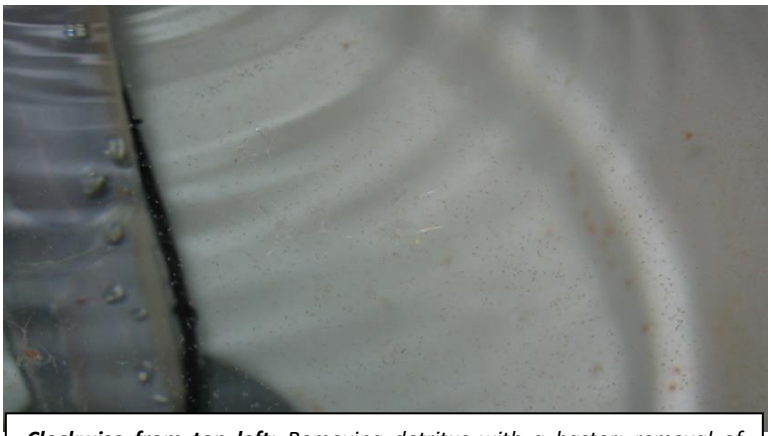
- ***Out flow filters (daily).*** Filter mesh will foul with old *Artemia* nauplii, cysts and detritus and this will need to be cleaned in a daily basis. This can be done during *Artemia* removal by resuspending the detritus on the mesh (jiggle the filter) and allowing it to be removed via the filter-siphon.

- ***Baseplate (daily).*** Dead areas within the tank, i.e. around the edge of the baseplate, will collect detritus such as unhatched *Artemia* cysts and dead larvae. If this is not removed regularly, then the water quality in the tank will decrease quickly. The siphon will not remove this, and the best way to remove it is by hand, using a baster. Place the waste into a beaker, dilute it and check for any live larvae which can then be returned by decanting or with the baster.

- ***Waterline (daily).*** If any waste has collected above the water line, this can be gently wiped off using a paper towel. Occasionally, in densely stocked tanks, many moults will be found on the surface. These are too large or buoyant to be removed by the siphon. Gently and slowly, force a beaker underneath the surface to collect any floating debris as the water pours inside. Check for live larvae and return as above.

- ***Hopper (weekly?)***. A thorough cleaning of hopper surfaces is recommended, as a biofilm will develop over time and could increase bacterial loading. This was done every 6 days, since the larvae were removed for regular counting, but it may be possible to increase this duration if data resolution is unimportant. Briefly, after transferring the larvae to a bucket, the standpipe filter is removed, cleaned and quickly disinfected. The central stand pipe can then be removed gently and is cleaned; the culture water is allowed to drain away. The surfaces of the hopper are quickly scrubbed and flushed. Ethanol can be sprayed with an atomiser if particularly dirty. After allowing this to evaporate, the hopper can be refilled.

  This may not be achievable at scale for a commercial hatchery, with an obvious minimum length of time between cleaning *ca.* 15 days at optimum temperature (i.e. from starting the hopper, to removing the Z3 to Aquahive plates). This time period may be short enough to maintain hygiene, however this would be dependent on rapid water turnover and good water quality in the hatchery. Previous studies have lost larvae due to bacterial or fungal infection, although this was not an obvious cause for mortality at CSAR.



Clockwise from top left: *Removing detritus with a baster; removal of standpipe, flushing and scouring of hopper; moults on the water surface; removal of floating detritus with a jug.*

Record sheets. As with broodstock, it is useful to keep up to date records when rearing *Nephrops* larvae, as this assists retrospective data collection (mortalities, feeding rates, temperature, etc) and helps anticipate resources and busy weekdays that hopper(s) may be started, Zoea 3 transferred, cleaning day, etc. Appendix 3 provides further examples of record sheets.

4. Post Larvae (PL)

4.1. Metamorphosis and nurturing PL

- ***Metamorphosis.*** At 15°C, the first Z3 should start to moult to PL stage IV at approximately day 19, perhaps 4-5 days after Z3 have been transferred from the hopper to the Aquahive plates. It takes approximately 7-8 days for the batch to complete metamorphosis, so with the initial PL appearing at day 19, one would expect the majority to have moulted by day 26. In a hopper stocked over 4 days, the majority of the larvae would therefore be between *ca.* 22 – 26 days old (or post hatch) when they metamorphose.

  From our initial studies that recorded metamorphosis in hoppers, metamorphosis occurred in the late afternoon, dusk and overnight. The majority of PL were observed at the end of the working day during larval collection in broodstock tanks, with some discovered the following morning. Few were observed during the day when illuminated, perhaps an adaptation to reduce predation when particularly vulnerable.

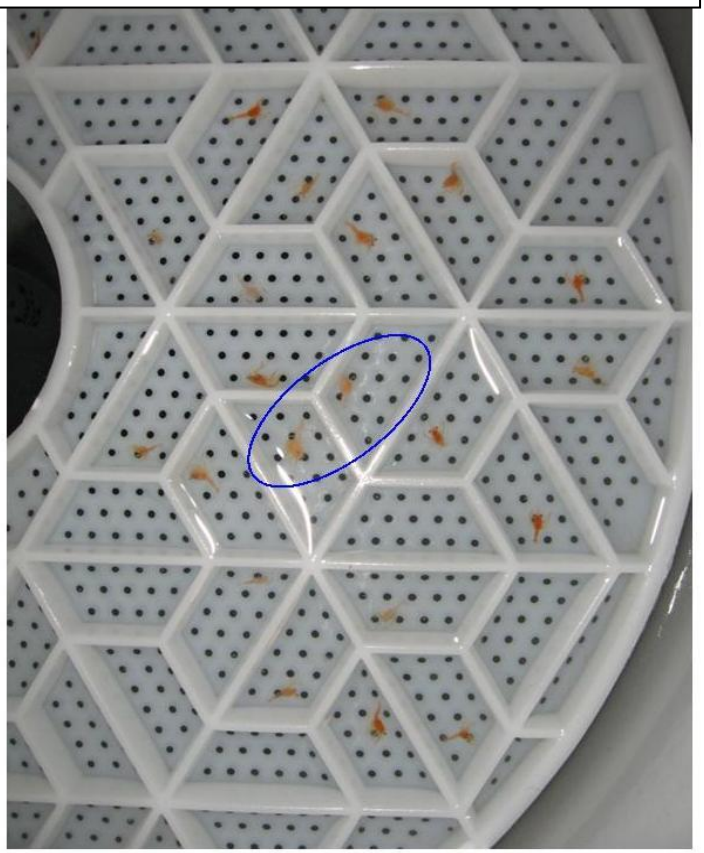
  Over the 7-8 day metamorphosis period, PL recruitment occurs as a bell shaped curve, with the majority of PL appearing between day 21 and 24. The overall metamorphosis period would likely be shortened if the larvae are accrued over less than 3 or 4 evenings; rearing at 15°C also reduces the moulting period duration (and expedites the onset of metamorphosis and improves moulting success, compared to lower temperatures).

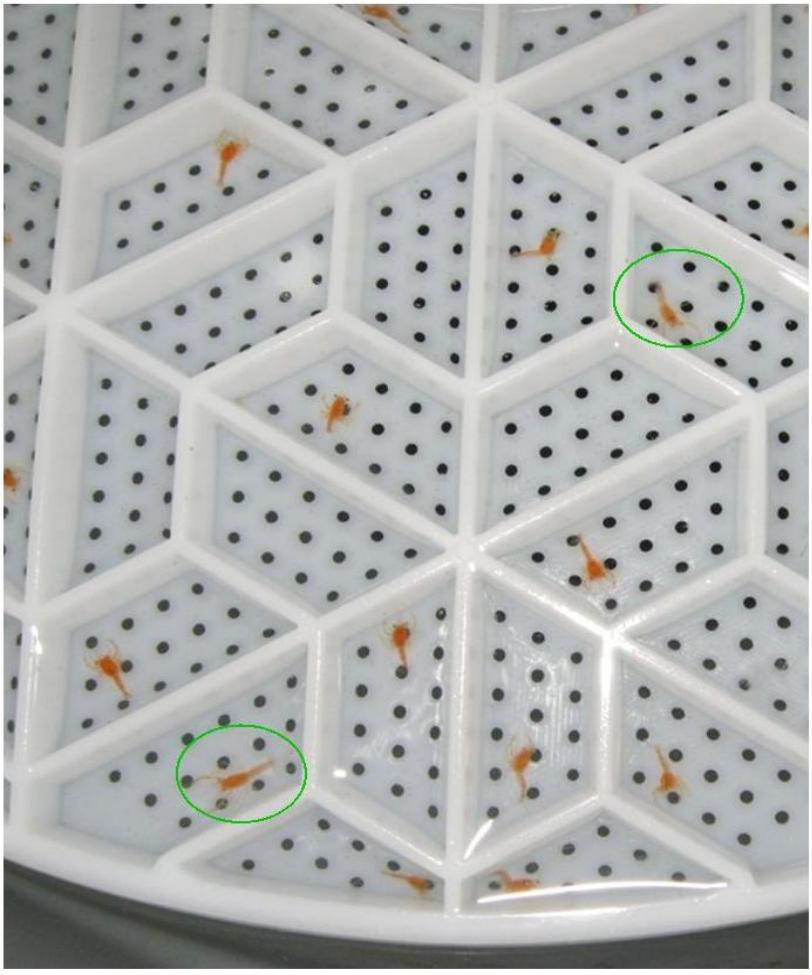
  Another observation showed that the small proportion of Z3 that metamorphosed late tended to get stuck in the moult. However, using the larval rearing procedure and Aquahive plates as outlined above, survival to PL1 was typically between 40-50% and perhaps as high as 55%.

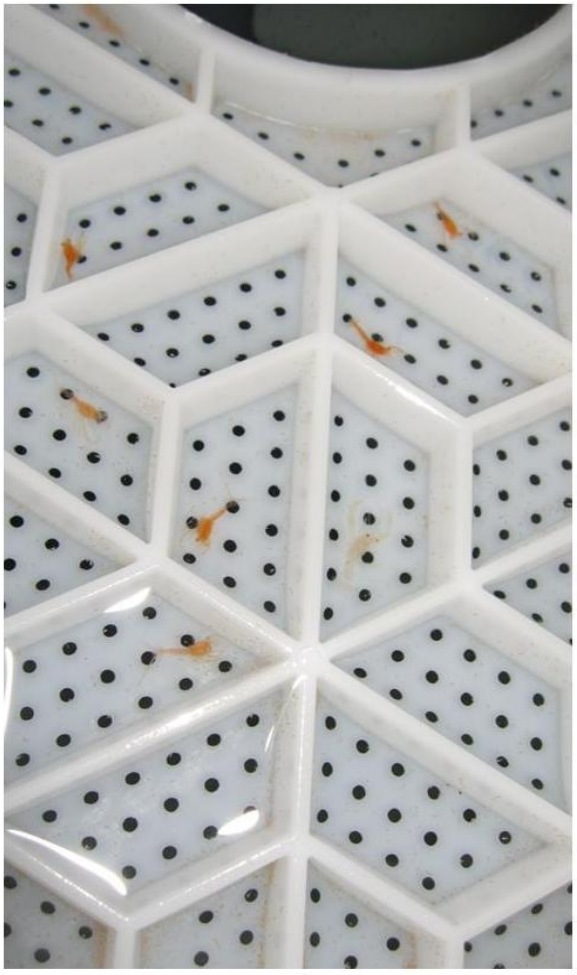
- ***Husbandry.*** During the metamorphosis period – day 15 to day 25 – there is no reason to open the Aquahive plates, provided the plates have been placed precisely over each other and are well secured. Opening and closing the plates is relatively high risk, and should only be attempted if the hatchery wishes to make high frequency survival checks. Therefore, husbandry should continue as normal, (i.e. removal of previous days' feed, cleaning and re-feeding, inflow and aeration checks).

  At day 25, it is worthwhile checking progress to estimate how many, if any, of the larvae remain at the Z3 stage. Appendix 3 provides a suggested record sheet for the Aquahive tray. It is challenging to note larval progress in every individual cell, although perhaps an outline of particular areas of the tray could be drawn to define certain stocking days, morts, etc. if required. If the logistics of the hatchery require that the Aquahive plate needs to be moved to another hopper, this can be done swiftly so that the PL are very briefly emersed for a few seconds. This was performed a number of times to assist cleaning, larviculture etc. and did not appear to cause any mortality.

Top left. *PL stage IV (circled) amongst Zoea 3 in hopper.* ***Top right.*** *Dead/moribund Zoea 3 (circled).* ***Bottom right.*** *Colourless PL morph.* ***Bottom left.*** *PL stage IV (circled) amongst live Zoea 3.*





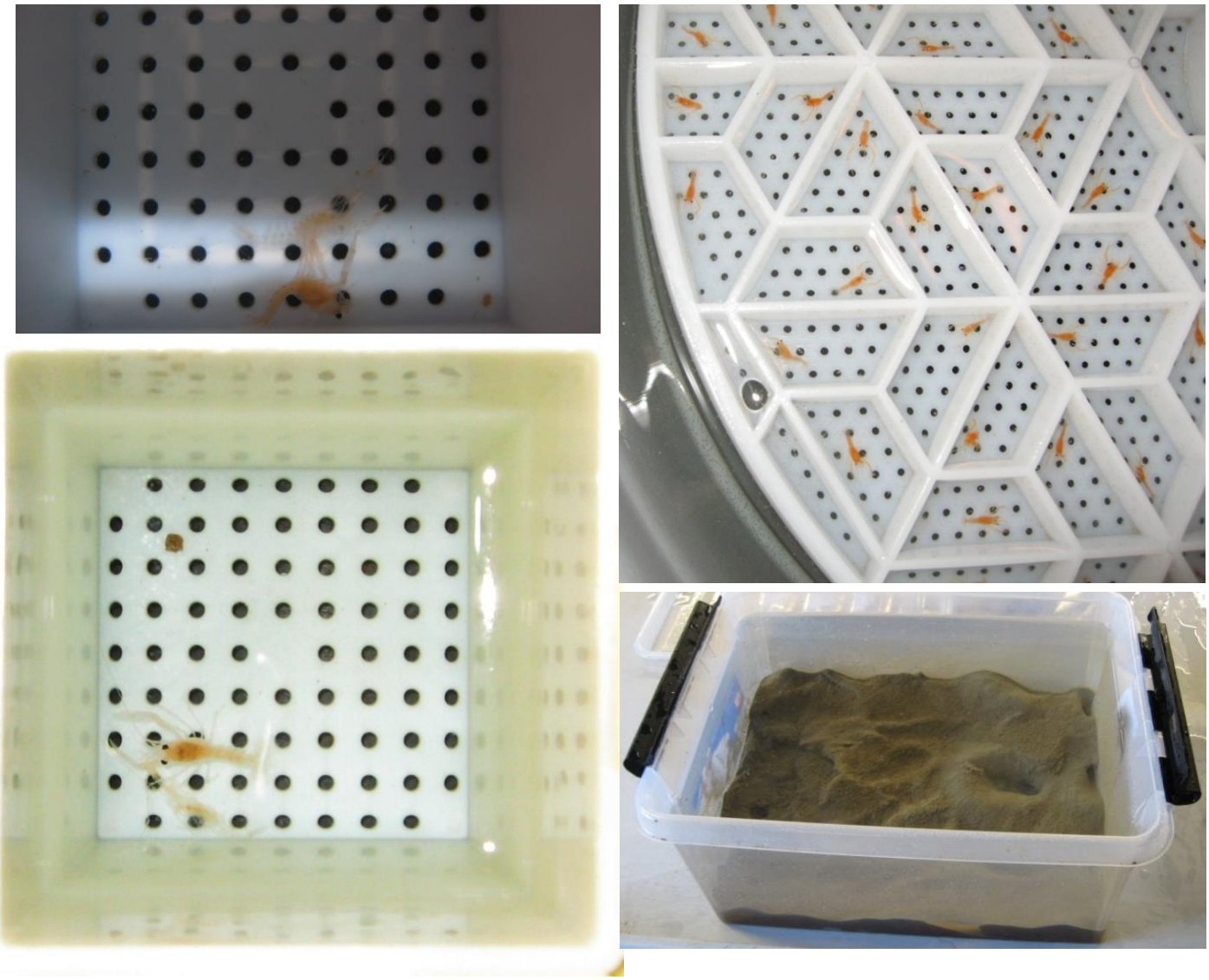


- ***Ongrowing.*** Historically, juveniles have been grown out of sediment and fed a variety of wild caught live feeds. Survival to PL stage V has been approximately 30-40%, which is lower than current *Homarus sp.* hatcheries. Mortalities typically arise during the moult from stage IV to V, which is likely to occur between 16-20 days after metamorphosis (*ca.* day 35-45 post-hatch) with high survival for the first 7-10 days (de Figueiredo MJ. (1979).

  Our recent work has shown that this cannot be improved easily; feeding novel formulated feed in Orkney cells, or *Artemia* and sterilised *Calanus* preparations in Aquahive plates, has not yielded higher survival. Additionally, there is some evidence to suggest that juveniles accrued from late spawning females (i.e., those in captivity for 2 months onward) had higher mortality than those recruited earlier in the season. The table below provides an overview of how to check for mortality.

  Rearing communally in sediment filled tubs (5-30L volume), a more naturalistic medium that allows burrowing, does allow some lobsters to reach PL stage V and beyond. This is likely to be workable for R&D, but much more challenging for a commercial hatchery. At the time of writing it is unclear whether this may be due to reduced stress, improved nutrition (e.g. phospholipids), commensal bacteria, or perhaps the sediment improves friction allowing the old moult to be removed.

Parameter	***Dead PL***	***Live, moulting or moribund PL***
Naked eye:		
Movement	None obvious on very close inspection.	In Turkey baster under light, flickering of swimming limbs.
Position	Any – normal, on the side, upside down.	Moulting/premoulting animals often on side.
Colour	Faded orange translucent. Totally white and opaque after several hours.	Remains translucent and orange, orange colour may fade in older or PL2 animals.
Gut/stomach fullness	Usually empty.	Usually partially full.
Moult	None.	If moulting not obvious, could be pre-moult or carapace just starting to lift.
Under microscope:		
Movement	None obvious on close inspection.	Flickering of limbs, antennules; heart/gills may be visible and moving.
Gut/stomach fullness	Usually empty – check.	Usually partially full – check.
Moult	None.	If not obvious, check carapace.
Presence of detritivores	Usually harpacticoids. Potentially ciliates, nematodes on animals dead for some time. May be evidence of other post mortem pathogens (hyphae) or damage (melanisation).	Not usually present.



Left top and bottom. *PL2 recently moulted or moulting – these will be on their side and immobile when moribund.* ***Top right.*** *Aquahive tray full of recently metamorphosed PL1, potentially ready for release.* ***Bottom right.*** *PL 3 raised communally on sediment (just visible near burrow).*

4.2. Potential release strategy

Juvenile mortality remains unacceptably high in the likely intensive systems desired by commercial hatcheries (i.e., using formulated feed or natural feed, out of sediment in Orkney cells or Aquahive). Since PL stage IV exhibit a benthic, exploratory habit, there seems to be only one sensible approach given the current state of the art: release *Nephrops* at this stage.

Keeping PL stage IV in Aquahive plates reduces husbandry, and does not allow the lobsters to associate a shadow with a feeding event. This is essential after release, i.e. the released lobsters do not leave shelter when a predator is near. The plates may also be more convenient to move, in water, to a release site, and to perform the release itself (whether by diver or a remote release system; Burton, 2003 outlines potential release mechanisms for *Hommarus sp*).

Recent work at UGOT suggests that lower salinity rearing of PL (i.e. below 30ppt) led to improved survival in PL populations – while this needs to be confirmed, it does suggest that release from just above the surface could be possible, since the PL may be sufficiently robust to pass through stratified layers as they fall through the water column.

Another recommendation is release during darkness; this will assist reduction of predation during and after release, as burrows are created or located.

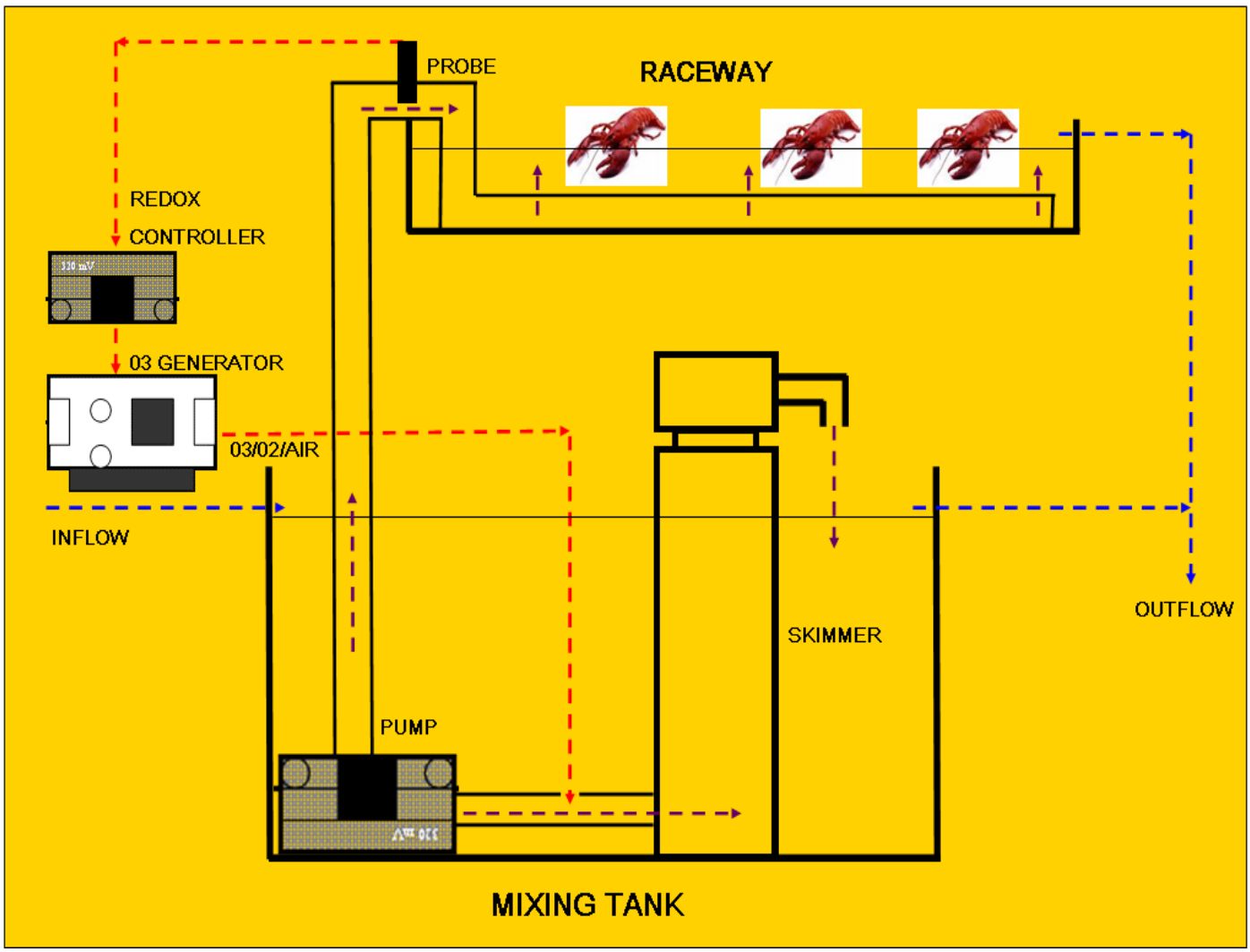
5. Appendices

Appendix 1 – Ozone system

Direct application of ozone is not commonly used in hatcheries, although short term exposure could assist maintenance of hygiene for large numbers of incoming females.

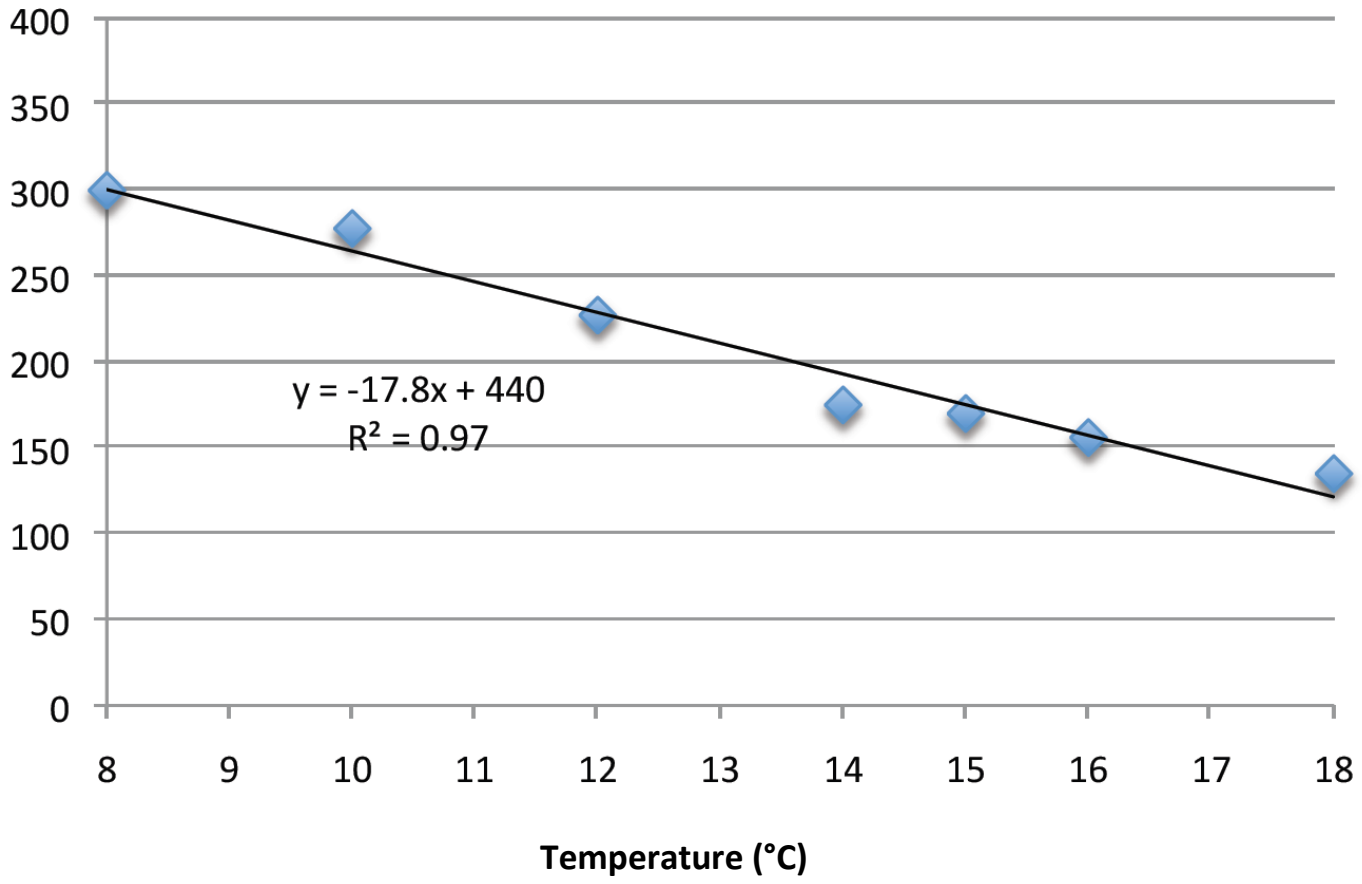
Incoming water was circulated through a protein skimmer (Schuran Aquaflotor, Tropical Marine Centre, UK), with ozone added at this stage using an ozone generator (Certizon, Sander, UK). The skimmer was kept clean otherwise the accumulated organic material increasingly reacted with the ozone and less residuals were available downstream in the culture water. Ozone dosing was measured and controlled by observing the potential difference in oxidation–reduction potential (ORP). Calibrated probes were placed in the inflow to the brood tanks and connected to an ORP controller (Aqua Medic, Bissendorf, Germany), itself connected to the ozone generator. Ozone was then dosed into the system according to the "set point" of the ORP controller.

In addition to ORP control, periodic spectrophotometric water assays (DR/2500 spectrophotometer; DPD method; Hach Company, Colorado, USA) were also performed, to measure the total residual ozone (TRO) dissolved in the system water.



Appendix 2 – *Nephrops* egg degree days

(Powell and Eriksson, 2013)



Appendix 3 – Record sheets

Nephrops Broodstock - daily husbandry

Date: **Day:** **AM Check:** **PM Check:**

Tank	Opening number	Morts	Closing number	Feed (g)	Tank cleaned	Temp (°C)	Comments:	Larvae number

Date: **Day:** **AM Check:** **PM Check:**

Tank	Opening number	Morts	Closing number	Feed (g)	Tank cleaned	Temp (°C)	Comments:	Larvae number

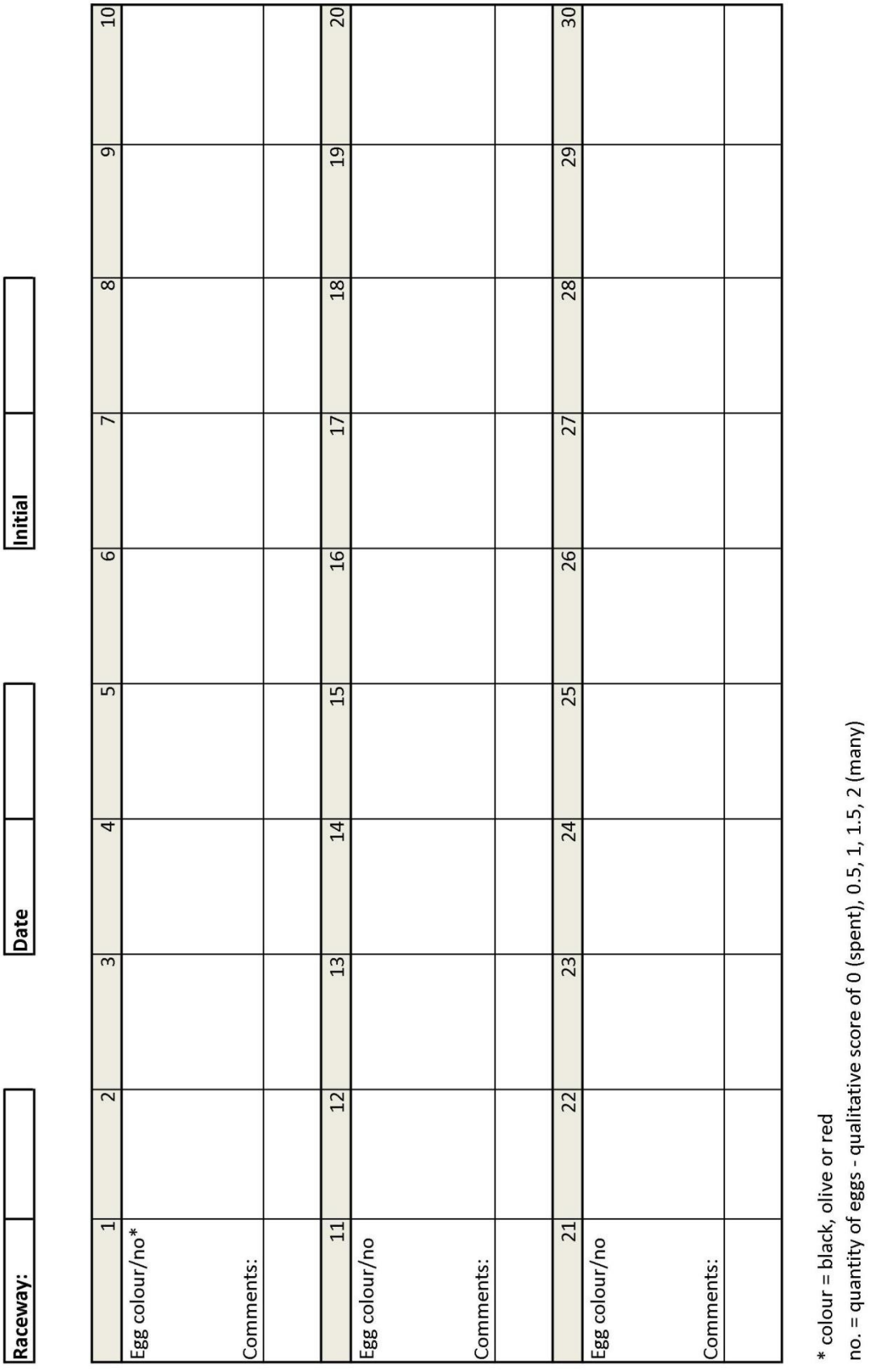
Date: **Day:** **AM Check:** **PM Check:**

Tank	Opening number	Morts	Closing number	Feed (g)	Tank cleaned	Temp (°C)	Comments:	Larvae number

Date: **Day:** **AM Check:** **PM Check:**

Tank	Opening number	Morts	Closing number	Feed (g)	Tank cleaned	Temp (°C)	Comments:	Larvae number

Nephrops Broodstock - weekly egg maturation



Raceway:		Date		Initial						
1	2	3	4	5	6	7	8	9	10	
Egg colour/no*										
Comments:										
11	12	13	14	15	16	17	18	19	20	
Egg colour/no										
Comments:										
21	22	23	24	25	26	27	28	29	30	
Egg colour/no										
Comments:										

* colour = black, olive or red
no. = quantity of eggs - qualitative score of 0 (spent), 0.5, 1, 1.5, 2 (many)

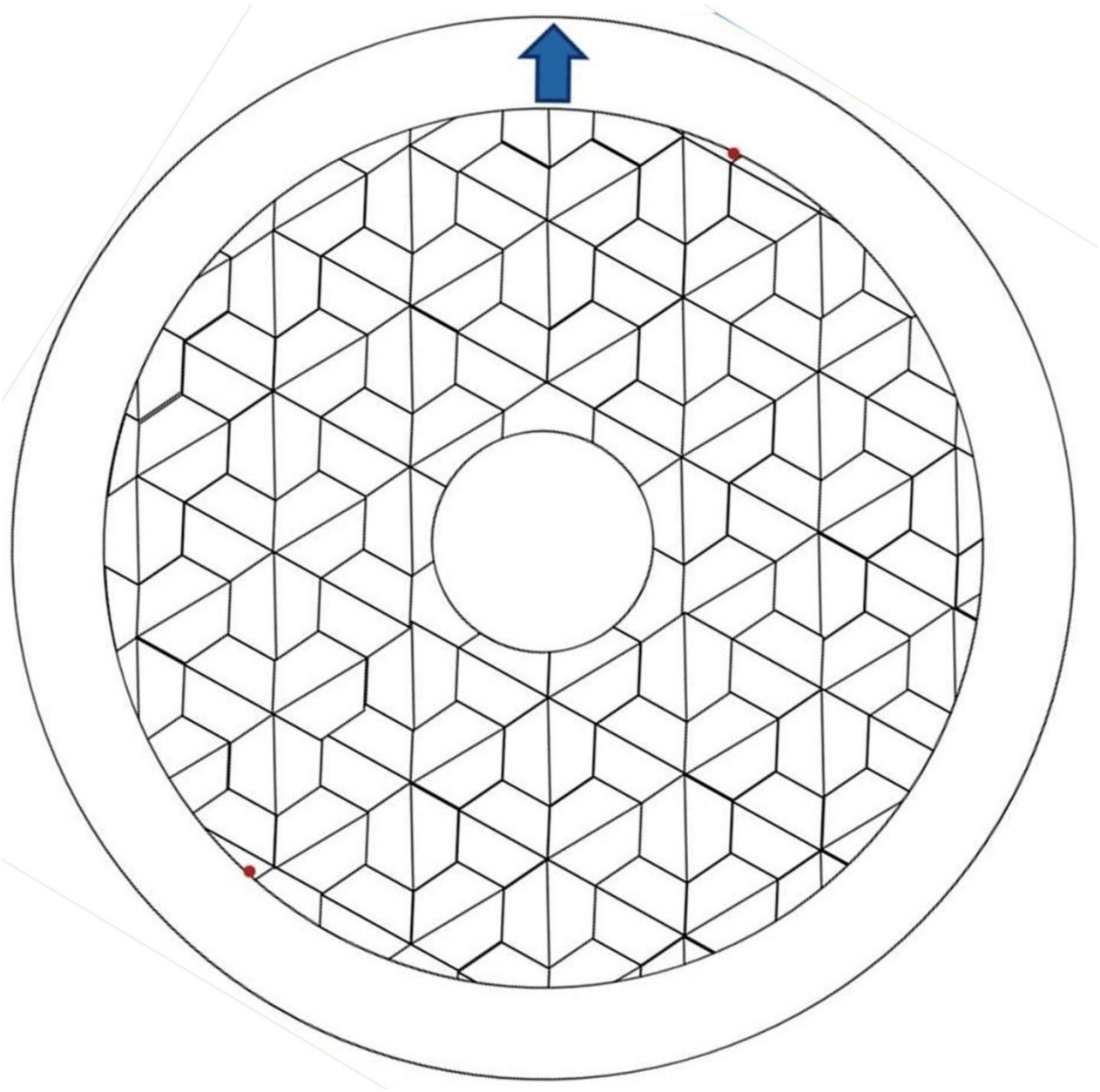
Nephrops Larvae – daily husbandry

Larval hoppers

Date:		**Day:**	**Check**	**am:**		**pm:**	**Temp:**
Hopper	Larvae #	DOH*	Feed		Type of Food	Comments/data	
			am:	pm:			
Date:		**Day:**	**Check**	**am:**		**pm:**	**Temp:**
Hopper	Larvae #	DOH	Feed		Type of Food	Comments/data	
			am:	pm:			
Date:		**Day:**	**Check**	**am:**		**pm:**	**Temp:**
Hopper	Larvae #	DOH	Feed		Type of Food	Comments/data	
			am:	pm:			
Date:		**Day:**	**Check**	**am:**		**pm:**	**Temp:**
Hopper	Larvae #	DOH	Feed		Type of Food	Comments/data	
			am:	pm:			
Date:		**Day:**	**Check**	**am:**		**pm:**	**Temp:**
Hopper	Larvae #	DOH	Feed		Type of Food	Comments/data	
			am:	pm:			

*DOH=Date of Hatch; comments/data can include counts for survival %, ratios of Zoea stage, day of hopper, etc…

Nephrops Z3 and PL1 – daily husbandry



Date	Day	Z3 (number)	PL (number)	Morts	Total	Comments

Appendix 4 – Hopper system

The pictures below provide an overview of how the baseplates (A) were connected to the hopper base, including the inflow and outflow pipework. The baseplate is 20mm thick, 200mm diameter, with a 40mm diameter central hole. The apertures are 2mm diameter, cut at a 35° angle away from the centre and at 35° away from the vertical.

This design of base-plate accommodates a 32 mm central standpipe, requiring a 32 mm socket (B) which the base-plate slots over (the external diameter of the top half of the socket was reduced using a lathe, to form a lip for the baseplate to sit on).

The hopper base is drilled; all the pipe-work is glued or screwed together, making a very firm structure for the baseplate margins and standpipe base. Marine grade silicone sealant was used around the sides of the baseplate to help secure it to the side of the tank and to ensure there are no gaps around its edge. This is important because it means water will be forced up through the holes drilled in the plate, rather than gaps at the margins.

Left: Pipework (removed from hopper) used to connect base-plate to hopper and drain. ***Right:*** Location of pipework and baseplate situated inside the hopper base. **A,** bespoke u-PVC base-plate with angled sides to match angle of tank; **B,** adapted, lathed 32 mm uPVC socket; **C,** Length of 32 mm uPVC pipe; **D,** 32 mm/1" threaded uPVC socket; **E,** 32 mm/1" uPVC tank connector; **F,** 32 mm uPVC union. NB. The mix of imperial and metric connectors was required as some elements were only available in one system. Image manipulation courtesy of Serpentine Design.

Appendix 5 – Artemia protocols

Top Left: Baseplates (top and bottom view) with lathed socket and short length of pipe in between.

Top Right: Multi-aperture baseplate – this appeared to be more successful, as the apertures were 10-15mm apart reducing deadspaces between water jets, allowing larvae to be caught in flow and circulated more efficiently.

Left: Approximate position of 32mm standpipe, filter and aeration. The filter is constructed from a *ca. 40*mm pipe and an appropriate reducing brush at the base.

DECAPSULATION OF DRIED CYSTS

Materials:

10 litre bucket
125 µm mesh
Gloves and safety goggles
Sodium hypochloride (bleach)
Sodium thiosulphate
Sea water
Sodium hydroxide (NaOH)
Thermometer
Ice

Method:

- Hydrate the cysts for 2-3 hours in a bucket of 10-20 litres of seawater with strong aeration.
- Use a 125 µm mesh to filter the hydrated cysts. Wash the cysts down to the bottom of the mesh so that the result is a brown clump of dry cysts.
- Re-suspend the cysts in a bucket with the proper amount of sea water (Table 1) always maintaining strong aeration. For doing this, reverse the mesh letting the brown ball to fall in the bucket, and rinse the mesh with the seawater in order not to lose too many cysts.
- Add the proper amount of bleach and NaOH 40% solution to the bucket (Table 1). Be careful during this stage; wear goggles and gloves and ensure area is well ventilated. The reaction is exergonic (will release heat) so keep ice at hand.
- Add ice when the temperature rises up to 25 degrees, it should not pass 40 otherwise the embryos will be damaged.
- When the colour of the solution changes from dark brown/grey to an orange/brown colour the reaction is complete.
- Filter and rinse thoroughly with sea water in order to eliminate chemical residuals and return pH to neutrality.
- Re-suspend the embryos with in the correct amount of sodium tiosulphate solution (Table 1) and aerate from 30 minutes.
- Filter the cysts again using the 125 µm and rinse thoroughly with sea water.
- Re-suspend the cysts in a 10 litre bucket. The decapsulated cysts will sink while the ones that are not will float. Discard the floating capsulated cysts.
- Repeat the procedure at least three times
- At the end filter and store in the dark and cold places (< 10 degrees).

Table 1. Relative volumes of chemicals used to decapsulate different weights of dry Artemia cysts.

Dry Cysts (g)	40% NaOH (ml)	Sodium hypochloride (ml)	Sea water (ml)	Sodium thiosulphate solution at $1gL^{-1}$ (ml)
50	16.5	415	270	1000
100	33	830	540	2000
200	66	1660	1080	4000
300	99	2490	1620	6000
400	132	3320	2160	8000

STORAGE OF DECAPSULATED CYSTS IN HYPERSALINE SOLUTION

After decapsulation, the embryos can be stored in either a "dry" condition in a sealable container in the fridge (at about 4°C) or suspended in a hypersaline solution in a cool dark place (< 10°C). This second option reduced the growth of bacteria around the decapsulated cysts meaning they can be stored for a longer period of time (up to one month). The hypersaline solution can be made by following the quantities in Table 2.

Table 2. Salt and sea water quantities for hypersaline storage of decapsulated Artemia cysts (assumes a sea water salinity of 30ppt).

Dry Cysts (g)	Salt (g)	Seawater (ml)	Final volume (ml)
50	50	160	310
100	97	320	620
200	190	650	1240
300	295	960	1860
400	380	1300	2490

HATCHING DECAPSULATED ARTEMIA CYSTS

For obtaining newly hatched *Artemia* nauplii (ZI stage), fill a cylindro-conical vessel with seawater and warm to a temperature of ≈ 28°C and add the decapsulated cysts. The vessel should be filled with the required volume of seawater in order to maintain a concentration 2 g dry cysts for each litre of water. To ensure the cysts are kept in suspension, the seawater should have medium/strong aeration. A direct source of light needs to be present over the tanks during the hatching process and subsequent filtration of hatched nauplii. The hatching process takes about 18 hours

1. **Filtering**

The day after the incubation of the embryos, newly hatched nauplii should be collected as follows:

- Take out the airline and turn off the aquarium heater (it is extremely important as otherwise it will begin to melt when it is not submerged)!
- Wait 10-15 minutes before filtering in this way the hatched nauplii will separate from the not hatched ones that will sink on the bottom.
- Discard the fraction of unhatched cysts at the bottom of the tank and then start to filter the hatched nauplii (ZI) or metanauplii (ZII). Position a bucket filled with sea water under a 125 µm mesh. This ensures the nauplii remain in water when filtering and prevents the *Artemia* from getting damaged or dying.

2. **Enriching *Artemia***

There are many different enrichments available with different enrichment times and processes. Be sure to follow the instructions of the specific enrichment in order to get the full benefit.

For enriching hatched *Artemia* nauplii and to let them develop to metanauplii (ZII):

- Place the previous hatched nauplii into cylindro-conical tanks filled with seawater in order to maintain a concentration of 300 nauplii ml^{-1}.
  For example, if you had 1.52 million nauplii, they would require about 5 litres of seawater (1,520,000/300 = 5 L). Obviously you can fill with more water, but in this case you will waste more enrichment and therefore more money.
- Suspend the nauplii with an airline with medium strong aeration.
- Place a direct source of light over the tanks as *Artemia* is phototactic and you will use this feature filtering the *Artemia*.
- When harvesting the enriched nauplii, the process is that same as with the newly hatched *Artemia*. However, you must be sure to rinse the filtered *Artemia* thoroughly to remove any excess enrichment and lipid films which can be transferred to the larval rearing tanks.

CALCULATION OF VOLUME OF ARTEMIA STOCK TO ADD TO HOPPER

Harvested, enriched *Artemia* should be added quickly to the hopper, or placed in an aerated bucket on ice for later use.

The volume of harvested *Artemia* stock solution to be added to the hopper, can be calculated using the equation below.

$$V_S = (V_T \times D_T)/D_S$$

Where:
V_S is the volume of harvested Artemia stock solution (in ml) to be added to the hopper.
V_T is the volume of the larval tank (a constant, in ml)
D_T is the required density of *Artemia* in the larval tank (a constant, *Artemia* ml^{-1}).
D_S is the density of the harvested *Artemia* stock solution (*Artemia* ml^{-1}).

D_S is calculated by counting 1ml samples of the stock solution under a dissecting microscope. The samples are spotted across a Petri dish and the numbers per spot are tallied.

Appendix 6 – Main equipment – supplier list

A non-exhaustive list of suggested suppliers is found below.

Product	Supplier	Web address
Hoppers	Mailbox (Stamford Products Ltd)	www.mailboxproducts.co.uk
Artemia cysts and enrichment	ZM Systems (Zebrafish Management Ltd)	www.zmsystems.co.uk
PVC pipe & fittings	Plastic Pipe Shop Ltd	www.pisces-aqua.co.uk
Aquahive	Shellfish Hatchery Systems Ltd	www.aquahive.co.uk
Nylon mesh	Normesh Ltd	www.normesh.co.uk
Black plastic for tank lids (corrugated polypropylene)	Cut Plastic Sheeting Ltd	www.cutplasticsheeting.co.uk
PVC sheets - brood cells matrix	The Plastic People (Barkston Plastics Ltd)	www.theplasticpeople.co.uk
Airline and fittings	Hydrogarden Wholesale Supplies Ltd	www.hydrogarden.com

6. Acknowledgements

This work was supported by the "NEPHROPS" project **(**EU Framework 7, Research for the benefit of SME Associations, grant number 286903). The authors would like to thank all project colleagues and technical staff, particularly Sara Barrento, Alex Keay, Andre Perez, Craig Pooley, Maria Scolamacchia (CSAR), Daniel Simonsson, Linda Svanberg (Department of Biological and Environmental Sciences-Kristineberg), David Mackenzie, Dawson Shearer (North Bay Shellfish Ltd). The authors are also indebted to Julian Kivell and Phillip Hopkins (Department of Physics, College of Science, Swansea University) for assistance with baseplate construction. Mr Richard Land (Shellfish Hatchery Systems Ltd) assisted with Aquahive installation, hopper hydrodynamics, and recommendations for broodstock acquisition and transport. Broodstock were sourced with the kind assistance of Tarbert Shellfish Ltd and D.R. Collin and Son Ltd.

7. Key References

Burton CA. (2003). *Lobster hatcheries and stocking programmes: An introductory manual.* Sea Fish Industry Authority Aquaculture Development Service. Seafish Report, SR552.

de Figueiredo MJ. (1971). *Sobre a cultura de crustaceaos decapodes em laboratorio:* Nephrops norvegicus *(Lagostim) e* Penaeus kerathurus *(Camarao).* Bol inform. Inst. Biol. Marit. Lisboa 1, 1–17.

de Figueiredo MJ. (1979). *Artificial culture of* Nephrops norvegicus (L.). *II—some studies on the growth of early post-larvae of* Nephrops norvegicus *(L.) reared from the egg.* Bol. Inst. Nac. Invest. Pescas 1, 13–23.

Farmer ASD. (1974). *Reproduction in* Nephrops norvegicus *(Decapoda: Nephropidae).* J. Zool. 174, 161–183.

Nicosia F and Lavalli K (1999). *Homarid Lobster Hatcheries: Their History and Role in Research, Management, and Aquaculture.* Mar. Fish. Rev. 61(2), 1-57.

Powell A, Eriksson SP. (2013). *Reproduction: Life Cycle, Larvae and Larviculture.* In Magnus L. Johnson and Mark P. Johnson, editors: Advances in Marine Biology, Vol. 64, Burlington: Academic Press, pp. 201-245.

Santucci R. (1926). *Lo sviluppo e l'ecologia post-embrionali dello "Scampo"* Nephrops norvegicus *(L.) nel Tirreno e nei mari nordici.* Mem. R. Com. Talassogr. Ital. 125, 1–36.

Smith RSM. (1987). *The biology of larval and juvenile* Nephrops norvegicus *(L.) in the firth of Clyde.* Ph.D. Dissertation, Glasgow University, Glasgow.

Notes