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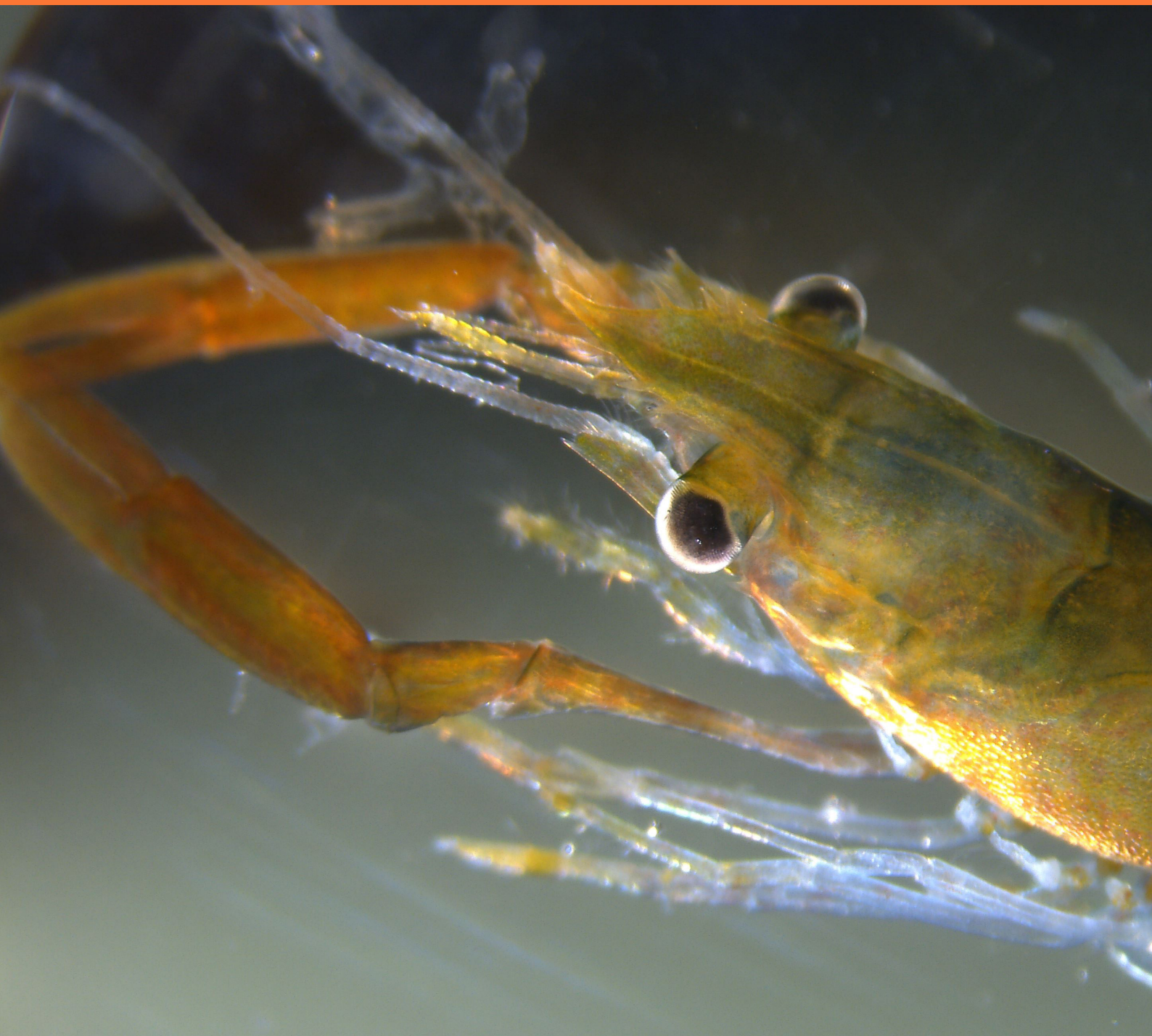

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Colophon

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Preface

This report gives an extensive literature survey on reproduction, production methodology and rearing of European lobster (*Homarus gammarus*) larvae and postlarvae – and provides recent results from a series of externally funded projects to DTU Aqua on aspects of egg development; water quality, larval feeding & nutrition providing directions for optimizing survival and growth of this species.

The projects were funded by:

- ENV Foundation (Energi Nord, Hjørring, Denmark).
- FLAG Nord .
- The EU Framework Programme Horizon 2020 (EUROSTARS-2) and Innovation Fund Denmark, 2021-2023, Project: E114944 AutoHatch
- Nordic Council of Ministers; Nordic Working Group on Fisheries (AG Fisk). Project (225)-2021-Lobster COE, 2021-2022.

Content

Summary

Broodstock of European lobsters are usually obtained from the wild. Berried lobsters are typically caught during spring and summer or caught in autumn and kept overwintering until spawning. Berried females are kept separately to avoid fighting and to diminish possible stress and injuries. The fecundity is variable, between ca. 5000 and 40,000 eggs per female, with normal maturity attained between 80 and 140 mm carapace length. Breeders can be held at ambient temperature of water can be cooled or heated to promote slower or faster development of eggs. During development, the color of the eggs changes from greenish black to black to orange and eventually to dark red along with the embryos absorption and development. The eye index measurement can give a good indication of when larvae will hatch.

Berried lobsters are typically disinfected before entering a hatchery to minimize risks of diseases. When in the system they are fed daily with mussles, shrimp or fish.

Upon larvae hatching, they are removed and reared in separate incubators/ tanks and typically fed with krill, mysis or other planktonic feeds: Feeding with dry feeds or just simple overfeeding is problematic and often leads to bacterial outbreaks, that can cause high mortalities. Live Artemia is typically a more secure option, as it will not organically pollute thus avoiding blooming and proliferation of bacteria. Improving hygiene ozone, UV and probiotics have been tested in lobster hatcheries as a sustainable, preventative strategy to reduce opportunistic infections, which may impact upon survival and growth. Compared to unozonated and un- UV controls, lobster larvae yielded poorer growth during the short pelagic period although they experienced reduced bacterial loading of larvae and culture water.

Postlarvae (st IV) can be obtained within 8 days after hatching, but typically from around 12 days post hatching. Larvae are normally reared communally in tanks having a size of 50-120 L. After reaching the postlarvae stage the larvae are reared individually, for instance in a Aquahive, to avoid cannibalism. Higher temperature speed up the moulting process, but temperatures above 22°C have proven negative in terms of total survival. Stocking density seems not related to larval mortality (cannibalism).Cannibalism is the single biggest issue for survival and in communal rearing systems often more than 90% are dying due to cannibalism before reaching postlarvae stage. Attempts to mass produce newly hatched larvae if kept individually have failed, - even though laboratory trials at DTU Aqua by keeping larvae individually in specific designed units have proven a high survival rate.

Much of the larval mortality in hatcheries arises from cannibalism in communal rearing systems; while cost effective methods are sought to reduce this (use of clay, algae etc), however without proving successful.

Use and growing of live adult *Artemia* have recently been successfull compared to use of *Artemia* nauplii and has significantly improved survival and reduced moulting time to postlarvae both for larvae reared individually and in communal systems.

While use of formulated dry feeds for larvae has not proven successful, trials at DTU Aqua have gained significant knowledge on nutritional requirements for postlarvae and juveniles.

1. Broodstock and reproduction

An impediment to commercial farming of European lobster is lack of control over reproduction and without it, a steady supply of seed cannot be insured, nor can selective breeding be applied. Only relatively limited work on European lobster reproduction or selective breeding have been carried out and a major block of hatchery reared or wild caught American broodstock lobsters have been successful insemination and successful egg extrusion (1). Egg bearing European lobsters for reproduction in aquaculture are thus typically obtained from the wild by fishermen or merchants with a permission to bring these to land or from areas with a legal catch. Catch of egg- bearing females is illegal in several European countries (i.e. Norway, UK). In Denmark fisheries is only allowed in some marine water bodies – South East part of Kattegat while banned in most others (Other parts of Kattegat incl. the fjords) and North Sea. In western part of Skagerrak a ban on berried females was introduced in 2022 after an initiative from local fisheries organisations in Northern Jutland. Egg bearing females caught in pods may ensure a better egg quality as compared to lobsters caught in gill nets or trawled, where loss or damage of egg are typically observed (author, pers. observations)

From late autumn and during winter and until March biological activity and thus lobster catches are very low (2). Most egg bearing lobsters in Danish waters are caught during early spring, over summer and at early autumn, where they are most active due to higher sea temperatures, but still within these periods catches in same fishing grounds can be very varying, indicating unknown intra-seasonal activity (Pers. comment H. Lund, lobster pod fisherman from Kattegat). Lobsters are in general more active at night time (2), than at day time where they may also be observed at more shallow waters.

Egg bearing females caught during early autumn (September) are typically holding eggs for next breeding season with eggs having a greenish to dark black colour indicating low development (Figure 1).

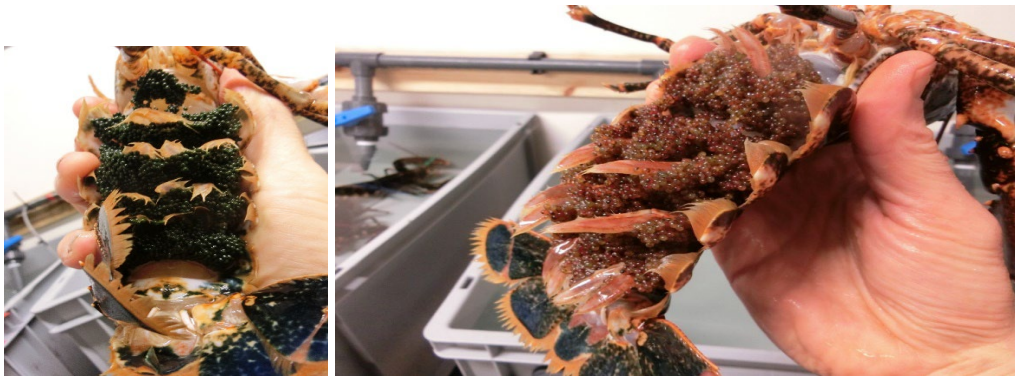


Figure 1. Picture of lobster females with eggs at different developmental stage. Photo DTU Aqua.

2. Transport and holding

It is important after catch to secure optimum transport and protection of egg bearing lobsters to avoid damage of eggs. At shorter transportation with a duration of some 5-8 hrs insolated cooling boxes (with freeze elements) can be a good solution (authors experience). Lobsters are thus packed in wet towels individually with tails bended under the cavity and cooled down to avoid individual interactions and movements. For longer transport distances, transport tanks with water and aeration (oxygen) may be an option, the fixation of claws by rubber bands is needed to avoid fighting, stress and potential egg damage. Build up of ammonia and deterioration of water quality may become a problem if no water exchange. If applied correctly none of the methods above have resulted in negative implications for later egg development (authors observations).

3. Fecundity and spawning

Female lobsters extrude fertilised eggs underneath their abdomen, where they remain attached to the swimming legs and then develop slowly over the winter. The fecundity is very variable, between ca. 5000 and 40,000 eggs per female, with normal maturity attained between 80 and 140 mm carapace length.

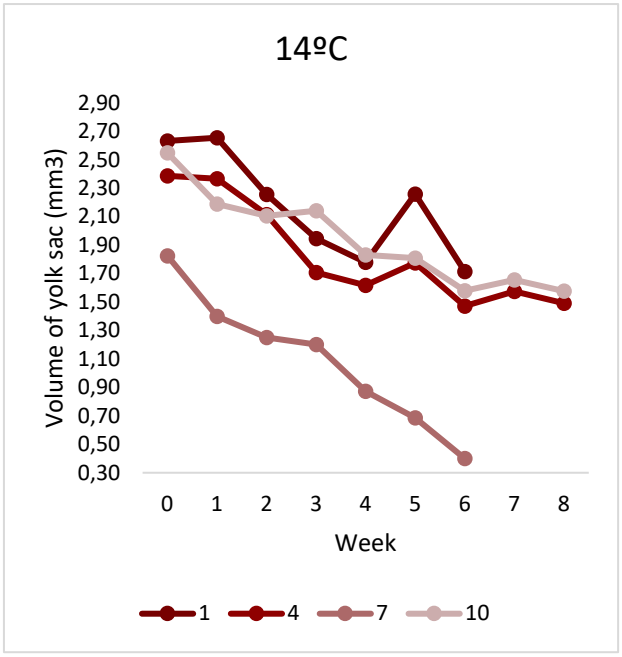
Clutch size is proportional to carapace length and thus age, but may vary with geographic location and sea surface temperature (3–5)

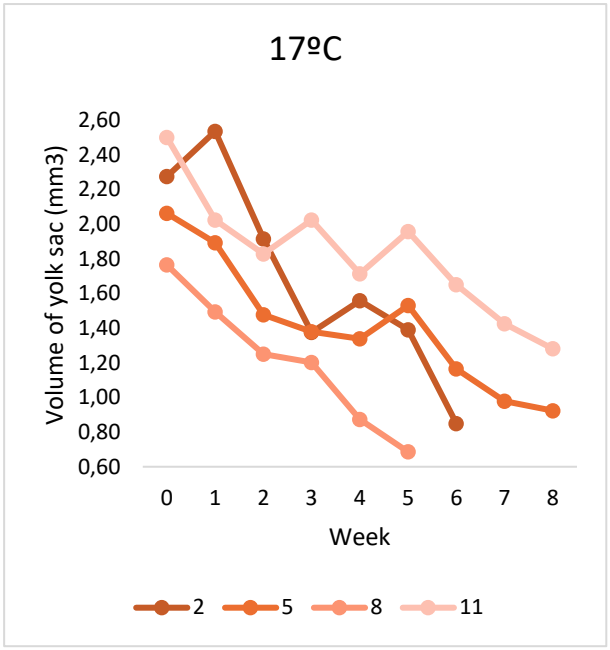
During development, the colour of the eggs changes from greenish black to black to orange (Figure 1) and eventually to dark red along with the embryos absorption and development. Advanced embryo stages will finally hatch and are released directly into the water column at dusk or early morning over several successive evenings and days and can extend over a week or more, dependent also on the temperature.

The egg development period is long from early gonad development to spawning of eggs may take 12 months and additionally 9-11 months for egg to ripe and hatch). Spawning has been reported from January to September from egg bearing individuals kept under semi natural temperature and light conditions in captivity (authors, personal observations). Usually wild females spawn in March to July – August (In Denmark) and during this period, it is more likely to obtain females with ripe eggs. The Spawning season in a hatchery may be extended by gradually decreasing the temperature, however this is mainly recommended for lobsters with unripe (greenish or black) eggs and not for lobster with eggs close to hatching.

Such management of environmental water temperature for egg bearing breeders can advance or delay embryo development, larval release and hence extend hatching season. However, in *H. americanus* a cold thermal regime during incubation may improve viability of embryos and larvae (6).

In a study at DTU Aqua the effect of temperature on egg development of berried lobsters was examined over a period of 8 weeks with lobsters caught in October and thus having overwintering eggs. Eggs were weekly sampled from individual lobsters held at three temperature regimes; 14, 17, 20 °C during incubation. Results indicated a faster egg development at 20° C, than at 17 °C and 14°C, with an increase in egg volume and a decrease in yolk sac. (Figure 1). Eye index similarly progressively increased with increasing temperature, and to a certain degree matched an eye index equation (7) (Table 1)





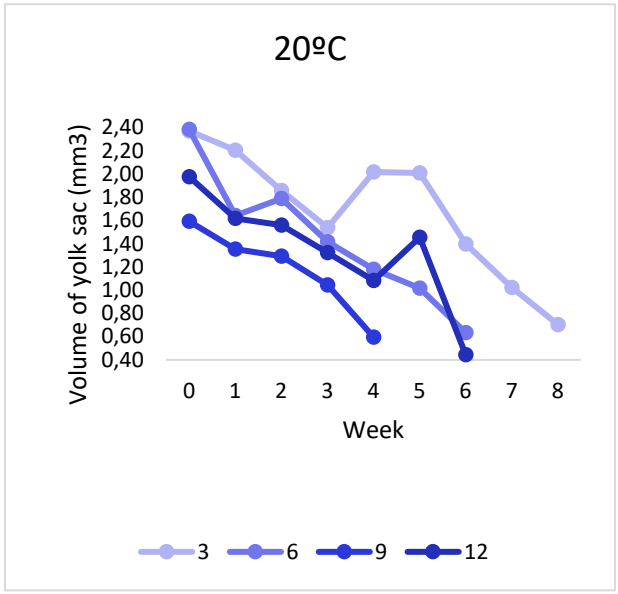
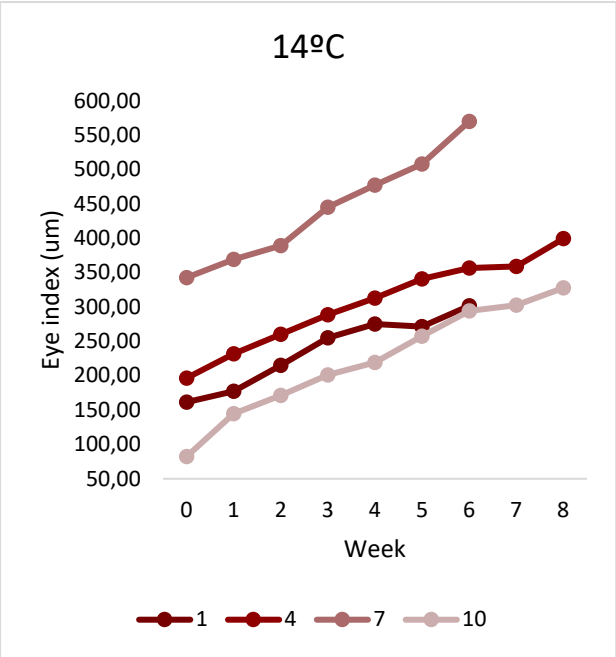
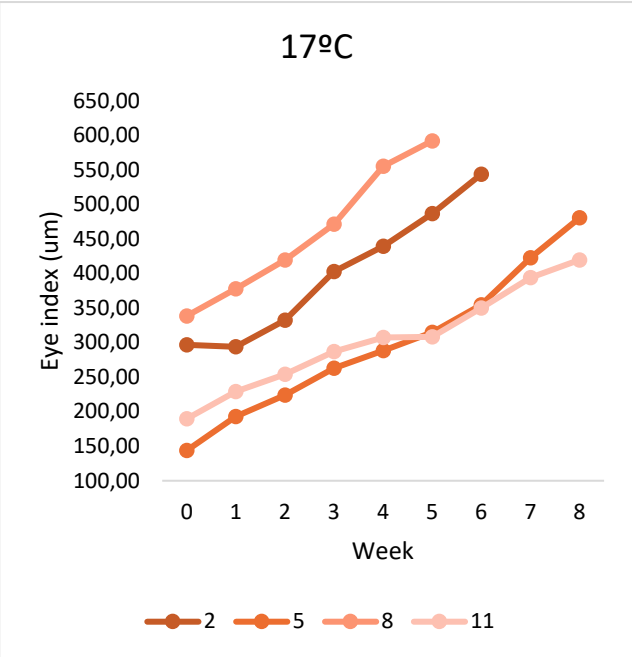


Figure 2A. Mean volume (mm^3) of egg yolk sac of European lobster female eggs at different temperatures (14, 17 and 20ºC) during a 8 weeks trial *(From report of Andreia Sophia, Erasmus Student DTU Aqua, 2021).*





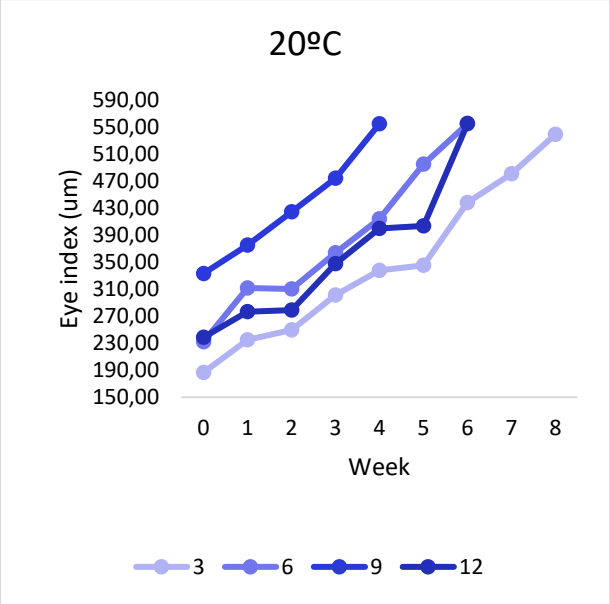


Figure 2B. Eye index of European lobster female eggs at different temperatures (14, 17 and 20ºC) during a 8 weeks trial *(From report of Andreia Sophia, Erasmus Student DTU Aqua, 2021).*

Table 1. Eye index development and time to hatch based on (1).

Eye Index(E) (microns, μm)	Time to hatch (T_h) (in weeks ± 2 weeks)
50 – 100	15
100 – 150	14
150 – 200	13
200 – 250	12
250 –300	10
300 – 350	8
350 – 400	7
400 – 450	5
450 – 500	4
500 - 550	2
600 - 620	Hatch imminent
640 - 680	Hatch imminent

Adapted from Richards & Wickins (1979) and Charmantier & Mounet-Guillaume (1992)

Table 1a. Time (weeks) until estimated hatching (HI) based on eye index measure at week 0 and the actual hatching (Hatch) of eggs of berried lobsters held at (14, 17 and 20 ºC (x4 replicates)) during 8 weeks experiment.

	14				17				20			
Week	1	4	7	10	2	5	8	11	3	6	9	12
0	13	13	8	15	10	14	8	13	13	12	8	12
1	13	12	7	14	10	13	7	12	12	8	7	10
2	12	10	7	13	8	12	5	10	12	8	5	10
3	10	10	5	12	5	10	4	10	8	7	4	8
4	10	8	4	12	5	10	2	8	8	5	2	7
5	10	8	2	10	4	8	HI	8	8	4	Hatch	5
6	8	7	HI	10	2	7	Hatch	7	5	HI		HI
7	Died	7	Hatch	8	Hatch	5		7	4	Hatch		Hatch
8		7		7		4		5	2			

4. Feeding and holding broodstock

Egg bearing females readily accept feed after being caught and typically they are fed to keep them nutritionally fit until spawning, but limited information exists on how this may affect egg quality. Typical broodstock feeds used in hatcheries are deshelled frozen blue mussels, Atlantic Northern shrimp, industrial fresh fish (sprat etc) or extruded pelleted diets. For natural sources of feeds, frozen feeds are preferred to avoid introduction of diseases. Broodstock close to hatching may not be fed as to avoid organic waste remainings and potential bacterial bloom - or other contamination of newly hatched larvae.

4.1 Hatchery holding conditions and larval quality

Broodstock is typically maintained individually in cages or tanks and the attached eggs allowed to hatch naturally, optimally under temperature and light regime control or under natural conditions. As hatcheries typically have wild ovigerous broodstock of unknown provenance natural variation occur including female size, nutritional status and egg reserves that likely is influencing larval quality, size and quantity (8-10). Selection of oviger breeders can be an important parameter for the later survival of larvae. In a report by Browne et al, 2009 (57) it was suggested to only take in egg bearing lobsters with a carapace length larger than 90 mm. A Norwegian experiment demonstrated positive correlation between size of wild caught egg bearing lobsters and egg size as well as larval survival (9).

Although lobsters should be able to deposit sperm from one mating and use this to fertilize several clutches of eggs, female lobsters kept during 2 years after first spawning in tank systems at DTU Aqua have not deposited new eggs when kept in tank systems. Whether this is due to nutritional and or environmental factors need to be elucidated. Nevertheless, optimising broodstock husbandry is beneficial to foster health and welfare of both broodstock and larvae. There is a potential loss of eggs between extrusion and hatching both due to biological causes (e.g. disease or infertility) and stressors such as animal capture and storage, which may also reduce fitness of the resulting larvae (4,5,11). Increasing habitat complexity in hatcheries (e.g. providing refuges) or compartmentalisation is a straightforward and cost-effective way to reduce female aggression, dispense with claw banding for breeders kept in communal tanks. Further optimisation of hatchery design may include reducing stressors such as light, sound and olfaction, which elicit physiological changes (12,13). Although ovigerous females may only be captured and maintained in hatcheries for a matter of weeks until larvae have been collected, some procure broodstock at the end of the previous season and overwinter for several months. It is therefore likely that exemplary husbandry remains important for prolonged broodstock and embryo development in captivity.

At the North Sea Centre, Hirtshals, Denmark a broodstock holding unit for 24 breeding lobsters was refurbished in 2023. The system consists of a shelving system with 1x 24 holding units which are supplied by cold natural seawater and additionally 1x 24 units with water passing a heat exchanger or cooler set to a predetermined temperature to eventual speed up or slow down maturation process and spawning. This has worked well, however one incidence with nitrogen supersaturation was experienced by heating water from 8 - to 12°C and caused broodstock mortalities, so precautions were taken to avoid this i.e. installation of degassing before heated water was led to tanks.

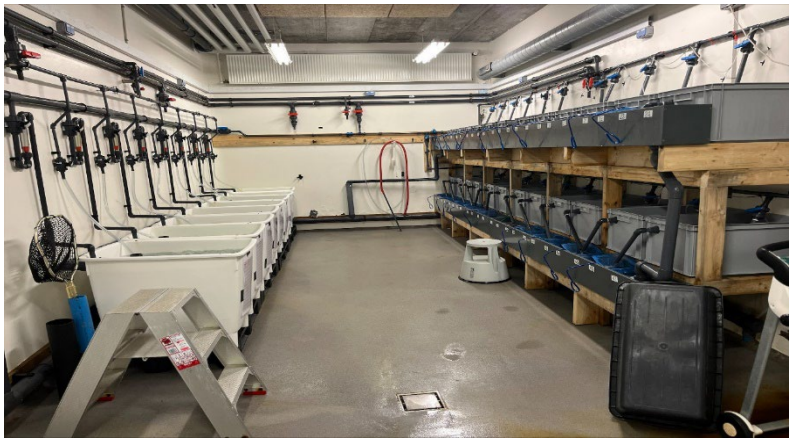


Figure 3. Picture of breeding facility at North Sea Centre with 48 boxes (split in 2 separate sections) (right) for hold of berried females. To the left communal tanks for larvae rearing st. I-III. Photo Ivar Lund, DTU Aqua.

4.2 Desinfection, hygiene precautions and infections

As breeders are typically obtained from the wild, hygiene management and disease control is very important. Strict microbial management of culture water, tank surfaces, larvae and feeds are mandatory (14) to increase larval lobster survival and growth (15,16). “Tail dipping’ of incoming broodstock abdomen and egg clutches, using standard aquaculture antimicrobials has been used to promote biosecurity and to avoid gill damage and is routinely performed using standard aquaculture antimicrobials (e.g. Buffodine or Kick-start (15,17), although Halamid has been employed to dip the entire animal (National Lobster Hatchery, pers. comm. 2021). It has though been suggested, that tail dipping may reduce embryo viability directly or interfere with a symbiotic relationship between egg microflora and commensals (such as the worm Histriobdella homari) (18, 19).

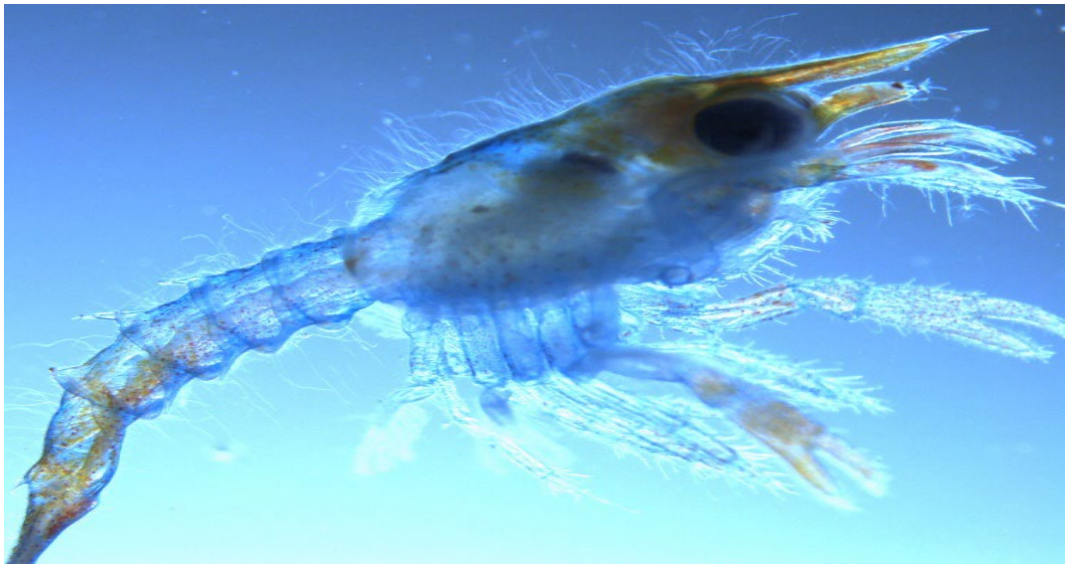


Figure 4. Picture of lobster larvae heavily infected with a filamentous bacteria on all surfaces. Photo DTU Aqua.

At DTU Aqua and North Sea Centre the following protocol has been used: Dipping in a 1% solution of Braunol (10ml Braunol per litre seawater) – submerge most of the body (including claws and walking legs) except for the mouth parts and gills of the animals (as the iodine is toxic to lobsters) in the solution for approximately 2 minutes. After being bathed in the Braunol solution, the females are rinsed in a water bath of seawater and placed into the individual hatching units. The procedure can be repeated but it has been observed, that eggs that are very close to hatching should not be submerged in iodine solutions as this may result in some mortality of the larvae.

In larval rearing tanks, high bacterial loading is most obvious following blooms of filamentous bacteria (e.g. Leucothrix mucor) (20). Experience from DTU Aqua shows that this bacteria can rapidly proliferate in rearing tanks and foul larval appendages, restricting movement, feeding (Figure 4) and cause massive mortalities. In addition to larval mortality, sub-lethal effects can impact on larval performance, for example microbial infection of gill surfaces, potentially impairing gas transfer (21). In addition to robust biosecurity and disinfection, systemic or water-borne antimicrobial treatment with ozone and UV light (17,22,23) and probiotics and nutritive supplements (14,24) have reduced larval bacterial loading and increased larval survival. Recently, juvenile *H. Gammarus* raised in SBCC possessed a more diverse gut microbiome compared with those from a conventional RAS hatchery (25). This could have important ramifications on the success of long-term ongrowing of juvenile lobsters, potentially improving resilience to current and emerging disease agents (26, 27).

5. Larval development and morphology

Public reports and hatchery handbooks are available detailing general lobster hatchery operation, facility specifications and husbandry techniques (15,28,29). Although general rearing approach remains similar, techniques and technology differ between hatcheries.

Three successive larval stages exist, which increase in size from about 8 to 14mm total length, and demand moulting of the exoskeleton between each stage (Figure 5 & 6). Intermoult duration increases slightly between successive stages; rate and success depend primarily on temperature but also on food quality and quantity.

Figure 5. Moulting larvae stage II with old exoskeleton still attached. Photo Ivar Lund, DTU Aqua.

Late stage 3 larvae become increasingly benthic and eventually metamorphose into “post-larvae” (or “PL”) which resemble very small adults. Juvenile *Homarus gammarus* are cryptically for several years, and appear to be relatively site-specific, before being recruited into the fishery. Survival in the wild, from larvae to adult, is likely to be very low adulthood (potentially <1 in 15,000 eggs (30); but depends on the habitate, predators etc.

An adult female may release up to 20,000 larvae during hatching. Developed larvae are released from a clutch over a period of 2–3 weeks, with the majority during a central 10-day period (15,31).

A proportion of the embryos develop on the clutch as pre-larvae, which remain attached to the female until dusk. The female adopts a ‘tail extended’ hatching posture and creates rapid pleopod movement, causing pre- larvae to moult to a stage 1 larvae (15). Larvae are motile, pelagic in the water column and moult over three successive larval stages (Stages I–III) (31). Larval development requires ca. 250 degree days, which may require several weeks in the wild, or <15 days in hatcheries which maintain a steady rearing temperature of 18–22°C (32,33). Stage III larvae develop into the initial post-larval stage, referred to as Stage IV. (Figure 7).

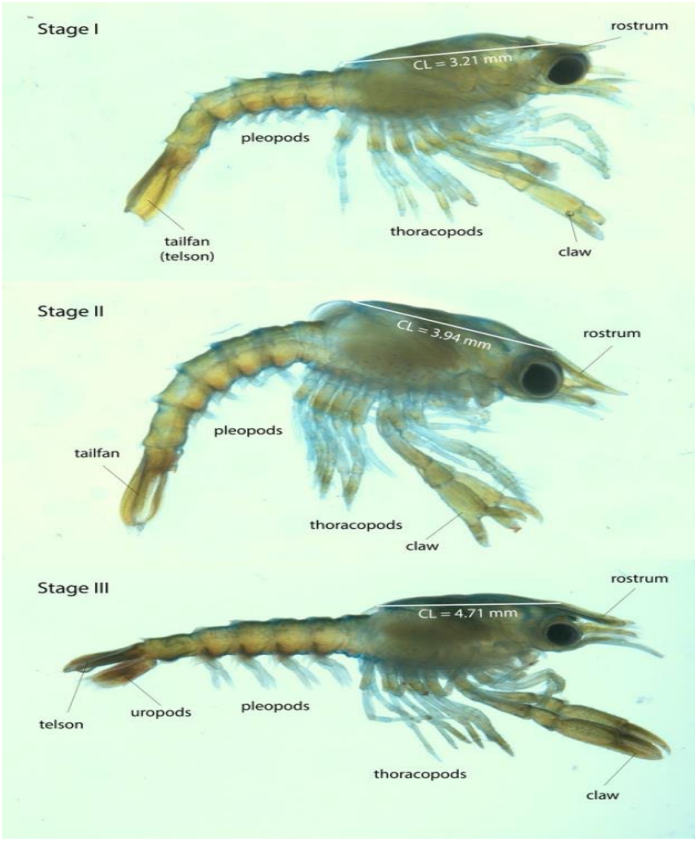


Figure 6. Larval stages (I to III) of *Homarus gammarus* in lateral view. Larvae photographed under a stereomicroscope (MC125 C, Leica). Photo Renata Goncalves, DTU Aqua.

Stage IV lobsters demonstrate an obvious change in both behaviour (becoming exploratory and benthic), and also morphology (development of abdomen, telson, rostrum and antennae (32).

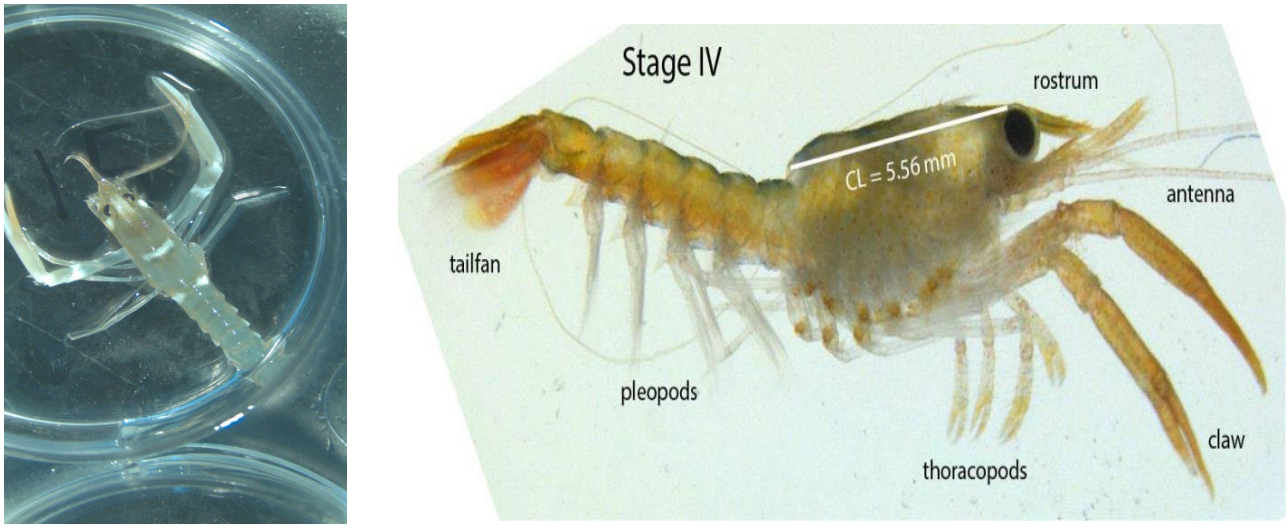


Figure 7. Postlarvae stage IV in petri dish and in a lateral view, Photo, Ivar Lund, Renata Goncalves, DTU Aqua.

Subsequent moult stages are referred to as stage V, VI, VII etc. As they grow, they increase the calcification of their integument and become young juveniles (Figure 8). H. gammarus juveniles seek and

establish refugia, but unlike *H. Americanus* remain cryptic during an extended early benthic phase (34).

Figure 8. Juvenile lobster stage VIII ready for release. Photo Ivar Lund, DTU Aqua.

6. Larval tanks and rearing conditions

The technical aims of lobster hatcheries have been to improve larval and PL survival and growth by reducing predation, promoting access to food, and by accelerating development via securing optimal, steady water temperature and quality.

Larvae are allowed to hatch naturally, and best practice suggests that larvae are isolated from broodstock soon after hatching into separate rearing systems containing feed, to eliminate maternal cannibalism (15,35). Stage I-III larvae are generally reared communally in upwelling (white) cylindro-conical vessels ca 60-100 L (also known as tubs, pots, incubators, hoppers, or Hughes Kreisels (36) with design varying between hatcheries and technology development (17, 37). They are removed at stage III–IV and then cultured individually in various rearing units, and often in separate cell-like rearing containers (known as Aquahive and invented by Northbay Shellfish Ltd, Scotland, UK). (Figure 9).

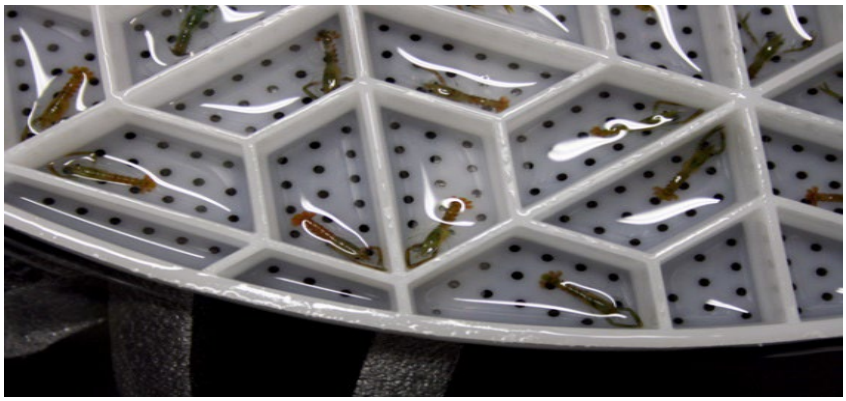


Figure 9. Postlarvae stage IV in Aquahive tray. photo, Ivar Lund, DTU Aqua.

Pelagic larvae are motile, following diurnal vertical movements in the water column (corresponding to light intensity) although horizontal currents will also distribute larvae. White tank colour makes larvae easily visible by eye and seems not to have a negative effect on larval behaviour. The tanks are well aerated with a high water turnover to promote water quality, planktonic feed distribution, and to reduce cannibalism. Alternatively, larger mesocosm (“green water”) systems also exist.

6.1 Use of microalgae

Operation varies between hatcheries during larval phase and according to larval availability, although ‘green water’ microalgal mesocosms have been generally phased out for more convenient ‘clear water’ production, which have instead provided either enriched *Artemia*, sterilised plankton or small feed pellets (17,33.38). DTU Aqua in 2022 evaluated the effect of green water in communal rearing systems. In a triplicate set up conical tanks of 50 L each (Figure 10) with use of clear water was compared with use of green water by daily addition of *Nannochloropsis S.*

Figure 10. Experimental up-flow 50 L tank at DTU Aqua for research.

All tanks were fed with newly hatched BF *Artemia* on a daily basis and larvae were counted by emptying each tank every 3 days over a sieve and counting the alive larvae. An initial density of 5 larvae L^{-1} tank $^{-1}$ was used for the experiment. All larvae were harvested from 1 female broodstock on the day of larval release. The duration of the trial was 22 days or until all larvae had either died or metamorphosed into PL. Results of progressive survival are shown in Figure 11 below.

Mortality decreased gradually in all tanks and only in tanks with green water larvae managed to moult into PL, although the percent survival was low and at max 3 %. The mechanism behind the better survivals is unknown but “a green water approach” is known to have a positive effect on various first feeding marine fish larvae, acting as a probiotic, enrichment source to live prey or to optimize bacteria proliferation and to improve larval gut matureness (39,40,41). Algae and light may also increase visual contrast and thereby increase prey capture and thus feed intake 3 (40). However, in a similar study comparing pseudo green water and clear water conditions on lobster larval survival until PL stage IV, survival was a similar 3 % but for both treatment groups (42).

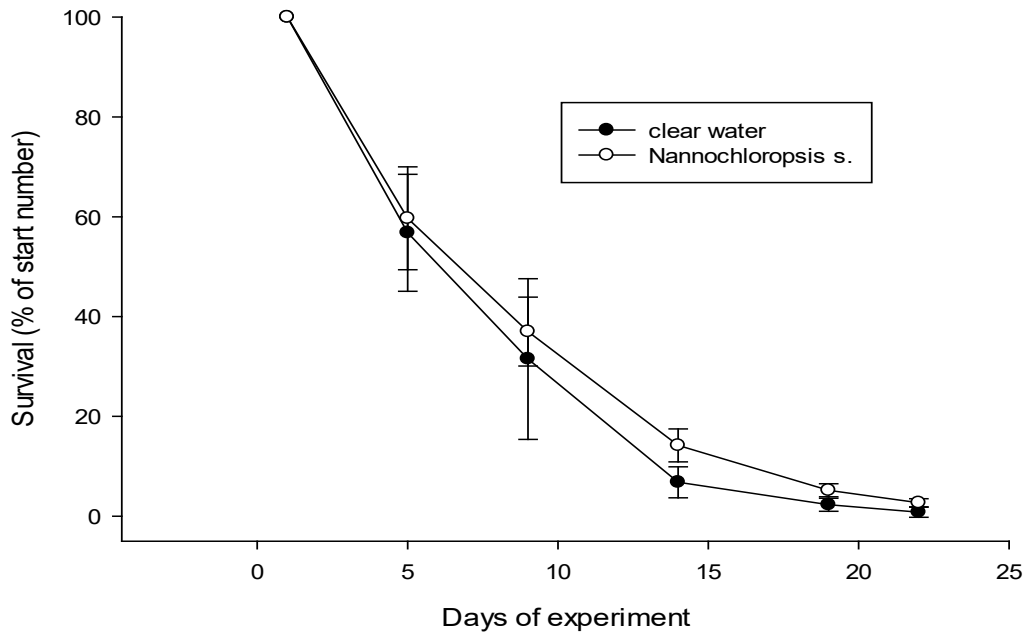


Figure 11. Progressive survival of larvae in tanks provided daily with microalgae and in clear water.

Regulating feeding frequency more evenly throughout the day, via timer controls rather than by hand, has been reported to improve larval growth and development (33). However, at DTU Aqua regulating feeding frequency or feed density with live feeds have had no obvious effects on survival with use of live feeds (*Artemia* nauplii), and a clearly negative effect as by use of krill, mysis, dry feeds as due to increase in organic loading, i.e. thus proliferation of especially filamentous bacteria which caused mass mortalities even by use of UV and high water exchange rate in flow through systems.

6.2 Use of UV on water quality

A specific experiment at DTU Aqua evaluated the effect of UV radiation on rearing water quality, survival and growth of European lobster larvae (34). While no effect of the UV on survival rate – surprisingly, larval growth decreased in the rearing tanks exposed to UV irradiation (Figure12), that may be caused by the high removal of also potential probiotic bacteria.

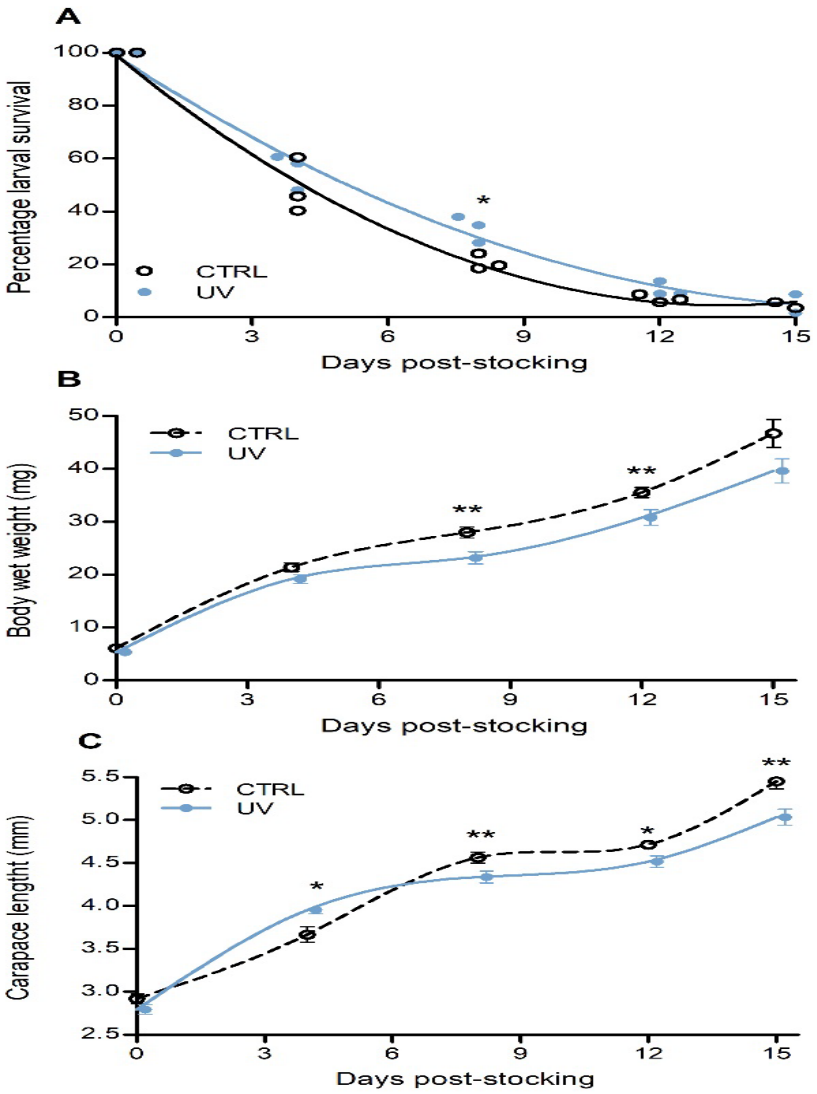


Figure 12. Survival (%), body weight (mg) and carapace length (mm) of lobster larvae reared with UV light (UV) or without (CTR) Mean cumulative mortality of lobster larvae reared at three different densities until 16 days after release and fed on EG *Artemia* nauplii.

The use of a high UV dose (577 mWs cm^{-2}) successfully reduced the microbial abundance in the rearing tanks of a flow-through system culturing *H. gammarus* larvae.

No significant impact was detected in several other biochemical water parameters, including organic matter content, water clarity, and waste nitrogenous compounds, except the higher nitrite-N level in the UV tanks. In general, the water quality was maintained in fairly good conditions in both treatments.

The impact of UV disinfection in the culture water and lobster larval microbiome composition should be considered in future studies as medium associated microbes may play an important role shaping the host gut microbiome with consequences for their health and growth.

6.3 Stocking density and rearing temperature

Stocking density has been suggested as a method of improving survival to PL. At DTU Aqua experiments with varying stocking density (e.g. 2, 7, 20 larvae/L) in 50 L conical tanks showed no improved

survival during larval rearing until PL, (Figure 13), only during the first days a lower mortality was observed at the lower stocking densities. For commercial purposes a higher stocking density is often used to utilise space more effectively and rear higher absolute numbers of juveniles (ca. 50 larvae/L (8). In another density related experiment at DTU Aqua with use of 8x60 3D printed single chambers (each 5x5 cm) and bottom up water flow in a RAS raceway were stocked with either 1 or 2 larvae (stage I) per chamber (in total 240 and 480 larvae) and fed with *Artemia* nauplii by an automatic dispenser 8 hours /day. Results are illustrated in Figure 14 and shows a high end mortality at day 18 for both larvae kept individually and larvae kept with 2 per chamber. Interestingly, in chambers with two larvae mortality was registered already from day 2, while for larvae kept individually almost no mortality was observed until day 8.

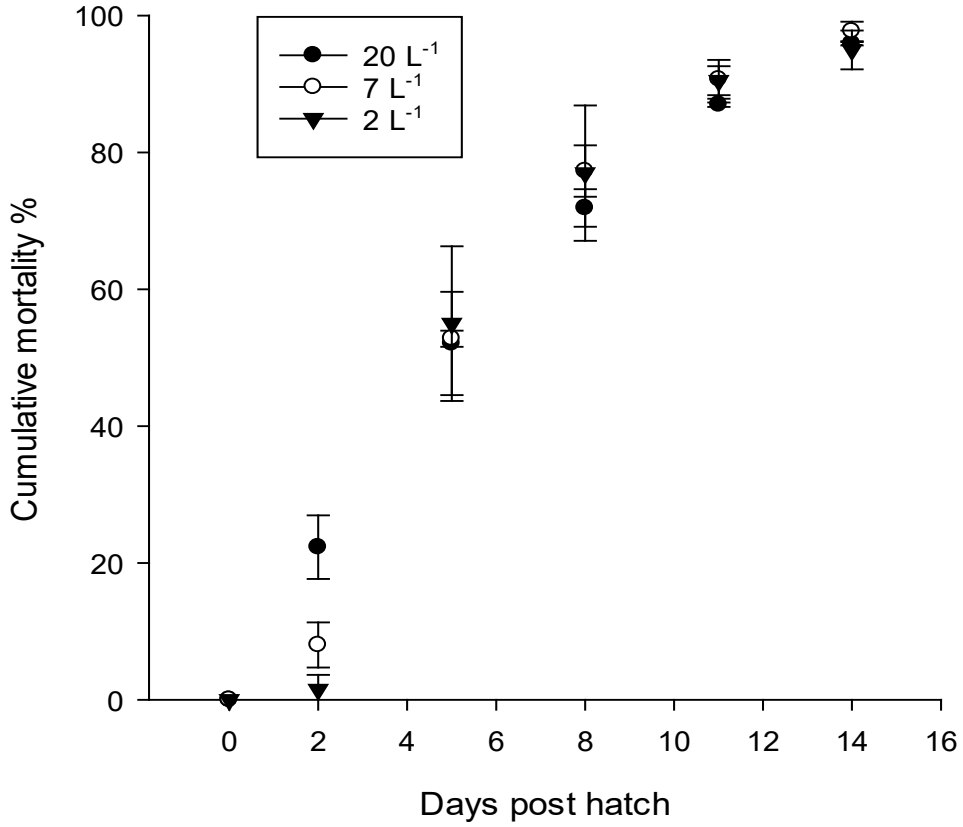


Figure 13. Mean cumulative mortality of lobster larvae reared at three different densities until 16 days after release and fed on EG *Artemia* nauplii.

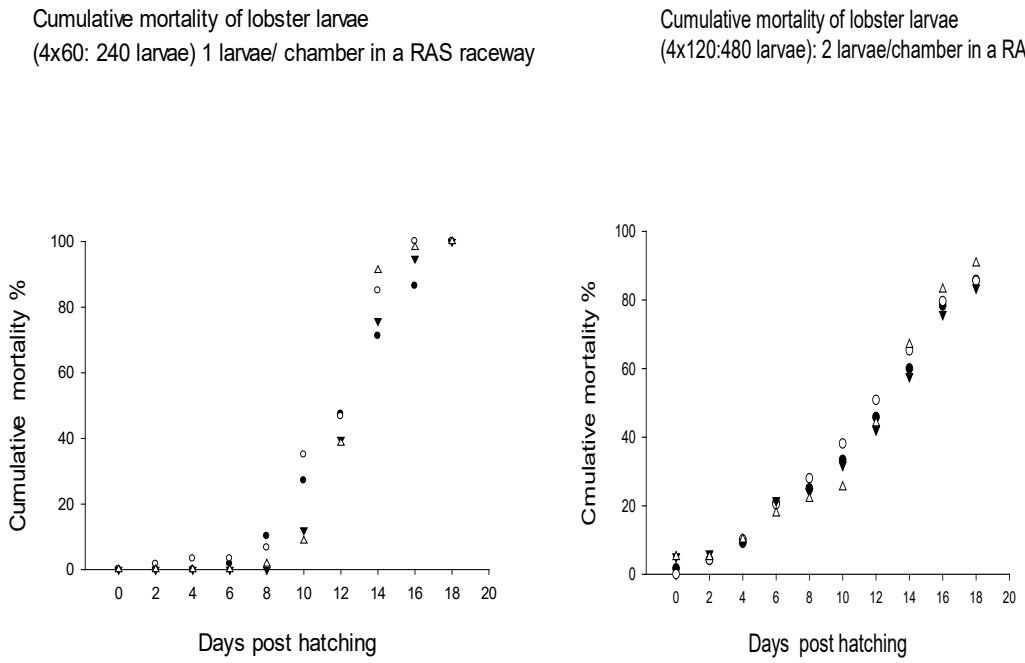


Figure 14. Progressive mortality of larvae in chambers with either 1 or 2 lobster larvae until day 18 post hatching.

Results indicate incidences of cannibalism in chambers with 2 larvae, while starvation may likely be the cause of mortality observed in chambers with individual larvae due to the sudden steep increase from day 8. Only very few larvae managed to develop into PL in both groups. Chambers construction was designed, so that water and live prey passed each chamber either from top and out from the bottom or vice versa (Figure 15), but obviously larvae performance and growth was not optimal.

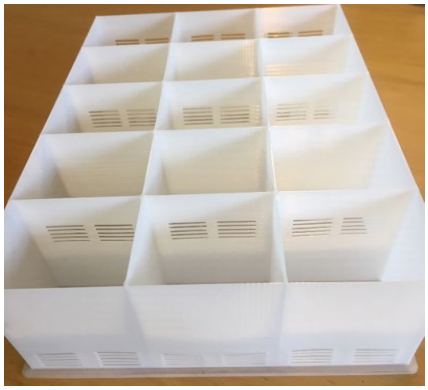


Figure 15. 3D printed unit with 15 chambers and perforated inlet and outlet for water flow.

Rearing temperature is typically 16–21°C, with some variability according to stock origin (15) enabling development to advanced stage III ca. 12 days after hatching at 21°C.

AT DTU Aqua three temperatures (12, 17 and 20.5 °C) were tested to determine the effect on the development and survival of lobster larvae until 18 DPH from newly hatched in a similar triplicate set up as shown for the microalgae study above. Results revealed an average survival of 8,5; 4,5 and 1.0 % respectively for the three tests groups. This indicates that temperature plays a role, but as growth of larvae was retarded at lower temperature, none of the surviving larvae at 12° C developed to PL

within 18 days of the experiment, which questions this rearing methodology also when considering the time related effect of cannibalism in communal rearing systems.

In a trial performed in spring 2023 at DTU Aqua, the effect of higher temperatures was tested to observe if that could influence survival and moulting time to stage IV postlarvae.

Three temperatures were tested 21, 23 and 26°C in a similar triplicate setup as above and larvae were fed Artemia nauplii (2-3 mL^{-1}) enriched by live Nannochloropsis microalgae.350 newly hatched lobster larvae from several breeders were stocked into each of 9 cylindriconical tanks of 50 L each divided into 3 RAS systems of 3 tanks each. Temperature was regulated by thermostats and heaters in each of the systems.

The trial was run until all larvae had moulted to stage IV or until no larvae left and all remaining counted every 3 days. Larvae that had moulted to stage IV were removed.

Results are shown in figure 16 and Figure 17. Results showed a general higher survival for the temp group 21°C as compared with higher temperatures at 23°C and 26°C. Some variability was observed in the temperature regimes due to the temperature of the incoming seawater.

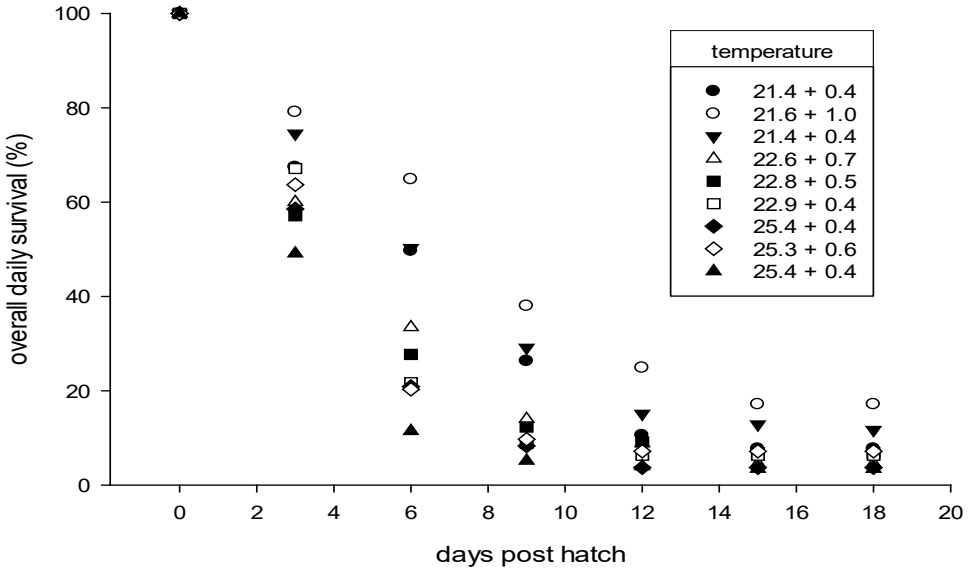


Figure 16. Overall cumulative survival at day 0,3, 6, 9,12,15, and 18 days post hatch.

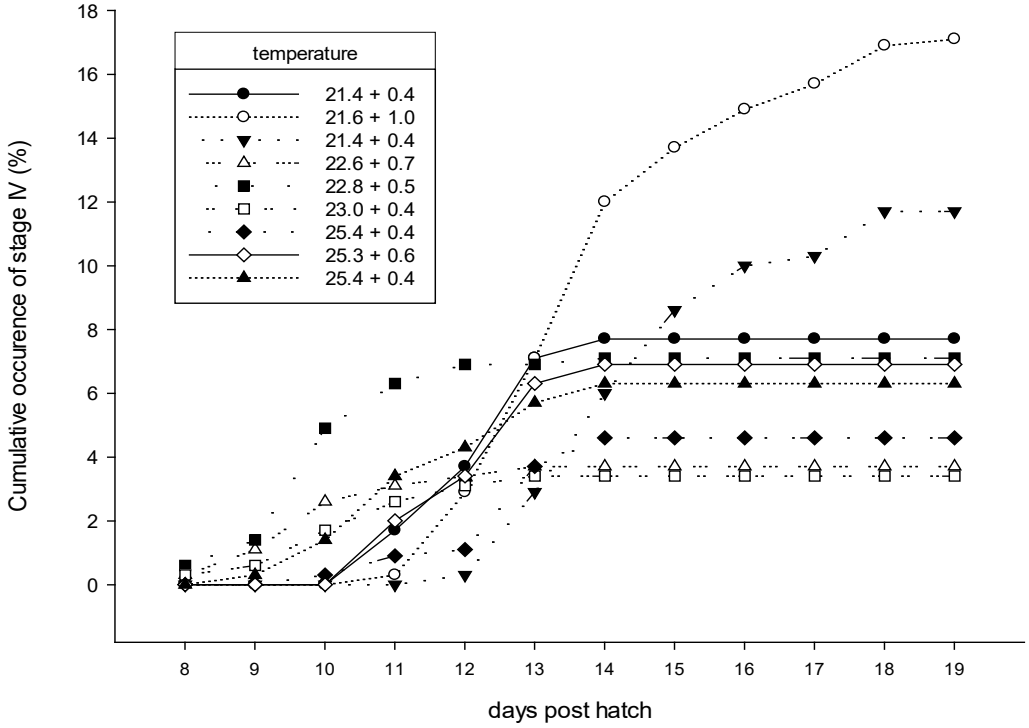


Figure 17. Overall cumulative occurrence of stage IV postlarvae.

Results (Figure 17) also illustrates, that time to first occurrence of postlarvae could be reduced by an increase in temperature, but despite this that it still caused a lower overall percent survival to this stage (mean 4.8% at 26 °C, 5.9% at 23°C and 12.2% at 21°C. Future experiments should test more narrow temperature regimes in the range 19° C – to 22° C, to find the exact optimal temperature for maximal survival in combination with *Artemia* metanauplii (see below).

Whilst larval survival is variable and is likely influenced by broodstock provenance, average seasonal European lobster larval survival between European hatcheries seems to vary considerably and has been generally reported to be between <1% to 20%, without much explanation for this observed variation. Even survival rates of up to 30-70% to PL have been reported in communal rearing systems (43), by rearing in 60 L hoppers at a density of from 8 to 113 lobster larvae L^{-1} (survival highest at lowest stocking density) and by provision of *Artemia* metanauplii and unicellular algae (*Isocrysis, Tetraselmis sp.*). In a cluster of universities and commercial companies, ELCE (European Lobster Centre of Excellence) having members from several EU countries and Norway, this has been discussed, but none of the members experience nearly any such high survival rates in communal rearing systems. At DTU Aqua several rearing techniques have been tried and some discussed above, including larval density, feeding frequency, feed density, green water; clay suspension to increase turbidity and test of influence of varying turbulence (air) without any obvious positive results of survival and thus with a typical survival to PL in the range 1-4%. Consequently, there is a great need, wish and potential to increase the survival to PL, to reduce and optimize work load in hatcheries, to decrease the number of berried females to be caught, to optimize release programmes and restocking efficiency and seek to prepare the ground for a commercially viable production of market size lobsters in aquaculture.

Isolation of stage I larvae in newly designed individual up flow chambers (Figure 18) at DTU Aqua has however, recently showed a potential for improved survival, resulting in up to 60 % of larvae reaching

PL when fed *Artemia* metanauplii /adults (length 4.7 mm) and a survival of about 20 % by feeding with *Artemia* nauplii (length 0.3 mm) (DTU Aqua, short report 2023 (see Annex A for details). This despite, that individually rearing of larvae put challenges to the lobster larval behaviour as due to the design of rearing chambers i.e. flow rate, space, feeding, etc,- factors that all may affect mortality. However, these recent results indicate also, that cannibalism is the single most important parameter for high mortalities and that size of *Artemia* live prey can have a significant importance (Figure 19).

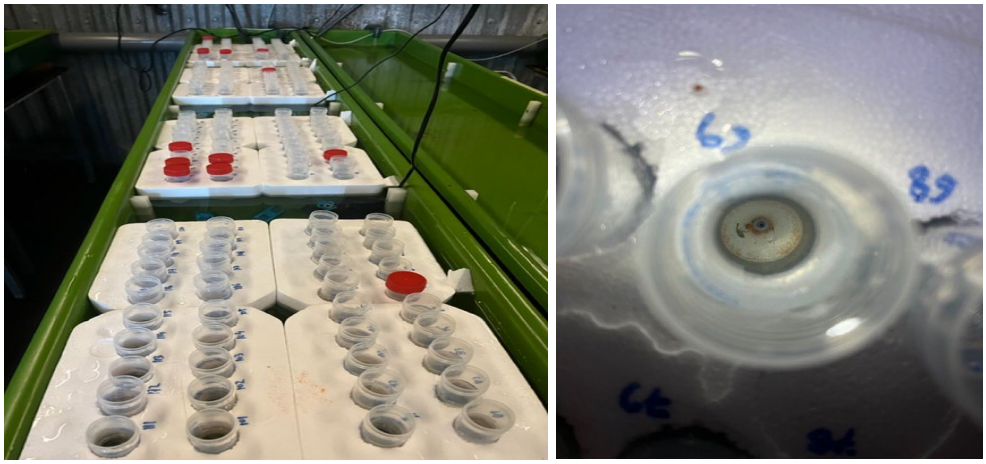


Figure 18. Individual rearing up-flow chambers in a RAS raceway.

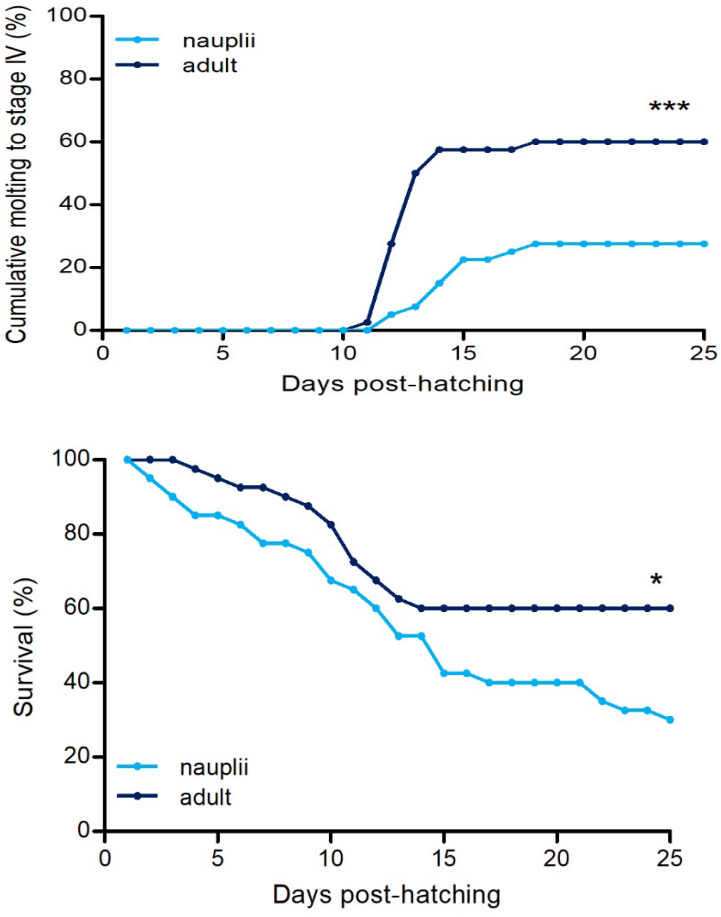


Figure 19. Effect of *Artemia* prey size (nauplii vs adult) on survival and cumulative moult (of initial numbers) to stage IV of *Homarus gammarus* larvae.

6.4 Importance of Artemia size

Based on the positive results on *Artemia* size with individual rearing this was evaluated in communal rearing tanks by the end of the project June 2023. 350 newly hatched stage 0 larvae were inserted into each of 6 fifty L conical tanks and reared for a period of 17 days, until more than 99% of all larvae had moulted to stage IV. Results are given below in Fig 20 & 21, Results indicate (Fig 21), that time to reach stage IV was much faster when fed with large adult *Artemia* than with *Artemia* nauplii, indicated by that already on dph 8 approximately 15-23% larvae had moulted to stage IV for lobsters fed on large artemia, while only a < 1 % for lobsters fed on *Artemia* nauplii. Similarly samples of larvae showed that within the same developmental stage (stage III or IV) larvae on large *Artemia* on average were more heavy than their conspecifics on *Artemia* nauplii (stage III: 21 mg vs 19 mg, stage IV 27.5 mg vs 24.5 mg). Overall survival to stage IV was significantly higher for lobsters fed on large *Artemia* (Figure 22) and reached 23-28%, while for lobsters on nauplii this varied between 13 - and 23%. In general survial was much higher than in previous trials for all tanks, which may be related to both an increased temperature (22-23C was provided), use of green water during rearing (*Nannochloropsis* sp) and that newly caught berried females were used for the experiment. Larvae and feeds will be analyzed to conclude further on the importance of feed size vs nutritional factors.

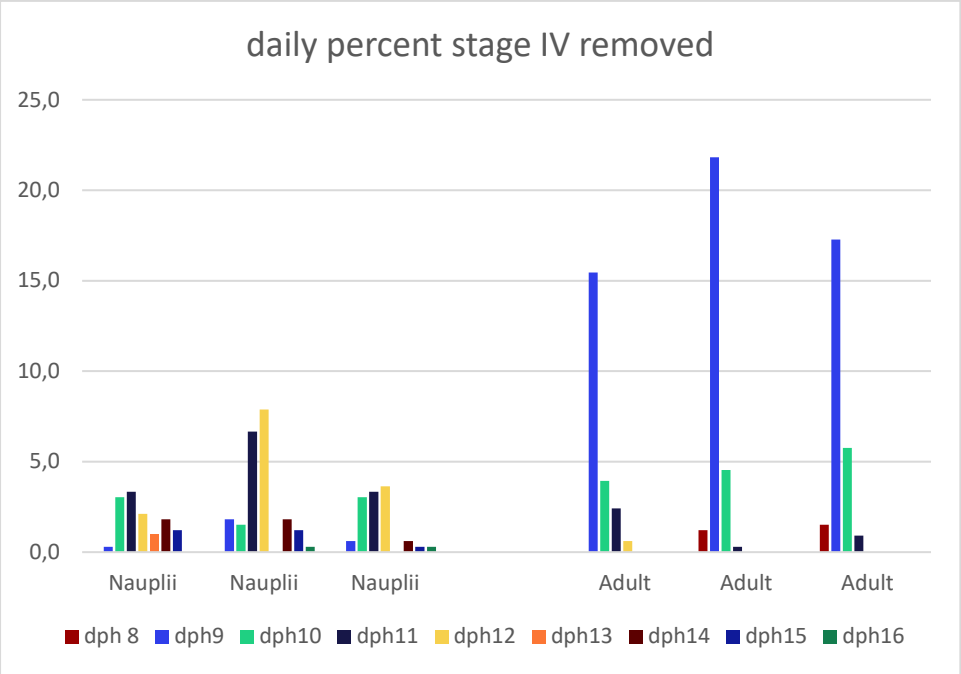


Figure 20. Dally occurrence of stage IV post larvae from DPH 8 by use of *Artemia* nauplii or adult *Artemia*.

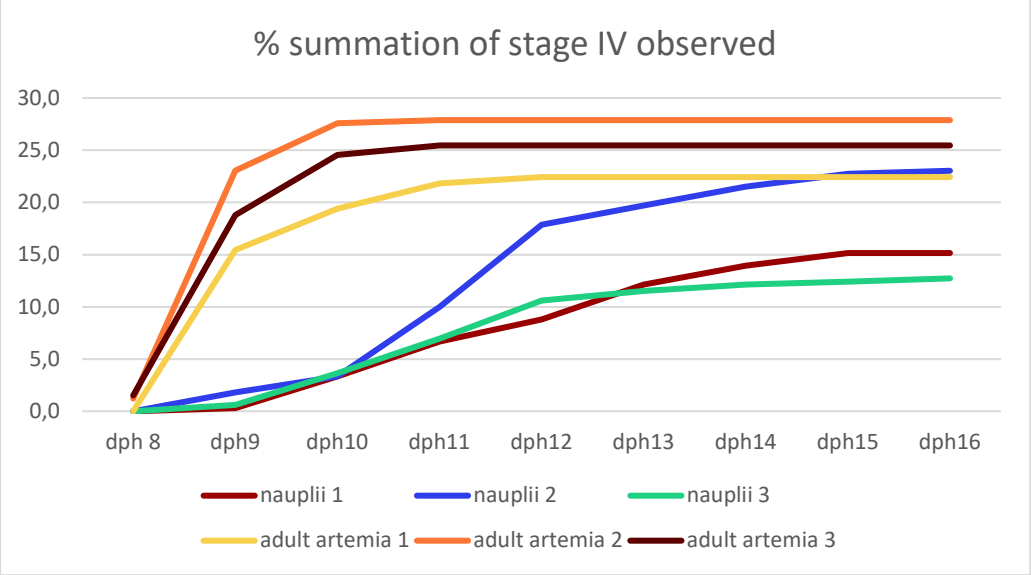


Figure 21. Survival to stage IV postlarvae.

7. Larval feed and feeding

Concerns about water quality, convenience and variation in composition of 'wet' feed led to the use and optimisation of 'live' feeds, principally brine shrimp *Artemia* salina (35). As reported the size of live *Artemia* likely influence positively on larval survival and may be related to a higher intake or energy yield, but the use of thawed non motile krill (same size or larger than *Artemia* adults) have not shown a similar effect on survival, which should be further examined. Whilst motile live feeds may stimulate feeding response, variations in nutritional quality, bacterial contamination and increased operational expense are incurred during hatching, enrichment and onward feeding. Although efforts have been made to optimise live feeds, such as biotic supplementation to optimise lipid levels and stabilise bacterial assemblages associated with feed cultures (24,38,44) frozen and sterilised plankton preparations have become popular due to convenience (22,33).

Tests at DTU Aqua not reported here have examined live prey, (*Artemia* nauplii (enriched/unenriched), vs use of "wet feed (Krill, mysis, shrimp waste meal) and extruded micro feeds, but none of these have had a significant positive impact on survival rates to PL in communal rearing systems, although some results with wet feed and shrimp waste meal (Figure 22) have been blurred by mortalities due to infection with filamentous bacteria due to the organic loading from these feeding sources.

Figure 22. Larvae of European lobster (ST III) handling & eating a piece of dried shrimp waste offal.

Commercially available fine grade-formulated feed pellets have been incorporated, providing additional convenience for feed preparation, storage, cost and feeding regime, and control over feed composition, size and hygiene (20, However, these feed types add an organic load to the tanks, that may outweigh the positive effects and cause bacterial bloom and affect larvae negatively as described. Besides wild lobster larvae likely consume a variety of different prey items, and since formulated feeds are not designed for Homarus spp., larvae require supplementation with A. salina (45) or a 'mix' of dry and frozen preparations may be an option (National Lobster Hatchery, pers. comm. 2021).

DTU Aqua have examined various sustainable ingredients and compounds to formulate feed pellets to lobster larvae (St I-III) in recent years. Thus a feeding experiment was conducted in which newly-hatched *H. gammarus* were fed one of four diets during their larval development. The formulated diets consisted of a control extruded feed using krill and fishmeal as main protein sources (CTRL) and two alternative diets replacing 15% of dietary protein by shrimp waste meal (SWM) or black soldier fly

meal (BSF). The three formulated diets were benchmarked against a conventional live feed composed by *Artemia* nauplii (ART). Test results included growth, survival, digestive capacity and energy metabolism.

The inclusion of BSF and SWM (which have a high chitin content that may be important for the moulting success of larvae) led to an increase in the exo- and endochitinase activities compared to the CTRL group. Exo- and endochitinase activities were minimal in the larvae fed ART. Digestive enzyme activities were modulated by the diet received suggesting a regulation according to the availability of substrate. Trypsin and amylase activities increased while lipase activity decreased in lobster larvae fed ART compared to those fed on the three formulated diets An increased low DNA to high DNA bacteria ratio (LNA/HNA) was recorded in the rearing tanks of larvae fed ART suggesting lower nutrient loading in those tanks (Figure 23). Despite the significant dietary impact in the digestive response, glycogen reserves, and water quality summarized above, the diets showed no effect on growth and survival during lobster larval development (Figure 24). However, the impact of larval feeding regimes at later postlarvae and juvenile stages needs to be assessed to understand the consequences of the observed physiological responses in the longer-term

Similarly, individually maintained larvae with access only to dry feed did not develop to PL stages. Together, this suggests further improvement of larval feed composition and attractiveness is required (33).

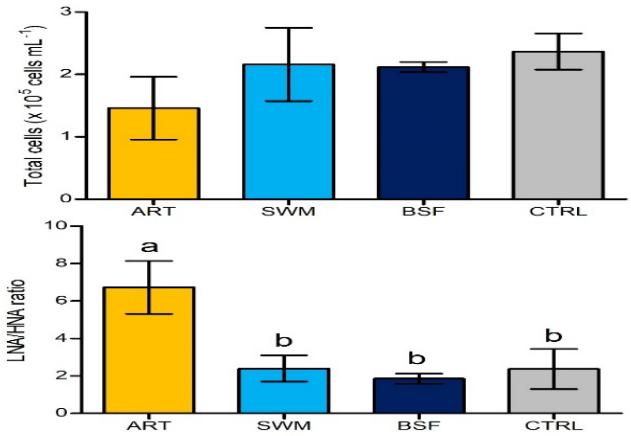


Figure 23. Water chemistry and bacterial loading as indicated by total bacteria cells and LNA/HNA ratio.

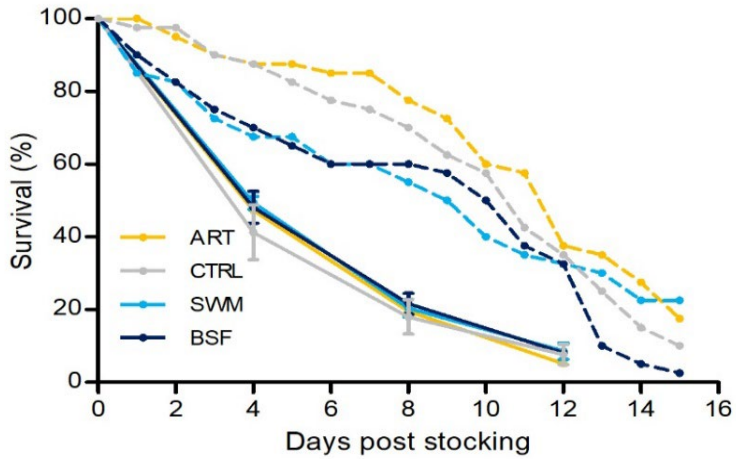


Figure 24. Survival of lobster larvae fed extruded pelleted diets where 15 % of fishmeal /krill meal was substituted with 15% shrimp waste meal or 15 % Black soldier fly meal and compared against a control diet with use of only fish meal/krill meal or a live *Artemia* nauplii feed.

8. Larval nutritional requirements

Although poorly understood, the specific nutrient requirements of different larval stages and ontogenetic development in digestive capacity are among the most important aspects for the hatchery production of this species. At DTU Aqua a three- year Ph.D. thesis was published (46) on the metabolism and nutritional requirements of *H. gammarus* early stages and thus provide solutions to nutritional challenges faced by hatchery production units.

Results from this thesis provide new insights into the metabolism and nutritional requirements of the early stages of *H. gammarus*. Results demonstrated that protein is a key nutrient for all stages examined, while lipids are of particular importance during larval development and dietary carbohydrate requirements increase after metamorphosis. Lipid sources of richer phospholipid content might improve *H. gammarus* lipid digestion and assimilation and, therefore, its inclusion in feeds for *H. gammarus* early stages deserves further investigation. Phospholipids (i.e. a class of lipids) as well as long chain fatty acids (LC PUFAs) especially eicosapentenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) are important for a range of physiological processes including neural development, membrane functionality and eicosanoic production. Studies have revealed that in tissue of wild lobsters 7.7 % and 18% of total fatty acids were EPA and DHA (58). As for many marine fish species, lobsters are having a limited ability to synthesize these LC PUFAs from shorter chain FA precursors and therefore need to be provided in formulated diets.

Thus a subsequent study at DTU Aqua further investigated the requirement of phospholipids in early stage lobster larvae (stage I-III). The effects of replacing live feed (*Artemia* nauplii) with two formulated extruded diets with different content of phospholipids and LC PUFAs were demonstrated for larval culture of *Homarus gammarus* obtained by supplementation of soy lechithin, arachidonic acid (Croda) and fish oil (DHA 70). The isonitrogenous and isoenergetic diets (P1 and P2) had a phospholipid content of 3% and 9 % and a LC- PUFA content of 1% and 4.8 %, respectively. Newly hatched larvae were distributed into triplicate upflow hoppers of 50 L each with 250 larvae per tank. Two additional groups one fed with *Artemia* (AF type) and one group left unfed were used as control. Temperature was 19.5 °C. Lobster larvae were fed twice daily for 17 days. Every three days, all larvae in each tank were counted and 10 larvae per tank sampled to determine ontogeny and subsequently frozen at -80°C for subsequent amino acid composition, FA composition and lipid composition. Survival was significantly different between groups and lowest for unfed larvae. No significant difference in ontogenetic development between groups. The highest number of stage IV post-larvae were obtained by *Artemia* followed by diets P2 and P1. Lipid and FA composition in larvae were significantly related to dietary composition. Results showed, that dietary lipid composition can influence on survival to post-larvae and are of importance in early larval stages. Cannibalism persisted as an influential factor in larval rearing of this species and affected overall survival.

Further recent work in 2023 has examined the incorporation, recovery and reallocation of 14C –labelled fatty acid 18:3n-3; 18:2 n-6 and 20:5 n-3 (LA, ALA, and EPA) to examine how long chain fatty acids can be metabolised in lobster larvae. This was carried out placing lobster larvae in flat-bottom 6-wells tissue culture plates (Sarstedt AG & Co., Nümbrecht, Germany) containing 10 mL. Incubations were performed in triplicate (n=3) for 5 hours, at a density of 10 larvae per well, with gentle stirring and 0.2 µCi (0.3 µM) of [1-^{14}C] labelled fatty acids (free FA molecule, labelled with ^{14}C in its first carbon from the carboxyl head), including either 18:2n-6 (LA), 18:3n-3 (ALA) (PerkinElmer, Inc., Waltham, Massachusetts, USA),and 20:5n-3 (EPA) (American Radiolabelled Chemicals, Inc., St. Louis, Missouri, USA). For each dietary treatment group, larvae incubated without ^{14}C substrate were also

assessed for lipid composition determination (n=3). Transformation of incubated [1-^{14}C]FA by desaturation/elongation processes was determined using pre-coated TLC plates G-25 (20 × 20 cm; Macherey-Nagel GmbH & Co. KG) pre-impregnated with a solution of 2 g silver nitrate in 20 mL acetonitrile (Reis et al., 2019). Incorporation of total radioactivity into pikeperch lipids, was determined in a LKB Wallac 1214 Rackbeta liquid scintillation ß-counter (PerkinElmer Inc., Waltham, Massachusetts, USA).

The recovery rate was significantly affected by both the developmental stage and the substrate used in the incubation. Please refer to Figure 25 for the results.

The percentage of recovery decreased until stage III, but then increased substantially after metamorphosis (stage IV). This suggests that de novo synthesis, elongation, or/and desaturation capacity increases until stage III and then decreases dramatically after metamorphosis.

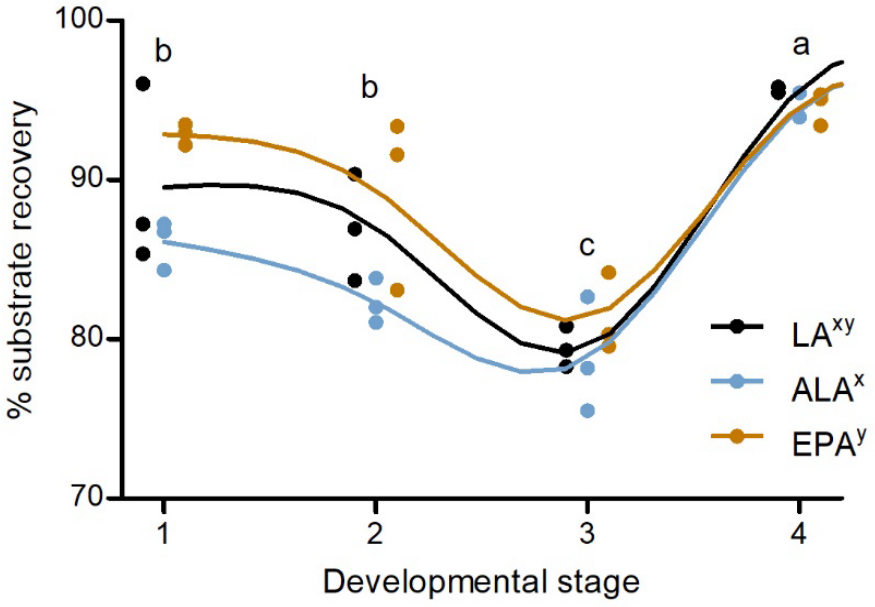


Figure 25. Recovery rate (%) of C14 labelled FA substrates provided.

Data are be published when all results have been evaluated and will provide insight and first ever information about uptake and metabolism of polyunsaturated fatty acids in European lobster larvae and postlarvae.

9. Juvenile nutritional requirements

Whilst research effort has been employed to develop feeds for juvenile *H. americanus* (47,56) species-specific composition for *H. gammarus* is now also becoming established. Formulations at DTU Aqua with a protein, carbohydrate and lipid ratio (ca. 50:25:12) supported comparable growth rate and moulting frequency for juvenile *H. gammarus*, compared with a control diet of krill (*Euphausia superba*) (48,49,50) and feeds with a high protein content (50%) were more suitable than low protein content (40%) for juveniles (48).

Substitution with alternative sustainable protein ingredients is a future way to minimize costs of feed and utiliza waste resources in an Experiment at DTU Aqua that substitution of 28 % protein with shrimp waste meal significantly enhanced survival of juvenile lobsters (51). Incorporating Atlantic prawn (*Pandalus borealis*) at a similar protein and lipid inclusion rate also supported growth of juvenile *H. gammarus*, with an added benefit of reducing feed production cost, or adding value to waste streams within a circular economy (52).

Feeds deficient in carotenoids also produce unpigmented lobsters, requiring supplementation to promote wild-type colouration and assist camouflage following release (53) or to improve market appeal for RAS production (53). Carotenoids also improve survival, growth, reproductive capacity, disease and stress resistance in crustaceans (54,55). Although there are general and species-specific knowledge gaps in terms of inclusion level, absorption and utilisation (51,53) pilot results with *H. gammarus* juveniles suggest carotenoid provisioning at 100 mg/kg can increase survival and growth by alleviating oxidative stress (National Lobster Hatchery, pers. comm. 2021).

AT DTU Aqua an experiment was performed to evaluate the coloration of lobster juveniles after feeding with either Antartic krill (AK), *Artemia nauplii* (ART) or an extruded feed (FEF) supplemented with Carophyll Pink 10% - Astaxanthin (at 0.150 %) inclusion rate. The extruded diet was composed of an equal mixture of krill meal, fish meal and squid meal and had 58% protein and 7.5 % lipids.

The diet type affected the growth performance of the juvenile lobsters during the 10-week challenge period. Lobsters fed the AK diet performed significantly better than those fed the FEF feed in most of the indicators estimated (final body weight and carapace length, molt frequency and increment, and intermolt period). Animals reared on adult brine shrimp (ART) performed as well as those fed AK, but not better than those reared on the formulated feed.

After the dietary challenge of 10-weeks, juveniles were allowed an equal period for recovery, during which, all treatments were offered the AK diet. No significant differences were observed in none of the growth parameters estimated, suggesting that the juveniles previously reared on the FEF diet were able to partially recover in terms of growth.

9.1 Exoskeleton coloration

The exoskeleton color intensity measured by digital analysis in terms of red, green, and blue scores reveal that juvenile lobsters fed on the FEF diet developed a deviating color from their natural coloration becoming paler (higher R, G, B scores). The deviation was significant only 2 weeks after the start of the challenging period and its severity increased during the 10-week dietary challenge. However, color deviation was reverted within 8-weeks after the juveniles were re-introduced to the AK diet.

There were no significant differences between the AK and ART dietary groups in terms of absolute R, G, B scores (Figure 26).

Results showed that lobsters fed ART presented a significant increased content of canthaxanthin compared to those fed AK, higher astaxanthin than those reared on FEF. The overall total carotenoid level was higher in the ART compared to the FEF dietary group. Results also suggest that, astaxanthin supplemen from Carophyll Pink 10 % have a relatively low storage stability (within a month) and is partly lost even at cold storage. All data will be published.

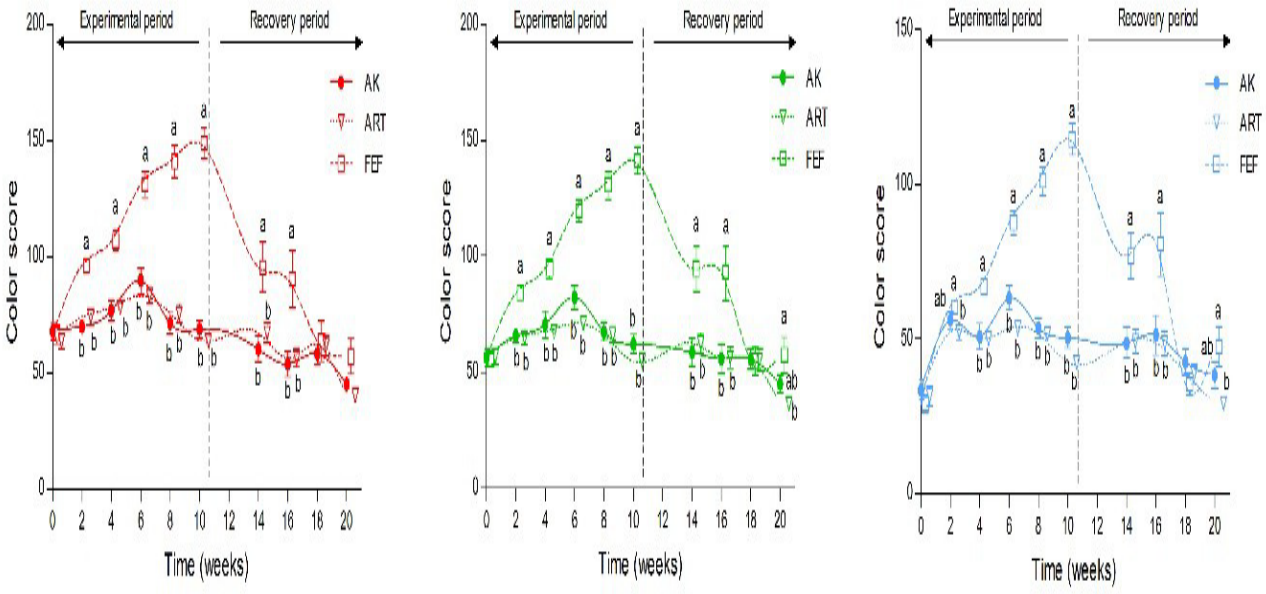


Figure 26. RGB color scores in the exoskeleton of juveniles. During the experimental period (week 0 to 10), the animals were divided in three groups, and each assigned a different diet (Antarctic krill – AK, adult Artemia – ART, and extruded feed – FEF). From week 10 to 20 (recovery period), the three dietary groups were fed with Antarctic krill (AK). Data represented as mean ± 1 SEM. Different letters indicate significant differences between dietary groups in each week.

10. Concluding remarks and future bottlenecks to be solved

Lobster larvae are aggressive and cannibalistic at both larval and PL stages. Communal rearing in hoppers results in survival many orders of magnitude higher than in the wild, but still may only yield in most cases a few percent survival to metamorphosis. Ongrowing of PL demands separate cells to prevent aggression and mortality. Whilst Aquahive technology (see below) has recently reduced the husbandry resources required to ongrow small PL until release, technology is still under development for a straightforward process to ongrow lobsters to “plate size” at commercial scales. Meanwhile, developing methods to decrease cannibalism at the larval stage, and reducing additional mortality via improved nutrition and hygiene, could decrease the need for adult broodstock. The underlying drive for sustainability also includes consideration of energy costs, the origin of feed components, reducing feed use, and treatment of waste.

In hatcheries, the life cycle is not yet “closed” at a commercial scale. One of the goals of farming is to domesticate the animal and create “lines” with desirable characteristics such as fast growth rate, high survivorship, attractive colour and shape, and potentially low aggression or ability to subsist on low protein feed. Whilst genetic and phenotypic screening of broodstock, and the resulting larvae and PL may be a goal for lobster farming, this is in contrast to the ethical goal of PL for release at sea. Securing genetic diversity, particularly with respect to local populations, demands the continual procurement of wild mated females. Released Juveniles also need to be reared in conditions that reduce behavioural naivety so that survival post-release is maximised; stakeholders in restocking programmes also need to be reassured that released lobsters remain local and are recruited to the fishery.

Replacement of an industry standard feed (wet, sterilised plankton) with a commercially available pelleted feed has not had a negative impact on larval performance. Indeed, use of a dry feeds specifically designed for *Homarus sp.* and without variations in natural quantity and composition could prove very beneficial. Dry feeds would also enable simple mechanical feeders to be used with less preparation, storage and refrigeration costs, allowing cost savings.

Much of the larval mortality in hatcheries arises from cannibalism in communal rearing systems; while cost effective methods are sought to reduce this, maintaining high survival and welfare have been investigated, for example by improving hygiene. Ozone, UV and probiotics have been tested in lobster hatcheries as a sustainable, preventative strategy to reduce opportunistic infections, which may impact upon survival and growth. Ozone and UV have been long used in aquaculture water treatment, although direct ozonation of livestock in situ requires additional care to maintain an effective dosage that is well within physiological limits. Compared to unozonated and un- UV controls, lobster larvae yielded poorer growth during the short pelagic period although they experienced reduced bacterial loading of larvae and culture water.
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References

1 Hedgecock, D., Moffett, W. L., Borgeson, W., & Nelson, K. (2009). PROGRESS AND PROBLEMS IN LOBSTER BROODSTOCK DEVELOPMENT. *Proceedings of the Annual Meeting - World Mariculture Society*, *9*(1–4), 497–506. https://doi.org/10.1111/J.1749-7345.1978.TB00269.X

2 Smith, I.P., Jensen, A.C., Collins, K.J. and Mattey, E.L. (2001) Movement of wild European lobsters *Homarus gammarus* in natural habitat. *Marine Ecology Progress Series, 222, 177-186*.

3. Tully O, Roantree V, Robinson M. Maturity, fecundity and reproductive potential of the European lobster (*Homarus gammarus*) in Ireland. *J Mar Biol Assoc U K*. 2001;81:61-68.

4. Lizárraga-Cubedo HA, Tuck I, Bailey N, Pierce GJ, Kinnear JAM. Comparisons of size at maturity and fecundity of two Scottish populations of the European lobster, *Homarus gammarus*. *Fish Res*. 2003;65:137-152.

5. Coleman MT, Porter JS, Bell MC. Investigating fecundity and egg loss using a non-invasive method during brooding in European lobster (*Homarus gammarus*). *ICES J Mar Sci*. 2019; 76:1871-1881.

6. Goldstein JS, Watson WH. Biochemical changes throughout early-and middle-stages of embryogenesis in lobsters (*Homarus americanus).* PeerJ. 2019; 7: e6952. Published online 2019 May 16. doi: 10.7717/peerj.6952

7. Perkins, H.C., 1972. Developmental rates at various temperatures of embryos of the northern lobster (Homarl~s americamls Milne-Edwards). Fish. Bull., Vol. 70, pp. 95-99.

8. Contarini G, Perrella N, Hickey J, Ballestrazzi R. Hatchery production of European lobster (*Homarus gammarus*, L.): broodstock management and effects of different holding systems on larval survival. *Ital J Anim Sci*. 2008;7(3):351-362.

9. Moland E, Olsen EM, Stenseth NC. Maternal influences on offspring size variation and viability in wild European lobster *Homarus gammarus*. *Mar Ecol Prog Ser*. 2010;400:165-173.

10. Leal MC, Pochelon PN, DaSilva TL, Reis A, Rosa R, Calado R. Variable within-brood maternal provisioning in newly extruded embryos of *Homarus gammarus*. *Mar Biol*. 2013;160(4):763-772.

11 Smith GG, Ritar AJ. Sexual maturation in captive spiny lobsters, *Jasus edwardsii*, and the relationship of fecundity and larval quality with maternal size. *Invert Reprod Dev*. 2007;50(1):47-55.

12. Skog M. Intersexual differences in European Lobster (*Homarus gammarus*): recognition mechanisms and agonistic behaviours. *Behaviour*. 2009;146:1071-1091.

13. Jezequel Y, Bonnel J, Coston-Guarini J, Guarini JM, Chauvard L. Sound characterization of the European lobster *Homarus gammarus* in tanks. *Aquat Biol*. 2018;27:13-23.

14. Goulden EF, Hoj L, Hall MR. Microbial management for bacterial pathogen control in invertebrate aquaculture hatcheries. In: Allan G, Burnell G, eds. *Advances in Aquaculture hatchery Technology*, 1st ed. Woodhead Publishing Ltd.; 2013:246-285.

15 Burton CA. Lobster hatcheries and stocking programmes: an introductory manual. *Seafish Report* 2003; SR552.

16. Powell A, Cowing DM, Scolding JWS, et al. *Nephrops norvegicus:* hatchery handbook. Accessed September 21, 2021. https://www.gu.se/sites/ defau lt/files/ 2020-05/Hatch ery%20man ual. pdf

17. Scolding JWS, Powell A, Bothroyd DP, Shields RJ. The effect of ozonation on the survival, growth and microbiology of the European lobster (*Homarus gammarus*). *Aquaculture*. 2012;364:217-223.

18. Lerch F, Uglem I. High density of *Histriobdella homari* Van Beneden (Anelida, Polychaeta) on ovigerous female European lobsters (Decapoda, Nephropidae). *Crustaceana*. 1996;69:916-920.

19. Uglem I, Uksnov LE, Bergh O. Chemical treatment of lobster eggs against epibiotic bacteria. *Aquacult Int*. 1996;4:1-8.

20 Hinchcliffe, J., Agnalt, A.L., Daniels, C.L., Drengstig, A., Lund, I., McMinn, J., Powel, A. 2021. European lobster Homarus gammarus aquaculture: Technical developments, opportunities and requirements. *Reviews in Aquaculture DOI: 10.1111/raq.12634*

21. Middlemiss KL, Urbina MA, Wilson RW. Microbial proliferation on gill structures of juvenile European lobster (*Homarus gammarus*) during a moult cycle. *Helgol Mar Res*. 2015;69(4):401-410.

22. Middlemiss KL, Daniels CL, Urbina MA. Combined effects of UV radiation, ozonation and the probiotic *Bacillus* spp. on growth, survival and general fitness in European lobster (*Homarus gammarus*). *Aquaculture*. 2015;444:99-107.

23. Attramadal KJK, Øien JV, Kristensen E, et al. UV treatment in RAS influences the rearing water microbiota and reduces the survival of European lobster larvae (*Homarus gammarus*). *Aquac Eng*. 2021;94:102176.

24. Daniels CL, Merrifield DL, Boothroyd DP, Davies SJ, Factor JR, Arnold KE. Effect of dietary *Bacillus* spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. *Aquaculture*. 2010;304(1–4):49-57.

25. Holt CC, van der Giezen M, Daniels CL, Stentiford GD, Bass D. Spatial and temporal axes impact ecology of the gut microbiome in juvenile European lobster (*Homarus gammarus*). *ISME J*. 2020;14(2):531-543.

26. Holt CC, Foster R, Daniels CL, et al. *Halioticida noduliformans* infection in eggs of lobster (*Homarus gammarus*) reveals its generalist parasitic strategy in marine invertebrates. *J Invertebr Pathol*. 2018;154:109-116.

27. Davies CE, Wootton EC. Current and emerging disease of the European lobster (*Homarus gammarus*): a review. *Bull Mar Sci.*2018;94(3):959-978.

28. Uglem I, Perez Benavente FG, Browne R. A regional development strategy for stock enhancement of clawed lobsters (*Homarus gammarus*)–development of juvenile lobster production methodologies. *NINA Report* 2006;211.

29. Jacklin M, Combes J. The good practice guide to handling and storing live Crustacea. SEAFISH report 2005. Accessed September 21, 2021. https://www.seafi sh.org/docum ent/?id=4b3b6 210-51d6-4e21-97b5-a6eab 65020a6

30. Allen EJ. The reproduction of the Lobster. *J Mar Biol Assoc U K*. 1895;4(1):60-69.

31. Nichols JH, Lawton P. The occurrence of the larval stages of the lobster *Homarus gammarus*, (Linnaeus, 1758) off the northeast coast of England in 1976. *ICES J Mar Sci*. 1978;38:234-243

32. Rötzer MAIN, Haug JT. Larval development of the European lobster and how small heterochronic shifts lead to a more pronounced metamorphosis. *Int J Zool*. 2015;2015:1-17. doi:10.1155/2015/345172s

33. Powell A, Hinchcliffe J, Sundell K, Carlsson N-G, Eriksson SP. Comparative survival and growth performance of European lobster larvae, *Homarus gammarus*, reared on dry feed and conspecifics. *Aquac Res*. 2017;48:5300-5310

34 Goncalves, R., Aalto, S. L., & Lund, I. (2023). The Effect of UV Irradiation on Rearing Water Quality, Growth, and Survival of European Lobster (Homarus gammarus, L.) Larvae. *Aquaculture Research*, *2023*, 1–10. https://doi.org/10.1155/2023/8668381

35. Fitzsimmons S, Saravanan S, Walden J, Arthur G. Effects of delay of onset of feeding and Artemia concentrations on the survival and growth of stage I European lobsters, *Homarus gammarus* (Linnaeus). *Aquac Res*. 2004;35:605-607

36. Hughes JT, Shleser RA, Tchobanoglous GA. A rearing tank for lobster larvae and other aquatic species. *Progess Fish Cult*.
1974;36:129-132

37. North Bay Innovations Ltd. Accessed September 21, 2021. https://
north bayin novat ions.co.uk/lobst ercub e/

38. Daniels CL, Merrifield DL, Ringo E, Davies SJ. Probiotic, prebiotic and symbiotic applications for the improvement of larval European lobster (*Homarus gammarus*) culture. *Aquaculture*.
2013;416:396-406

39 Giménez Papiol, G., Roque, A., & Estévez, A. (2018). Variations in Bacterial Community of Rearing Water and Gut of Common Dentex, Dentex dentex (Linnaeus 1758), Larvae Using Three Microalgae Management Approaches. *Journal of the World Aquaculture Society*, *49*(5), 953–963.
https://doi.org/10.1111/JWAS.12437

40 Papandroulakis, N., Divanach, P., Anastasiadis, P., & Kentouri, M. (2001). The pseudo-green water technique for intensive rearing of sea bream (Sparus aurata) larvae. *Aquaculture International*, *9*(3), 205–216. https://doi.org/10.1023/A:1016813623122

41 Rocha, R. J., Ribeiro, L., Costa, R., & Dinis, M. T. (2008). Does the presence of microalgae influence fish larvae prey capture? *Aquaculture Research*, *39*(4), 362–369.
https://doi.org/10.1111/J.1365-2109.2007.01746.X

42 ÖNAL, U., & BAKİ, H. (2021). The Growth and Survival of The European lobster (Hommarus gammarus) Larvae in Pseudo-Green Water and Clear Water Under Low Density Conditions. *Çanakkale Onsekiz Mart University Journal of Marine Sciences and Fisheries*.
https://doi.org/10.46384/JMSF.1028180

43 Contarini, G., Perrella, N., Hickey, J., & Ballestrazzi, R. (2008). Hatchery production of European lobster (Homarus gammarus, L.): Broodstock management and effects of different holding systems on larval survival. *Italian Journal of Animal Science*, *7*(3), 351–362.
https://doi.org/10.4081/IJAS.2008.351

44. Daniels CL. Optimisation of the rearing diets for early life stages of the European lobster, Homarus gammarus*,* to enhance growth, survival and health using biotic dietary supplements. PhD Thesis. University of Plymouth; 2011.

45. Fiore DR, Tlusty MF. Use of commercial Artemia replacement diets in culturing larval American lobsters (*Homarus americanus*). *Aquaculture*. 2005;243:291-303.

46. Goncalves I de sousa R. Ontogenic development and nutritional requirements in early life stages of the European lobster (*Homarus gammarus*, L.) 2021. PHD thesis, pp 1-151. Technical University of Denmark National Institute of Aquatic Resources.

47. Tlusty MF, Fiore DR, Goldstein JS. Use of formulated diets as replacements for Artemia in the rearing of juvenile American lobsters (*Homarus americanus*). *Aquaculture*. 2005;250:781-795.

48. Goncalves R, Lund I, Gesto M, Skov PV. The effect of dietary protein, lipid, and carbohydrate levels on the performance, metabolic rate and nitrogen retention in juvenile European lobster (*Homarus gammarus*, L.). *Aquaculture*. 2020; 525:735334

49. Goncalves R, Lund I, Gesto M. Interactions of temperature and dietary composition on juvenile European lobster (*Homarus gammarus*,*L*.) energy metabolism and performance. *Comp Biochem Phys A*. 2021;260:111019. doi:10.1016/j.cbpa.2021.111019

50. Goncalves R, Gesto M, Teodosio MA, Baptista V, Navarro-Guillen C, Lund I. Replacement of Antarctic krill (*Euphausia superba*) by extruded feeds with different proximate compositions: effects on growth, nutritional condition and digestive capacity of juvenile European lobsters (*Homarus gammarus*, L.). *J Nutr Sci*. 2021;10:e36 1–15.

51 Goncalves, R., Lund, I., Sousa, D., & Skov, P. V. (2022). Shrimp waste meal (*Pandalus borealis*) as an alternative ingredient in diets for juvenile European lobster (*Homarus gammarus*, L.). Animal Feed Science and Technology, 294, [115478]. https://doi.org/10.1016/j.anifeedsci.2022.115478

52. Kristiansen TS, Drengstig A, Berghein A, et al. Development of methods for intensive farming of European lobster in recirculated seawater. Results from experiments conducted at Kivitsøy lobster hatchery from 2000 to 2004. *Fisken Og Havet*. 2004;6:1-52.

53. Mynott S, Daniels C, Widdicombe S, Stevens M. Using camouflage for conservation: colour change in juveniles European lobster. *bioRxiv*. 2018;431692. doi:10.1101/431692

54. Wade NM, Gabaudan J, Glencross BD. A review of carotenoid utilisation and function in crustacean aquaculture. *Rev Aquac*. 2017;9:141-156.

55. Lim KC, Yusoff FM, Shariff M, Kamarudin MS. Astaxanthin as feed supplement in aquatic animals. *Rev Aquacult*. 2018;10:738-773.

56. Hinchcliffe J, Powell A, Langeland M, Vidakovic A, Sundell K, Eriksson SP. Comparative survival and growth performance of European lobster *Homarus gammarus* post-larvae, reared on formulated dry feeds. *Aquac Res*. 2020;51:102-113.

57 Browne, R., Benavente, P.,Uglem, I., Balsa, J.C.,M. an illustrated hatchery guide for the production of clawed lobsters, (using a green water technique) Aquaculture Explained, 23 (2009).

58. Budge et al. 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 59: 886-898.

Annex A
Effects of *Artemia* prey size on survival and growth of European lobster larvae

Effects of *Artemia* prey size on the survival and growth of European lobster (*Homarus gammarus*) larvae

Renata Goncalves May 2023

Introduction

Homarid lobsters, including the *Homarus gammarus*, are opportunistic scavengers classified as omnivorous or carnivorous (Cobb & Castro, 2006). In their natural environment, homarid lobster larvae feed on a variety of zooplankton, such as copepods, diatoms, gastropods, and decapod larvae (Nicosia & Lavalli, 1999). However, replicating these natural diets in captivity poses challenges, leading to the common use of live *Artemia* as a diet in *H. gammarus* hatcheries (Hinchcliffe et al., 2021). This is because *Artemia* is easily available in the form of cysts that can be hatched and cultured to different sizes.

H. gammarus larvae are highly responsive to the swimming behavior of *Artemia* and can effectively capture and extract nutrients from this prey (Conklin, 1995). Consequently, determining the optimal size of live prey is of utmost importance as it directly influences capture efficiency and, subsequently, the overall food consumed by the lobster larvae. Therefore, the main objective of this study was to examine the effects of feeding *H. gammarus* larvae with *Artemia* of different sizes on their survival and growth during the critical period from hatching to stage IV of development.

Material and Methods

Experimental animals:

The experiments were conducted at the Section for Aquaculture, DTU Aqua facilities in Hirtshals, Denmark. Lobster larvae used in the study were obtained from a mixed parentage of wild-caught females captured along the Skagerrak coast of North Jutland, Denmark. The newly-hatched larvae were collected from the broodstock holding tanks and transferred to 10L buckets where they were disinfected with 1ml m^{-3} of DIVOSAN forte (17.4% PAA and 21.9% H_2O_2) for 30 min.

Culture system:

The disinfected larvae were then rinsed in clean seawater and stocked into individual chambers within a raceway tank that received seawater from a semi-recirculated system (Figure A1). The sea water was heated to 20°C and irradiated with ultraviolet light before entering the individual chambers. Each individual chamber consisted of a 30ml cylindroconical transparent plastic container, featuring a bottom inlet and a top outlet with 8 drilled apertures (1.5 mm diameter) covered with a mesh filter (site 0.5 mm). Water was jetted through the bottom inlet of each chamber to facilitate larval

movement and mixing of *Artemia* in the water column. The water flow in each chamber was adjusted to approximately 120ml min^{-1}.

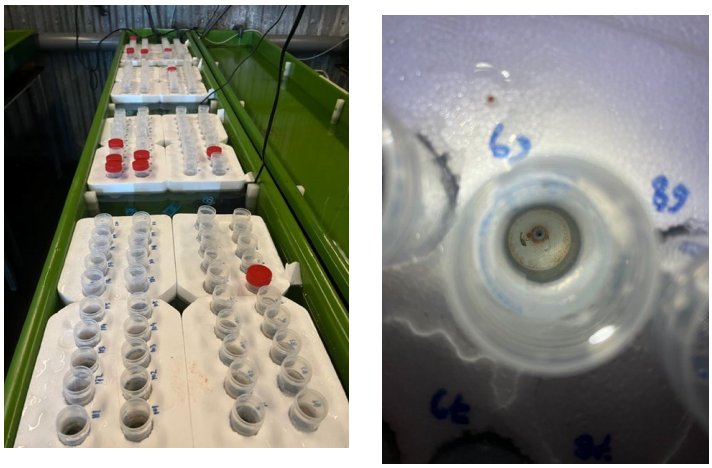

Figure A1. The experimental system consisted of individual chambers that were installed within a raceway tank supplied with seawater from a semi-recirculated system.

Artemia production:

Artemia cysts (A.F. grade, INVE, Belgium) were hatched daily at 28°C in seawater. After 16h incubation, the nauplii (≈ 0.3 mm) were collected, rinsed in clean seawater and used to feed the lobster larvae. Adult *Artemia* (≈ 4.7 mm) were grown on *Isochrysis* sp. algae, and their production was maintained throughout the trial. The size of the prey was estimated by measuring 10 individuals of each group (nauplii and adult *Artemia*) under a stereomicroscope (MC125 Leica, Germany) equipped with a digital camera (MC190HD Leica, Germany) (Figure A2).

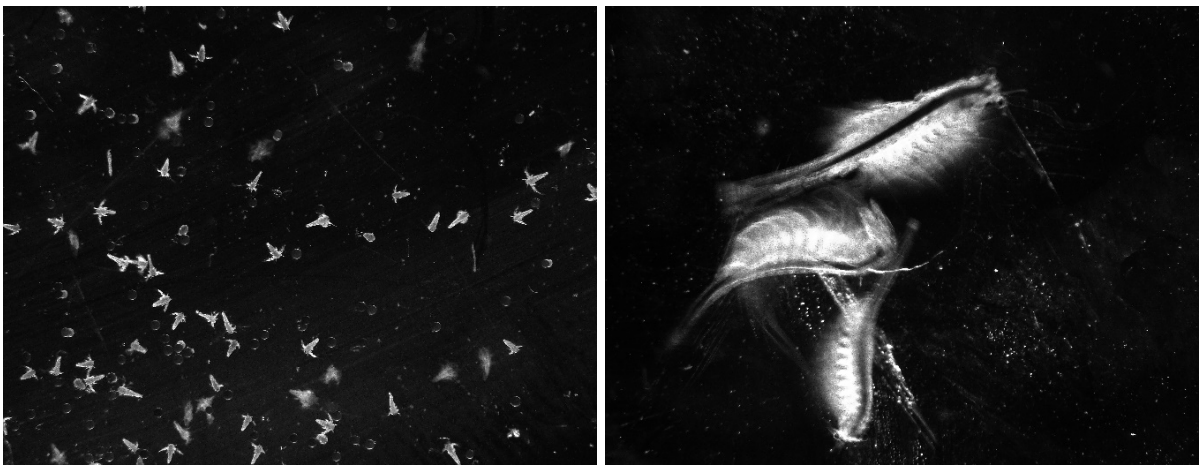

Figure A2. The left panel shows the nauplii, while the right panel shows the adult stage of *Artemia* used to feed.

Feeding Regimes:

This experiment examined the feeding of newly-hatched larvae until stage IV with one of the following regimes:

- 0.3 mm *Artemia* nauplii at ≈ 7 ml^{-1} (≈ 210 $chamber^{-1}$)
- 4.7 mm adult *Artemia* at ≈ 0.5 ml^{-1} (≈ 15 $chamber^{-1}$)

The larvae were provided with their respective diets twice daily at 9:00 and 16:00. Prior to each feeding, any uneaten food, waste, and deceased larvae were removed by siphoning the bottom of each chamber using a plastic air tube. At the beginning of the trial, a sample of 10 larvae was collected and their body weight, total length (measured from the anterior tip of the cephalic shield between the eyestalks to the posterior tip of the abdomen), and carapace length (measured from the base of the eye socket to the posterior edge of the cephalothorax) were recorded. The same measurements were taken for all the surviving individuals at the conclusion of the trial.

Statistical Analysis:

The collected data were assessed for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests, respectively. Parametric data were analyzed using Student's t-test ($p<0.05$) to compare time to metamorphosis, body weight, total length, and carapace length between treatments. Non-parametric data were analyzed using the Mann–Whitney test ($p<0.05$). Survival and stage IV moult occurrence were analyzed using the Kaplan-Meier procedure, and the significance was determined using the Log-Rank test ($p<0.05$).

Results

The survival rates of *H. gammarus* larvae varied between 30% and 60%. These rates were significantly higher ($\chi^2 = 5.846$, $p = 0.016$) in the group of larvae fed adult *Artemia* compared to those fed nauplii, as shown in Figure A3. Furthermore, larvae fed adult *Artemia* reached the postlarvae stage IV faster (Table 1) and in greater numbers (Figure A3, $\chi^2 = 10.939$, $p < 0.001$) compared to those fed nauplii (Figure A4).

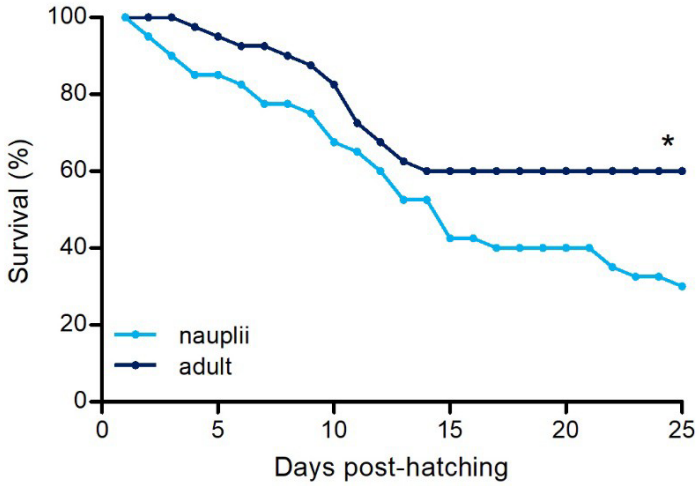

Figure A3. Effect of *Artemia* prey size (nauplii vs adult) on survival (% of initial numbers) of *Homarus gammarus* larvae.

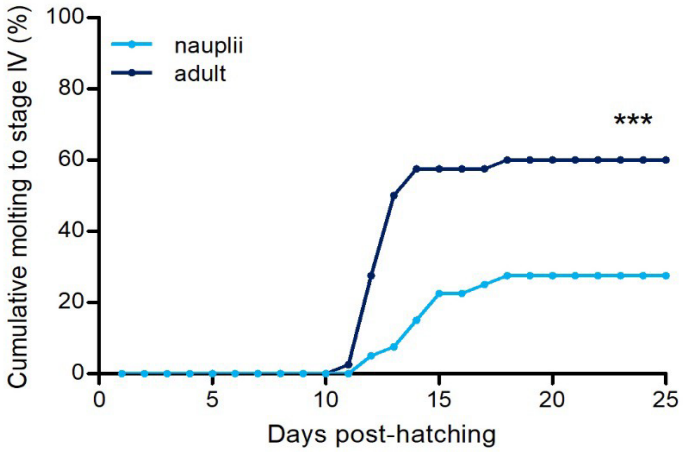

Figure A4. Effect of *Artemia* prey size (nauplii vs adult) on cumulative molt to stage IV (% of initial numbers) of *Homarus gammarus* larvae.

Interestingly, the size of *Artemia* prey did not have a significant impact on the size of the lobster larvae. There were no significant differences observed in the final body weight, carapace length, or total length between the groups fed different sizes of *Artemia* prey (Table A1).

Table A1. Effect of *Artemia* prey size (nauplii vs adult) on growth and development of *Homarus gammarus* larvae.

	Nauplii	Adult	Statistics
Initial body weight (mg)	7.9 ± 0.7		
Initial carapace length (mm)	2.7 ± 0.1		
Initial total length (mm)	9.3 ± 0.4		
Final body weight (mg)	44.0 ± 11.0	48.2 ± 7.1	$t(33) = 1.33$, p = 0.193
Final carapace length (mm)	4.9 ± 0.4	5.1 ± 0.2	U = 72.0, n=33, p = 0.061
Final total length (mm)	15.3 ± 1.3	16.0 ± 0.8	$t(33) = 1.97$, p = 0.058
Time to metamorphosis (days)	**14.5 ± 1.9**	**12.8 ± 1.3**	***t*(33) = 2.93, p = 0.006****

Conclusion

The present study provided evidence that feeding *H. gammarus* larvae with adult *Artemia* resulted in higher survival rates and accelerated development to stage IV compared to larvae fed with nauplii *Artemia*. These results indicate that the small size of nauplii *Artemia* may have hindered their capture by lobster larvae, leading to partial starvation. While it is possible that differences in nutritional composition between the two prey types may have influenced survival, previous research on spiny lobster larvae, *Jasus edwardsii*, has demonstrated that larger *Artemia* sizes are more suitable, regardless of their nutritional composition (Ritar et al., 2003), which aligns with our findings.

Our results emphasize the importance of prey size in the culture of *H. gammarus* larvae, with prey sizes greater than 4mm being more suitable than those smaller than 0.5mm. This information can be useful in developing artificial diets for this species, as well as optimizing feeding protocols for larvae in aquaculture settings.

References

Cobb, J. S., & Castro, K. M. (2006). Homarus species. In B. F. Phillips (Ed.), *Lobsters: Biology, Management, Aquaculture and Fisheries* (pp. 310–338). Blackwell Publishing Ltd.

Conklin, D. E. (1995). Digestive Physiology and Nutrition. In J. R. Factor (Ed.), *Biology of the Lobster Homarus americanus* (pp. 441–463). Academic Press. https://doi.org/10.1016/b978-012247570-2/50038-4

Hinchcliffe, J., Agnalt, A.-L., Daniels, C. L., Drengstig, A., Lund, I., Mcminn, J., & Powell, A. (2021). *European lobster Homarus gammarus aquaculture : Technical developments , opportunities and requirements*. *September*, 1–19. https://doi.org/10.1111/raq.12634

Nicosia, F., & Lavalli, K. (1999). Homarid lobster hatcheries: Their history and role in research, management, and aquaculture. *Marine Fisheries Review*, *61*(2), 1–57.

Ritar, A. J., Smith, G. G., Dunstan, G. A., Brown, M. R., & Hart, P. R. (2003). Artemia prey size and mode of presentation: Effects on the survival and growth of phyllosoma larvae of southern rock lobster (Jasus edwardsii). *Aquaculture International*, *11*, 163–182.

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