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Sea Urchin transportation systems for land, sea and air

(Activity A6.4.1 of the NPA URCHIN project)

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Report

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Summary/recommendation:

This report gives a brief introduction to the URCHIN project, funded by the Northern Peripheries and Arctic Programme (NPA) including the aims of this study. The authors recommend that the maximum period for transporting live sea urchins for use in subsequent roe enhancement trial, using the dry techniques described in this report would be between 14 and 44 hrs (depending on temperature). The results from the immersion transport trials show that transporting sea urchins in chilled, aerated seawater systems will be suitable for sea urchins for periods of at least 22 days. If ammonia-stripping (denitrifying) capacity is included into the transport system, then transportation times may be significantly longer. The average sea urchin unionized ammonia (UIA) production in chilled (6.6°C) and aerated seawater holding systems is 1.34625^{-5} kg urchins/day/litre. All sea urchins held in novel spray holding systems died within 4-5 days. Although spray-holding systems are effective for live holding of various species of shellfish the results of these short trials indicate that they are not suitable for live holding and transportation of sea urchins. If sea urchins are delivered alive and ready for processing or immediate consumption, then the airfreight packing techniques described in this report is sufficient for at least up to 44hrs transport. If sea urchins are required for storage in a holding system and remain alive for an extended period, then the techniques used in this trial are effective up to 34hrs transport.

Summary/recommendation in Norwegian:

Denne rapporten gir en kort innføring i URCHIN-prosjektet finansiert av *Northern Peripheries and Arctic Programme* (NPA), inkludert målene med studien. Forfatterne anbefaler at ved «tørr» transport av levende kråkeboller, som senere skal holdes levende for oppfôring, må ikke transporttiden være lengre enn mellom 14 og 44 timer avhengig av temperatur. Teknikker for tørr transport er beskrevet i rapporten. Resultatene fra forsøk med transport av kråkeboller i vann viser at når kråkeboller transporteres i nedkjølt godt luftet vann kan transporttiden være opp til 22 døgn. Hvis en i tillegg til lufting bruker filtersystemer som fjerner ammoniakk kan transporttidene være betydelig lengre. Gjennomsnittlig produksjon av UIA (uionisert ammonium) er 1.34625^{-5} kg kråkeboller/døgn/liter i nedkjølt (6.6°C) sjøvann. Alle kråkebollene som ble holdt i et eksperimentelt system med overrisling døde innen 4-5 døgn. Selv om overrislingssystemer fungerer bra for hold av diverse skalldyr viser forsøkene at de ikke egner seg til levendetransport og lagring av kråkeboller. Hvis kråkeboller skal leveres levende til marked for snarlig konsum eller prosessering er metodene for pakking for lufttransport, beskrevet i denne rapporten, tilstrekkelig for inntil 44 timers transporttid. Hvis kråkebollene skal holdes lenge levende i sjøvann etter ankomst til marked er metodene for transport beskrevet i rapporten tilstrekkelige for inntil 34 timers frakttid.

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Executive summary

This report gives a brief introduction to the URCHIN project, funded by the Northern Peripheries and Arctic Programme (NPA) including the aims of this study.

The results of from this report show that for short time periods (between 14 and 44 hrs dependent on temperature) there are simple and effective methods of transporting live sea urchins out of water (outlined in Section 3.1 of this report). Particularly when it is possible to keep the urchins at cool temperatures (1-5°C). Transporting sea urchins for longer periods (> 44 hrs) requires more sophisticated transport techniques and immersion in seawater. There are transport systems available for live seafood species that are also effective for transporting sea urchins. The trials conducted as part of the URCHIN project indicate that simple chilled, aerated seawater systems would be capable of transporting live sea urchins for considerable periods (at least 22 days) without affecting sea urchin mortality. Such systems would enable live sea urchins to be transported from peripheral areas of the NPA to international markets by sea (such as Greenland to Denmark).

The methodology and protocols for air transport are outlined in Section 4.1 of this report. A study conducted as part of the URCHIN project showed that transporting sea urchins by air (using the techniques described in this report) was possible for up to 34 hrs if the sea urchins are to go back into seawater post transport. When transport was extended to 44 hrs the mortality rate increased. However, in terms of getting sea urchins to market for sale or processing then 44 hrs would still be viable as the sea urchins were still alive and in relatively good condition when unpacked after 44 hrs, regardless of the quantity of sea urchins and gel ice.

The ability to transport sea urchins without mortality or loss of quality is an important factor when developing markets in peripheral areas such as the NPA. The methodologies and protocols outlined in this report give sufficient details to transport live sea urchins by air packed in polystyrene boxes (up to 44hrs) and by road by simply covering and protecting them (between 14 and 44hrs depending on temperature). For transport by road or sea for longer periods transport systems that immerse the sea urchins in chilled and aerated seawater are required. The results in this report indicate that live sea urchins can be transported for periods of at least 22 days with chilling and simple aeration. The results also indicated that sea urchins could be transported in immersion systems for longer periods if denitrifying equipment is installed as part of the transport system. Systems are available that are effective at transporting live sea urchins but issues such as back freight of empty containers, logistical requirements such as power supply and other factors also play a role in developing suitable transport systems and these must be taken into account when considering live transport of sea urchins over significant distances for extended periods.

It is also important to note that the transport limits defined in this report are for sea urchins that are not exposed to direct sunlight, excessive heat, wind or air movement of any type (e.g. when travelling on a boat) and excessive cold at any stage of the collection and transport process. If this occurs, then the sea urchins may die immediately or sometime after transport and it will not be clear when the stress event occurred (unless they all die immediately from a major stress event). Care must be taken during every step of collection (e.g. rough handling should be avoided), moving the sea urchins from one form of transport to another (e.g. boat to trailer) and during the transport itself.

1 Introduction

1.1 URCHIN project (Utilisation of the Arctic Sea Urchin Resource)

The URCHIN project aim is to utilise the sea urchin resource present in the northern arctic regions. The challenges of fishing, sustainable and responsible harvesting of stocks, legislation and supply chains for sea urchin products from isolated and environmentally harsh and challenging areas in the Northern and Arctic region need to be addressed and overcome through innovation and national and transnational technology transfer.

This project aims to gather the existing expertise from Norway, Iceland, Ireland and Greenland, together with knowledge from Canada to optimise the fishing of high value sea urchins in Northern and Arctic areas. Furthermore, roe enhancement technology from Norway for roe fattening to increase the value of low value sea urchins once they have been collected in the northern arctic regions will be developed in Greenland and Iceland. The project would also investigate sea ranching to repopulate areas that have been extensively overfished in the past in Ireland. Issues regarding the provision of adequate legislation and fisheries management will be identified and legislative organisations will be provided with the appropriate knowledge to provide sensible and sustainable management of sea urchin fisheries. The project will also estimate market needs for sea urchin roe as well as establishing logistic routes from the NPA to markets and methods of transporting live and processed sea urchins within and between countries.

1.2 Scope of this report

The aim of this report (Activity A6.4.1 of the URCHIN project: see Appendix I) is to outline the methods that are currently used for transporting live sea urchins both worldwide and within the Northern Peripheries and Arctic area (NPA) (see funding program website for details of the program area – http://www.interreg-npa.eu/). Investigate and test new and novel techniques for transporting urchins by road and/or sea to national and international markets. To enable the utilization of techniques for opening up new sea urchin fisheries such as Greenland and developing the fisheries in countries such as Norway.

1.3 Sea urchins species

All trials described in this report have used the green sea urchin *Strongylocentrotus droebachiensis.* The results are based on this species and additional testing would be required to test whether the same parameters/results applied to other sea urchin species.

2 Summary of transportation techniques used for sea urchin and other shellfish around the world

There is a paucity of published information regarding transportation techniques and protocols for sea urchins. This is surprising given that there are an estimated 70,000 tons of sea urchins consumed per annum in Japan alone and a high percentage of these are imported from other countries, often as whole, live sea urchins (Stefánsson *et al*., 2017). Within the NPA there are up to 300t of sea urchins exported from Iceland annually by air to European markets and yet there is no written information available on the transport techniques used within the NPA.

Sea urchins are relatively robust animals and if treated correctly are able to spend significant periods out of water without suffering from high levels of mortality or loss of quality, even after re-immersion in seawater. However, the lack of a detailed description for handling and transport techniques means that it is unclear what the 'correct' method of handling and transport is, or what the limits of existing transport techniques are. This report attempts to describe what is known about live transport of sea urchins and to provide a summary of transport techniques for live sea urchins by air, road and sea.

It is important to define the meaning of 'live' sea urchins. It is possible that a sea urchin exposed to adverse conditions will suffer delayed mortality for up to two weeks. Sea urchins transported by air could be alive on delivery at a processing facility but may be unlikely to survive re-immersion in seawater (e.g. for subsequent roe enhancement). In this report, we consider a 'live' sea urchin to be in good condition and to be able to survive (at least two weeks) being re-immersed in seawater.

2.1 Worldwide experience

The only references the authors found regarding live transport of any shellfish was an EU funded project called 'MusselsAlive' which ran between 2010 and 2013. This report summarises the best practices for handling and transportation of live mussels. These techniques are relatively simple and consisted of holding the mussels in mesh bags on ice (Barrento *et al*., 2013). The results are not relevant for sea urchins as mussels are capable of closing the shell and surviving for relatively long periods out of water. Sea urchins cannot be exposed to air in the same manner as mussels as they require a sealed, moist atmosphere (Dale, *et al*., 2005).

The only published study on related to handling and transport of sea urchins investigated the effects of rough handling during transport on subsequent roe enhancement (Dale, *et al.*, 2005). The conclusions from this study were that rough handling and air exposure had a significant impact on mortality during subsequent roe enhancement of the sea urchins.

In general, most sea urchin fisheries rely on transportation methods that minimize exposure to excess high temperatures, direct sunlight, wind and prolonged air exposure. This is generally avoided by keeping sea urchins in cool, well-oxygenated running seawater, or if removed from water transporting them in a completely covered and protected environment. Preferably with a moist lining to maintain a moist atmosphere during transport and some form of chilling. For air travel, the transport technique consists of packing sea urchins in sealed polystyrene boxes with gel ice to keep the temperature low. For road transport, this can vary but a simple and effective technique is covering the sea urchins with moist felt and tarpaulins to create a moist, protected environment. The latter is considered an effective method for transporting sea urchins for periods of at least 12 hrs (*per obs*.) (Currently the limits of this type of transport are unknown). Methods that are more complex are also available and include full immersion systems that circulate water and re-oxygenate the system (covered in Section 3.2).

3 Road/Sea transport trials conducted as part of the NPA URCHIN Project

3.1 Nofima road transport protocols (short distance/time)

The standard Nofima sea urchin transport protocols were used to transport sea urchins by road in the roe enhancement trial run in 2016 as part of the URCHIN Project. This technique is described in the Nofima Report, see James *et al.* (2017), and consists of the following:

Sea urchins were collected in catch bags and held in mesh bags in the sea until they were ready to be packed and transported. The mesh bags were then placed in the back of an enclosed pick-up vehicle (the compartment was enclosed so there was no effect from wind etc.). They were covered with wet (soaked in seawater) hessian sacks and a tarpaulin to ensure a dark, moist cool environment. They were then transported for approximately 3hrs. On arrival at the roe enhancement site, the urchins were transferred to a small boat, covered with the sacks and tarpaulin, and delivered to the sea-based holding facility.

This methodology for short-term road transport is well established but the maximum limits for this type of transport have not been measured or published.

3.2 Nofima dry transport trial (limits to road transport trial)

A trial was conducted by Nofima to test what the limits of survival are for sea urchins collected and transported out of water. This is applicable to the transport of large quantities (tons) of sea urchins for roe enhancement. The results primarily focus on survival of the sea urchins and we did not investigate the impact of roe quality.

3.2.1 Methodology (Trials 1 and 2)

Green sea urchins were collected from a site close to Tromsø and transported to a live holding system at Nofima, Tromsø. They were held in this system until the beginning of the experiment (approximately 6 hrs) when they were randomly allocated to one of two temperature regimes (ambient and 4°C up to 28 hrs in Trial 1) (5°C and 3°C up to 56 hrs in Trial 2). They were held in a covered, moist environment. This consisted of an insulated plastic container. Inside the container the urchins were covered with hessian sacks that had been soaked in seawater. The container was covered with a loose fitting lid and a tarpaulin to avoid any air movement (Figure 1). This method is suitable for larger quantities of sea urchins to be transported in vehicles, trailers and/or containers by road.

Trial 1: In Trial 1 the sea urchins remained in these conditions (at two temperatures) for 12 hrs, after which samples (ambient n = 20; 4°C n = 20) were removed every second hour (up until 28 hrs post removal from water) and returned to the seawater holding systems (Figure 2). The sea urchins were then held for a further two weeks in the seawater holding system to check for post transport mortality.

Trial 2: In Trial 2 the sea urchins remained in these conditions for 24 hrs (at two temperatures), after which samples (5°C n = 20; 3°C n = 20) were removed every fourth hour (up until 56 hrs post removal from water) and returned to the seawater holding systems (Figure 2). The sea urchins were then held for a further two weeks in the seawater holding system to check for post transport mortality.

Figure 1 *The transport containers used to simulate transporting live sea urchins by road. Clockwise from top left: the insulated container; batches of 20 urchins were collected in mesh sacks; and placed in container; the sacks were covered with hessian sacks soaked in seawater; a loose fitting lid was placed onto the container; the entire container was covered with a tarpaulin.*

Figure 2 *The holding systems used when the sea urchins went back into seawater.*

3.2.2 Results

The average temperature in the dry holding systems during the trials are shown in Figure 3A and B. The survival results for the dry transport trial are shown in Table 1.

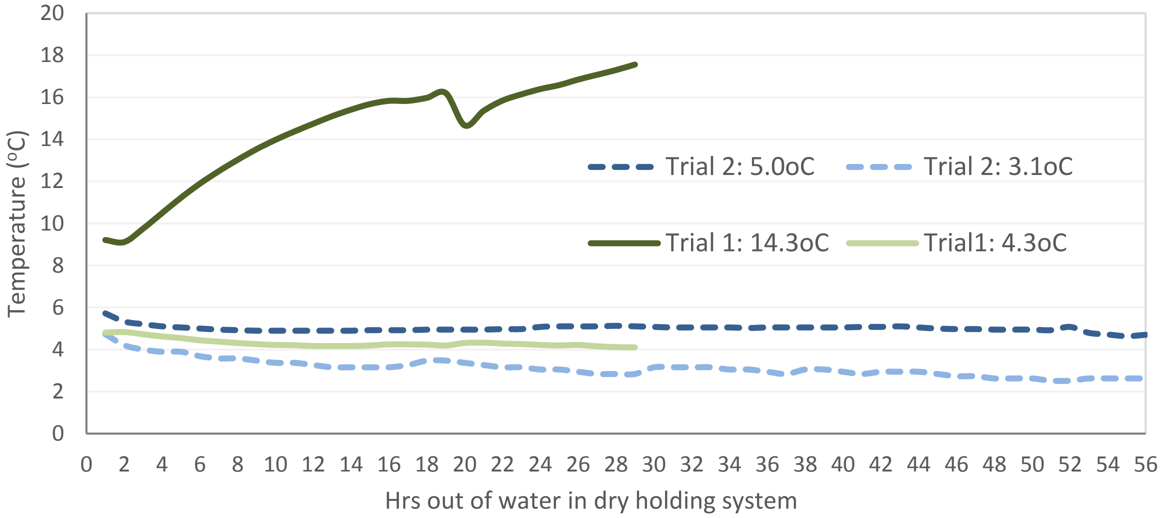

Figure 3 The temperature in the two treatments during: Trial 1) the 28 hour dry holding period at ambient (average 14.3°C) and 4°C and; Trial 2) the 56 hour dry holding period at 5°C and 3°C.

Table 1 Temperature and survival results for dry transport trial. Results in dark box and white text indicate they are blow the survival threshold of 90 %.

Trial 1							
Hrs out of water	**Ambient Ave. Temp**	**Cool Ave. Temp**	**Survival at re-immersion in seawater (%)**	**Survival 7 days post re-immersion (%)**		**Survival 14 days post re-immersion (%)**	
				Ambient	**4°C**	**Ambient**	**4°C**
12	15.1	4.2	100	100	100	95	100
14	15.6	4.2	100	100	100	95	100
16	15.8	4.2	100	100	100	**70**	95
18	16.2	4.2	100	100	100	**90**	95
20	15.3	4.3	100	95	100	**85**	95
22	16.1	4.3	100	**75**	100	**50**	95
24	16.6	4.2	100	**65**	100	**40**	100
26	17.1	4.2	100	**70**	100	**10**	95
28	17.6	4.1	100	**5**	100	**0**	100
Trial 2							
Hrs out of water	**5°C Ave. Temp**	**3°C Ave. Temp**	**Survival at re-immersion in seawater (%)**	**Survival 7 days post re-immersion (%)**		**Survival 14 days post re-immersion (%)**	
				5°C	**3°C**	**5°C**	**3°C**
24	5.1	3.1	100	95	100	95	100
28	5.1	2.8	100	95	95	95	90
32	5.1	3.2	100	95	100	90	100
36	5.0	2.9	100	95	90	90	90
40	5.1	2.9	100	95	95	**85**	90
44	5.0	2.9	100	90	100	**85**	100
48	4.9	2.6	100	100	**85**	**90**	**85**
52	5.1	2.5	100	**80**	**95**	**70**	**90**
56	4.7	2.6	100	**85**	**95**	**70**	**85**

3.2.3 Discussion

We have used a 90 % survival (two urchin in 20 died) after a two weeks post-harvest period in a seawater holding system as the survival threshold for subsequent roe enhancement trials. The survival results from Trial 1 show that the capacity for sea urchins to survive out of water transport is temperature dependent. When held at ambient temperatures (in this experiment this varied from 9.1°C to 17.5°C with an average of 14.3°C) sea urchins mortality passed the threshold after being exposed to 14 hrs out of water (14 day mortality results) and the mortality rate increased rapidly for exposure periods longer than this. In contrast, sea urchins held at 4°C did not pass the threshold in the experimental period of 28 hrs.

The survival results from Trial 2 show that the capacity for sea urchins to survive out of water transport is temperature dependent even at relatively low temperatures. When held at 5.0°C sea urchins mortality passed the threshold after being exposed to 36 hrs out of water (14 day mortality results) and the mortality rate increased gradually for exposure periods longer than this. In contrast, sea urchins held at 3°C did not pass the threshold until held out of water for 44 hrs.

Although we did not investigate the impact of transport on sea urchin roe quality, there were multiple observations of sea urchins spawning during the air exposure period in both trials (Figure 4) and this would certainly have had an impact on roe quantity and quality. Spawning events resulting from stress events such as dry transportation is well documented for sea urchins and the reproductive cycle of the sea urchins should obviously be taken into account when using this transport technique.

Figure 4 Sea urchins spawning spontaneously during simulated transport out of water (left is a female and right is a male sea urchin).

3.2.4 Conclusion

The maximum period for this transport technique is temperature dependent. The authors recommend (based on a 95 % survival rate threshold) that to transport live sea urchins for subsequent roe enhancement trials the maximum transport period for an average transport temperature of 14.3°C is 14 hrs, at 5.0°C is 36 hrs, and at 3.0°C is 44 hrs.

3.3 Long distance (time) road/sea transport

3.3.1 General introduction

The road transport technique described in Section 3.2 is temperature dependent and varies between 14-44 hrs. If the transport period is longer then it becomes necessary to transport sea urchins immersed in cool, seawater. This becomes important for countries such as Greenland where the transport and labour costs are expensive, air transport is not feasible and long distance shipping by sea becomes the most likely scenario for building a sea urchin industry. There are systems designed to carry live seafood in chilled shipping containers and although they are not specifically designed for sea urchins they may also be suitable for transporting sea urchins for long distances and over extended periods. As part of the URCHIN project, trials were conducted testing one of these systems (Aqualife system) (see Appendix II for trials conducted in Greenland).

Description of the Aqualife system (Norbury, 2013): This live seafood transporting system was initially designed to transport live shellfish, lobsters and crabs in specially designed tanks inside 40-foot chilled containers (reefers). The Denmark-based Aqualife Inc. and its partner Maersk Lines started this in 2010 as a joint venture. A significant investment was made in the technology and aquaports in Nova Scotia and the Netherlands, where shellfish would be loaded and unloaded. Development of the system has been delayed since 2010 for various reasons but it is now in the process of further development and expansion into transportation of other species. Animals transported in the system are placed in tanks, complete with oxygenation and filtration systems, that fit 20 at a time into a 40-foot container (Figure 5). Each tank can hold up 300 kilograms of seafood (NOTE: this would be species specific and has not been tested for sea urchins), for a total capacity of 6,000 kilograms per container. From early 2011 to early 2012, Aqualife shipped about 35 to 40 containers across the Atlantic proving that the technology worked for specific species with minimal density dependent transport mortality.

Figure 5 The 'Aqualife' container systems designed to fit into especially designed chilled reefers for transport.

3.4 Nofima two day road transport trial using Aqualife system

In 2017 Nofima (in conjunction with an industry partner) ran a short trial transporting sea urchins in the Aqualife system from the north of Norway (Trollbukt) to the South (Oslo) by road transport in a chilled truck. A total of 25.8kg amount of sea urchins were harvested locally and stored at Trollbukt for approximately one week prior to transport in running, ambient, flow through seawater. The sea urchins were then packed in a single 'Aqualife' live transport container and transported from Trollbukt to Oslo (28 hrs) (Figure 6). When the transport container was opened in Oslo there was no mortality observed and all urchins appeared to be in excellent condition. Seawater samples were collected from the transport system when they arrived in Oslo and a sub-sample of 30 urchins were transported to Tromsø by plane (5 ½ hrs) in a Polystyrene box with chiller packs. These were then stored in recirculating system at Nofima, Tromsø (at 6°C) for two weeks to test for post-transport mortality. The sea urchins had an average gonad index (GI) of 10.1 % (Figure 7) and an average test diameter of 45.1mm. After 2 weeks in the recirculation system there was 100 % survival (one urchin had lost some spines from predation but was alive).

3.4.1 Results

The results from the seawater samples collected during the trial are presented in Table 2 below. The water samples were as follows:

1. Seawater sample collected at time of initial packing in Trollbukt.
2. Seawater sample collected from the 'Aqualife' container holding only seawater (no sea urchins) at time of unpacking in Oslo.
3. Seawater sample collected from the 'Aqualife' container holding sea urchins at time of unpacking in Oslo.

Table 2 The results of the water quality testing during and after the trial.

Sampled on 3/11/2017	Salinity ($^{o}/_{oo}$)	Oxygen (% DO)	pH	Temp (°C)	Ammonia (NH_4-N)	Nitrate (NO_3-N)
Initial packing sample	33.8		7,81		< 0.5mg/l	< 10mg/l
Unpacking sample (urchins)	33.4	99	7.74	3.9	0.6 mg/l	< 10mg/l
Unpacking sample (seawater)	33.4		7.949	3.9	< 0.5mg/	< 10mg/l

***NB:** Light grey shading indicates the reading was below the limits of the test, dark grey indicates the parameter was not measured.*

3.4.2 Discussion

The trial results showed that sea urchins (held at relatively low densities) were able to survive transport immersed in seawater in an aerated system for periods at least up to 28 hrs (plus air transport 6hrs) and arrive at their destination in very good condition and with no mortality. Further testing was required to determine whether a cooled and aerated holding system such as the 'Aqualife' system would be sufficient for higher densities and for longer transport periods. In addition, the ammonia levels in the holding system will increase significantly when transporting either higher densities of urchins or similar densities for longer periods and this also needed to be tested to determine the limits of transport with cooled, aerated seawater (without any denitrifying equipment).

Figure 6 *Urchins being transported in the 'Aqualife' transport system; clockwise from top left: sea urchins in baskets; baskets being loaded into Aqualife container; Aqualife container being loaded into refrigerated truck; sea urchins being unloaded after transport; sea urchins in excellent condition after transport.*

Figure 7 *The gonad quality of the best urchins opened during sampling at arrival in Oslo.*

3.4.3 Conclusions

The Aqualife system is a suitable system for live transport of sea urchins for periods of at least 28 hrs and most likely for significantly longer periods. Further testing is required to measure the limits of this type of transport system (cool, aerated seawater without any denitrifying equipment).

3.5 Nofima long term transport trial (22 days)

The Aqualife system trial showed that a container of seawater that circulates and re oxygenates the water within the system is suitable for live transport of sea urchins for periods of at least 28 hrs and most likely significantly longer. Siikavuopio *et al.* (2004) published a paper describing the effects of chronic ammonia exposure on gonad growth and survival of the sea urchin *S. droebachiensis* and concluded that both mortality and gonad growth were significantly effected in sea urchins exposed to long-term levels (42 days) of unionised ammonia above 0.016mg l^{-1}. There is no information on what the maximum ammonia levels should be for live sea urchins held in live transport systems. In order to investigate what the limits of a static, aerated sea urchin live transport system would be Nofima ran a trial testing various biomass of sea urchins to seawater ratios and the resulting increases in the levels of ammonia in the system over time.

3.5.1 Methodology

A series of nine tanks were used. Each tank held 100l of seawater and was aerated with 10cm of 'leaky hose' connected to an air blower. A ventilated plastic crate (60 x 40 x 20mm) was placed in each tank. The crate sat on two upstands that raised it 4cm from the bottom of the tank, allowing water circulation around the crate (Figure 8). The nine tanks were randomly allocated one of three density treatments: 1) 50 urchins/crate, 2) 100 urchins/crate and 3) 150 urchins/crate (Figure 9).

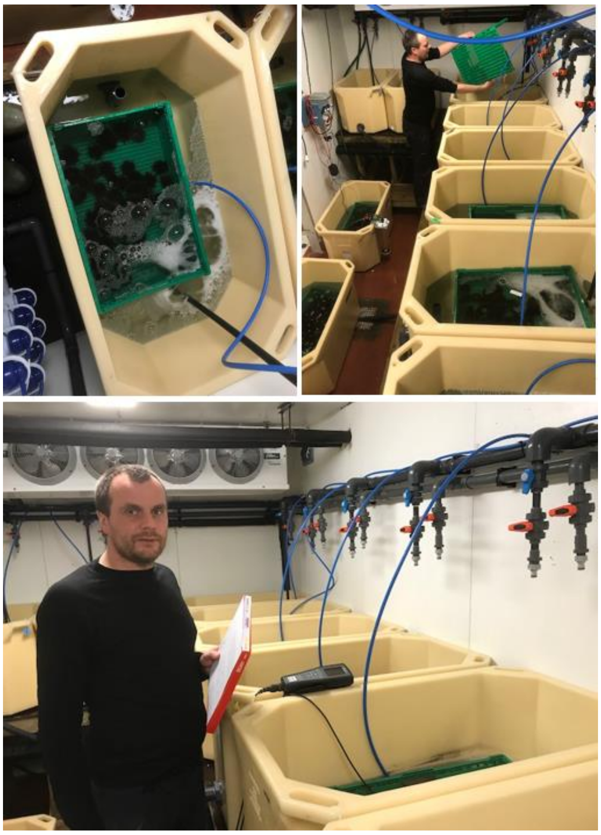

Figure 8 Clockwise from top left: 100l tanks with plastic crates and airlines; setting up experimental tanks; doing insitu seawater dissolved oxygen and temperature measurements.

Figure 9 The three density treatments (from left, 150, 100 and 50 sea urchins/basket).

Sea urchins were collected on 19 March 2018 from a site 20 minutes drive from the Nofima facility (average test diameter = 42.8mm). They were collected and transferred to the experimental tanks within 2 hrs of collection. Every day the dissolved oxygen and temperature was measured in each tank using a handheld WTW dissolved oxygen meter. Every second day pH and salinity were measured using a WTW Multi meter (3630 IDS) and unionised ammonia was measured using analysis kits and a WTW portable colorimeter pHotoFlex STD. The condition of the urchins was monitored throughout and any dead urchins removed. After 22 days a sub-sample of urchins from each crate (*n* = 20) were randomly selected and transferred to sea-based holding systems where they were held for 2 weeks to monitor post transport survival and condition.

3.5.2 Results

The survival of the sea urchins after 22 days in the experimental holding systems, and subsequently 14 days in a sea-based holding system in the various density treatments are shown in Table 3. The water quality parameters in the holding tanks throughout the 22 day experimental period are shown in Table 4. The average accumulated total Unionised Ammonia (UIAUIA) readings (mg/l^{-1}) recorded in the three density treatments over the experimental period (22 days) are shown in Figure 10. The average daily total UIA readings (mg/l^{-1}) production recorded in the three density treatments over the experimental period are shown in Figure 11.

Table 3 The survival of the sea urchins after 22 days in the experimental holding systems and subsequently 14 days in a sea-based holding system to test for post transport mortality.

Density treatment	**Survival after 22 days in transport system (% survival ± SE)**	**Survival after 14 days in sea-based holding system (post transport system) (% survival ± SE)**
150	98.3 (± 1.6)	98.3 (± 1.6)
100	98.3 (± 1.6)	98.3 (± 1.6)
50	100 (± 0.0)	100 (± 0.0)

Table 4 Water quality in the three density treatments throughout the 22 day trial period.

Day	150/basket				100/basket				50/basket			
	Temp.	**O^2 (%)**	**pH**	**Sal.**	**Temp.**	**O^2 (%)**	**pH**	**Sal.**	**Temp.**	**O^2 (%)**	**pH**	**Sal.**
0	5.1 ±0.17	99.1 ±0.71	7.89 ±0.02	34.5 ±0.17	5.1 ±0.17	99.1 ±0.71	7.89 ±0.02	34.5 ±0.17	5.1 ±0.17	99.1 ±0.71	7.89 ±0.02	34.5 ±0.17
2	6.8 ±0.43	99.4 ±0.71	7.79 ±0.03	34.3 ±0.24	5.2 ±0.13	102.7 ±0.56	7.90 <0.01	34.8 ±0.03	4.8 ±0.06	102.4 ±0.10	7.95 <0.01	34.9 ±0.03
4	5.1 ±0.06	102.7 ±0.21	7.87 ±0.02	34.4 ±0.15	4.8 ±0.05	102.9 ±0.05	7.89 ±0.02	34.7 ±0.10	4.6 ±0.03	103.1 ±0.10	7.93 ±0.02	34.9 ±0.05
6	5.1 ±0.06	102.2 ±0.12	7.86 ±0.02	34.5 ±0.15	4.8 ±0.33	102.7 ±0.05	7.91 <0.01	34.7 ±0.11	4.7 <0.01	103.2 ±0.06	7.97 <0.01	34.9 ±0.05
8	5.1 ±0.06	103.5 ±0.12	7.93 ±0.01	34.6 ±0.23	4.9 ±0.06	104.3 ±0.03	7.92 <0.01	34.7 ±0.17	4.8 <0.01	104.6 ±0.15	7.96 <0.01	35.0 ±0.05
10	5.1 ±0.33	99.1 ±0.88	7.93 ±0.01	34.8 ±0.12	4.9 ±0.06	99.2 ±0.03	7.91 <0.01	34.7 ±0.06	4.7 <0.01	99.6 ±0.17	7.95 <0.01	35.2 ±0.03
12	4.8 ±0.33	98.8 ±0.88	7.96 ±0.01	35.0 ±0.12	4.6 ±0.06	98.9 ±0.03	7.94 <0.01	35.0 ±0.06	4.5 <0.01	99.3 ±0.03	7.96 <0.01	35.2 ±0.08
14	4.3 ±0.03	98.7 ±0.11	7.98 ±0.01	35.1 ±0.16	4.2 ±0.05	99.1 ±0.05	7.94 <0.01	35.1 ±0.05	4.0 <0.01	99.5 ±0.18	7.98 <0.01	35.4 ±0.03
16	4.5 ±0.03	99.4 ±0.15	7.97 ±0.02	35.1 ±0.28	4.3 ±0.05	99.5 ±0.03	7.94 <0.01	35.1 ±0.08	4.2 <0.01	100.0 ±0.08	7.96 <0.01	35.5 ±0.03
18	4.8 ±0.03	99.0 ±0.12	8.02 ±0.01	34.9 ±0.27	4.6 ±0.03	99.1 <0.01	7.97 <0.01	35.1 ±0.05	4.6 <0.01	99.5 ±0.15	8.00 <0.01	35.5 ±0.03
20	5.1 <0.01	100.1 ±0.18	7.98 ±0.01	35.3 ±0.15	4.9 ±0.05	100.3 <0.03	7.95 <0.01	35.2 ±0.12	4.7 <0.01	100.3 ±0.12	7.97 <0.01	35.6 ±0.14
22	4.6 <0.03	100.2 ±0.03	7.98 ±0.02	35.3 ±0.33	4.4 ±0.06	100.4 <0.06	7.95 <0.01	35.5 ±0.08	4.2 <0.01	101.1 ±0.39	7.97 <0.01	35.6 ±0.03

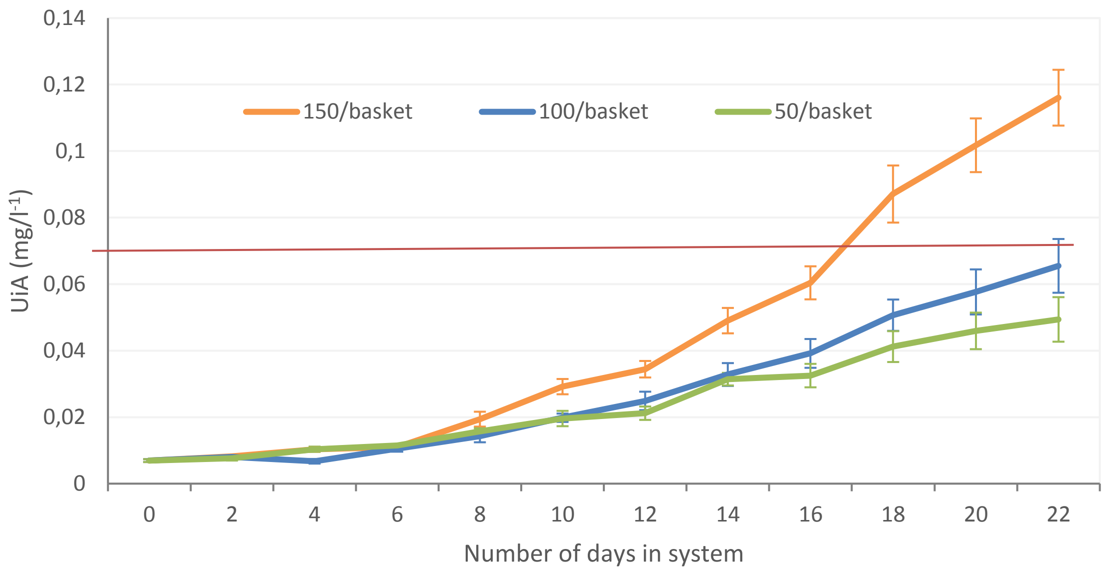

Figure 10 *The average accumulated total unionized Ammonia (UIA) readings (mg/l^{-1}) recorded in the three density treatments over the experimental period (22 days). The red line indicates the maximum recommended UIA for long-term holding from Siikavuopio et al., (2004).*

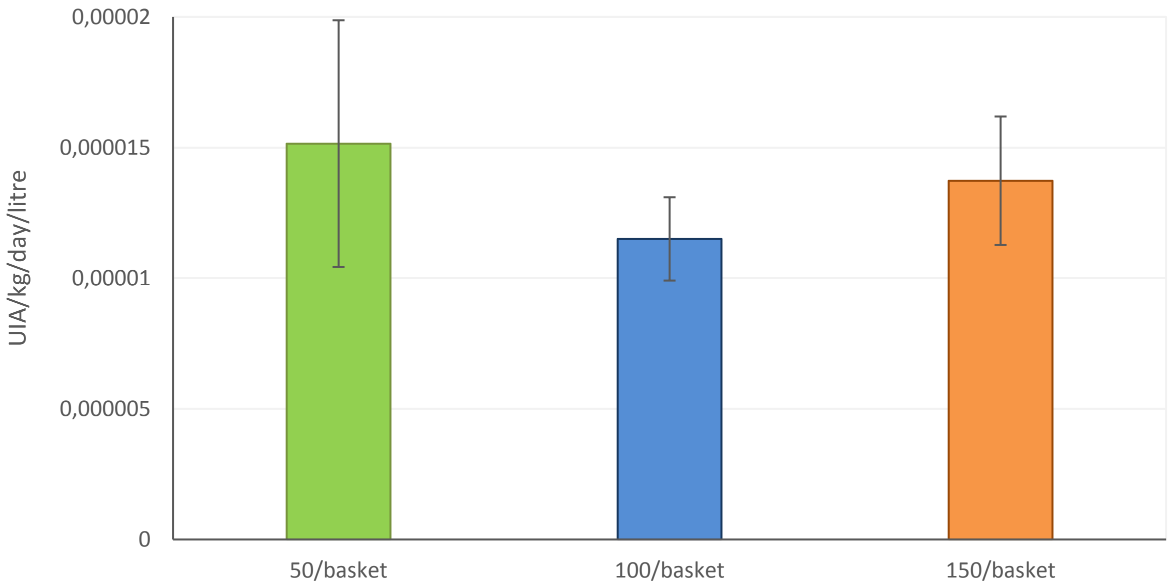

Figure 11 *The average daily total unionized Ammonia readings (mg/l^{-1}) production recorded in the three density treatments over the experimental period (22 days).*

3.5.3 Discussion

There were minor variations in temperature between the experimental treatments, but these were too small to have any significant impact on the mortality results. Similarly, there were little differences in the dissolved oxygen levels in the three experimental treatments. The aeration provided in the experimental tanks was sufficient that the minimum dissolved oxygen recorded in any of the tanks was 97.8 % during the experimental period.

There was a gradual buildup of Ammonia in the experimental system over time. However, the levels of UIA only exceeded long-term recommendations (from Siikavuopio *et al.,* 2004) in the high-density treatment after 16 days and did not pass threshold in low or medium density treatments. It should be noted that this is only a recommended threshold for long-term holding and there would most likely be no impact in the limited period (22 days) that the experiment was conducted from the UIA levels recorded during the trial. There were no differences between daily average production of UIA between density treatments. Average overall (all treatments combined) daily production of UIA was 1.34625^{-5} kg urchins/day/litre of seawater. This can be used to calculate the UIA accumulation in live transport systems if there is no denitrifying (ammonia removing) equipment included in the system. If there is denitrifying equipment this figure can be used to calculate the quantity of ammonia required to be removed by any given amount of sea urchins. This production figure will be temperature dependent and if transport temperatures vary significantly from those used in this trial (average seawater temperature during the trial was 6.6^{o}C) this would need to be taken into consideration.

The survival results indicate that this type of transport method will be suitable for sea urchins for periods at least up to 22 days and probably longer. If ammonia-stripping (denitrifying) capacity is included into the transport system, then transportations times may be significantly longer still. In the case of the NPA this would allow for transportation from Greenland to European markets by sea in relatively simple transport systems. Although the sea urchins appeared in very good condition at the conclusion of the experimental period (Figure 12), this study did not investigate roe quality and this would also need to be considered when shipping sea urchins direct to market using these techniques.

Figure 12 The sea urchins removed from the high-density treatment after 21 days were in good condition (spines erect and moving).

3.5.4 Conclusions

The results from the trial show that transporting sea urchins in chilled, aerated seawater systems will be suitable for sea urchins for periods at least up to 22 days. If ammonia-stripping (denitrifying) capacity is included into the transport system, then transportations times may be significantly longer.

Average sea urchin UIA production in chilled and aerated holding systems

= $1,34625^{-5}$ kg urchins/day/litre (at 6.6°C seawater temperature)

3.6 Nofima spray / immersion (air) / Recirculation holding system trial

In addition to the long-term transport trials in Section 3.5, Nofima investigated the potential of transporting sea urchins in limited water systems with a spray mechanism to create a 100 % saturated atmosphere within the transport container. This would require transporting significantly less quantities and weight of seawater than for full immersion systems.

3.6.1 Methodology

Trial 1:

Sea urchins collected from a site 20 minutes' drive from the Nofima facility were held in sea crates for 2 days to purge before transportation to Nofima. On arrival they were placed in six tanks (270l) connected to a recirculation system. Ten urchins were placed in each tank and these were randomly allocated to two holding treatments (three replicates of each):

1. Full immersion in in recirculating seawater.
2. Sea urchins held in trays and exposed to gentle seawater spray (urchins facing mouth upwards) (Figure 13).

The test was run for 5 days. After 4 days the sea urchins held in the spray treatment started to lose skin and looked dead (see Figure 14). After 6 days all sea urchins were transferred to sea for survival test. After 2 weeks the results were 100 % mortality in the spray treatment and 100 % survival in the immersion treatment.

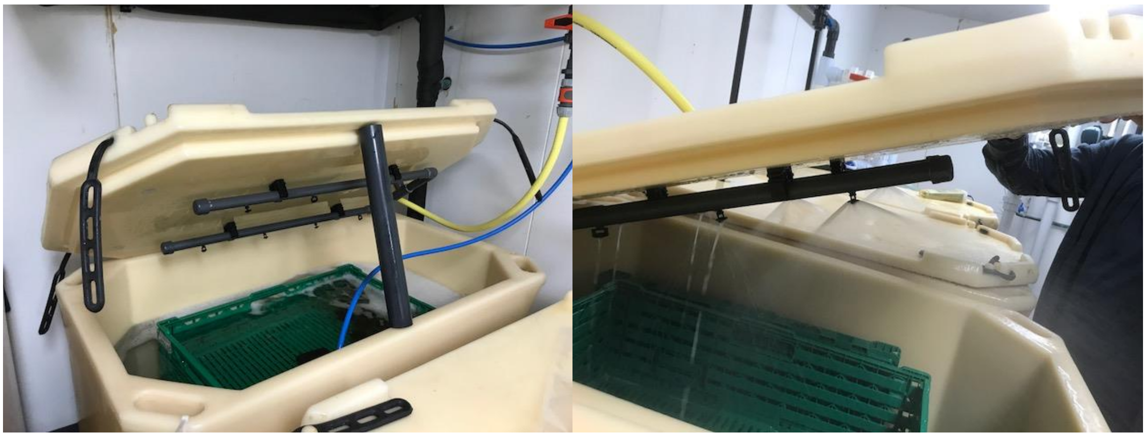

Figure 13 The spray system used to test the effectiveness of a spray-based holding system for live sea urchins.

Trial 2:

Sea urchins collected from a site 20 minutes' drive from the Nofima facility were transported to Nofima and placed in six tanks (270l) connected to a recirculation system. Thirty urchins were placed in each tank and these were randomly allocated to three holding treatments (two replicates of each):

1. Full immersion in recirculating seawater.
2. Sea urchins held in trays and exposed to gentle seawater spray (urchins facing mouth upwards).
3. Sea urchins held in trays and exposed to gentle seawater spray (urchins facing mouth downwards).

The sea urchins were held in the system for four days. After two days both the spray with mouth up and mouth down looked in poor condition. After 4 days both mouth up and mouth down spray treatments looked dead (Figure 14). All the urchins were then transferred to sea for survival test. After 2 weeks the results were 100 % mortality in the spray treatment and 100 % survival in the immersion treatment.

Figure 14 Sea urchins exposed to the fine spray treatment (left) compared to sea urchin held in full immersion in a recirculation seawater system (right).

3.6.2 Conclusion

All sea urchins held in the spray system (regardless of orientation) died within 4-5 days. It appeared as though the seawater spray removed the skin of the sea urchins even though it was a very fine misted spray. Although spray-holding systems are effective for live holding various species of shellfish the results of these short trials, indicate that they are not suitable for live holding and transportation of sea urchins.

4 Air transport of live sea urchins

4.1 Summary of existing techniques

The standard techniques for transporting sea urchins by air consists of packing sea urchins in polystyrene boxes; normally the boxes are filled to capacity (without squeezing the sea urchins into the space). Prior to packing the sea urchins gel ice packs are placed in the bottom of the container (no specific details of the quantity of gel ice are published) to keep the internal temperature cool. The gel packs are covered with an absorbent layer and the sea urchins are packed on top, the lid closed and sealed prior to transport (Figure 15). This is the technique used in most countries around the world (including countries in the NPA) to air freight sea urchins and although it is an effective method of transportation there is little information on the amount of gel ice required and how longer periods live sea urchins can be transported using this technique.

Figure 15 Traditional packing of live sea urchins in polystyrene boxes with gel packs (under the urchins) and absorber liners between the gel packs and urchins.

4.2 Nofima packing and transportation trial

The following describes an experiment undertaken by Nofima as part of the URCHIN project. It is a simulated transport trial for live sea urchins to investigate maximum transport times for live, whole, good quality sea urchins to a processors and overseas markets. The results of the simulation trial were used as a basis for packing and transporting sea urchins in a subsequent sea urchin roe enhancement trial sending live sea urchins to a processor in Japan.

4.2.1 Methodology

Sea urchins were collected and held in the sea in catch bags prior to packing in polystyrene boxes. The sea urchins were randomly allocated to one of six boxes (treatments) that were packed with the following sea urchin quantities and ice gel packs:

Urchin quantity and quantity of ice gel packs:

- 3 replicates of 1.6kg urchins/box, 4 gel packs (2kg)/box (Box 1,2 & 3)
- 3 replicates of 2.4kg urchins/box, 7 gel packs (3.5kg)/box (Box 4, 5 & 6)

The six boxes were then exposed to various transport periods:

- 24 hrs transport (Box 1 & 4)
- 34 hrs transport (Box 2 & 5)
- 44 hrs transport (Box 3 & 6)

During the transport periods, the polystyrene boxes were stored at approximately 10-13^{o}C. After 24, 34 and 44 hrs two boxes from each density treatment were opened and the condition of the sea urchins and any mortalities were recorded. Each box had a temperature logger to record the internal temperature during the trial period. Sea urchins from each box (treatment) were then placed separately (72 urchins / treatment) in plastic baskets in stacks in the sea at Kårvika for 14 days. During this period the sea urchins were fed the Nofima diet and at 7 days and 14 days checked for condition and survival.

4.2.2 Results

The results of the trial showed there was virtually no immediate mortality resulting from the transport periods used in the trial (only one sea urchin died during transport, Table 5). This is despite there being differences in internal temperatures depending on the quantity of urchins and gel ice (Figure 16). All the sea urchins appeared to be in good condition when removed from the transport boxes with only one recorded mortality at this stage of the trial. After seven days storage in the sea-water holding systems there was still very limited mortality ranging from 1.4 % in the 1.6kg packs held for 24 and 34 hrs to 6.9 % in the 2.4kg pack after 44hrs transport. After 14 days storage mortality levels increased and was considerably higher in the 44hr transport treatment, regardless of the amount of sea urchins packed in the box (16.7 % and 20.8 % for sea urchins in the 1.6kg and 2.4kg respectively) (Table 6). There was very little difference in mortality (2.8-5.6 %) in the 24 or 34hr treatments regardless of the amount of sea urchins in the packs (Table 6).

Table 5 Sea urchin mortality and condition, and gel pack condition immediately after transport period.

Box Number	Length of transportation	Appearance at end of transportation	Condition of freezer packs		Mortality at end of transportation (individual urchins)	
			4 freezer packs	7 freezer packs	1.6 kg urchins	2.4 kg urchins
1 & 4	24 hrs	Excellent	½ frozen	2/3 frozen	1	0
2 & 5	34 hrs	Very good	½ frozen	2/3 frozen	0	0
3 & 6	44 hrs	Very good	Defrosted	Slightly frozen	0	0

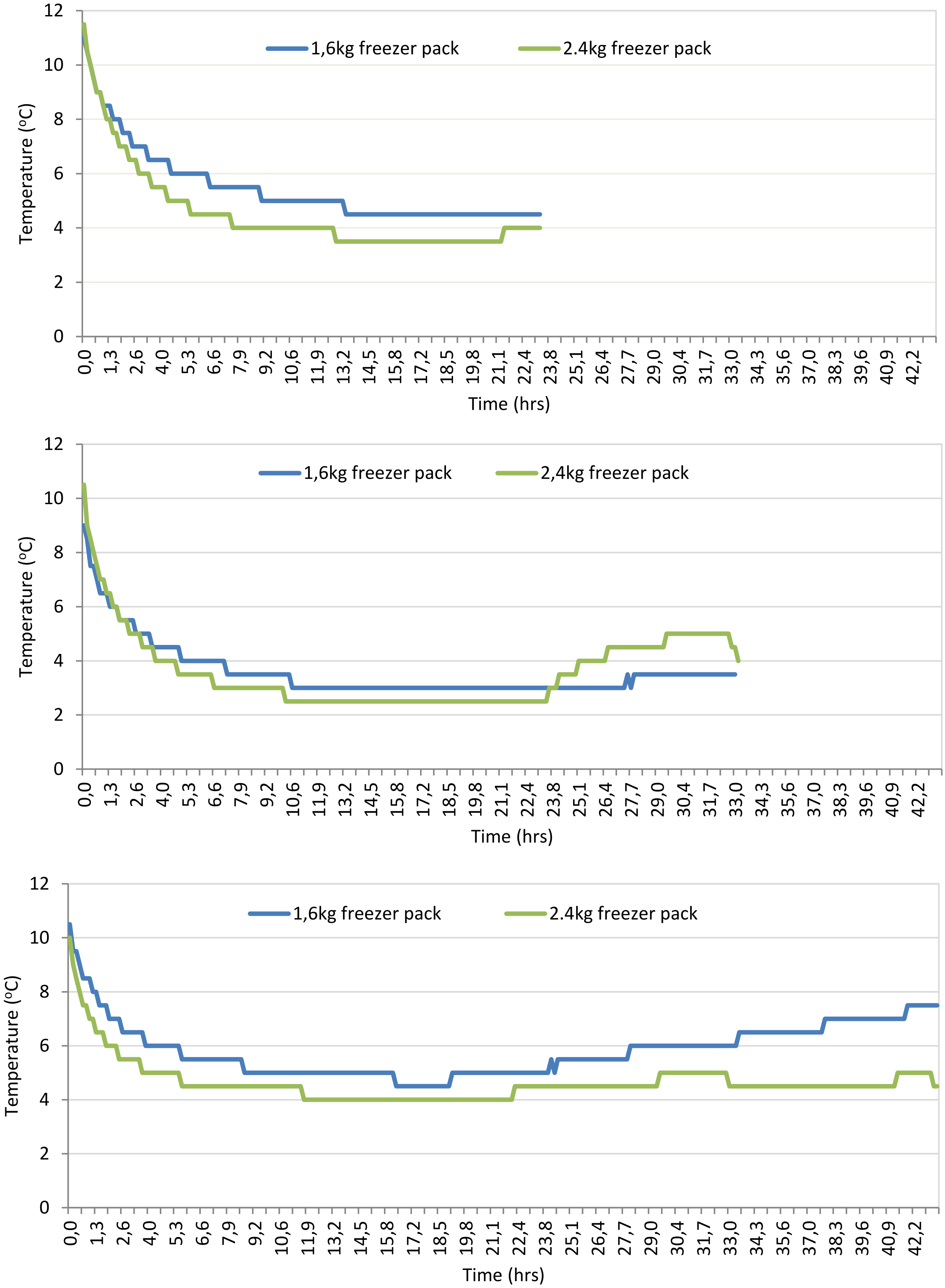

Figure 16 Internal temperatures in the polystyrene boxes during the transport trial.

Table 6 Sea urchin mortality from the six treatments after 7 and 14 days storage in sea-based holding systems.

Treatments				Post transport mortality			
				7 days		14 days	
Box number	**Number (weight) ice packs**	**Weight of urchins**	**Transport period**	**Actual number**	**Percentage of total**	**Actual number**	**Percentage of total**
1	4 (2.0kg)	1.6 kg	24 hour	1	1.4 %	4	5.6 %
2	4 (2.0kg)	1.6 kg	34 hour	1	1.4 %	2	2.8 %
3	4 (2.0kg)	1.6 kg	44 hour	5	6.9 %	12	16.7 %
4	7 (3.5kg)	2.4 kg	24 hour	2	2.8 %	4	5.6 %
5	7 (3.5kg)	2.4 kg	34 hour	4	5.6 %	4	5.6 %
6	7 (3.5kg)	2.4 kg	44 hour	5	6.9 %	15	20.8 %

4.2.3 Discussion

The results of the trial showed that there was a 34 hour window of transport for sea urchins if they are to go back into seawater post transport. When transport was extended to 44 hrs the mortality rate increased. However, in terms of getting sea urchin to market for sale or processing then 44 hrs would still be viable as the sea urchins were still alive and in relatively good condition for consumption or processing when unpacked after 44 hrs, regardless of the quantity of sea urchins and gel ice.

4.2.4 Conclusions

If sea urchins are to be delivered alive and ready for processing or immediate consumption then the packing utilised in the current trial is sufficient for at least up to 44 hrs transport. If sea urchins are required for storage in a holding system and remain alive for an extended period, then the techniques used in this trial appear to be effective up to 34 hrs transport with minimal mortality.

4.3 Nofima commercial test of air transport technique

Following the simulated packing trials 25kg of urchins were packed in 5 polystyrene boxes using the same technique described in the simulated trials (each container had 3.5kg of ice gel and approximately 5kg urchins) and sent to a processor in Hokkaido, northern Japan. The following summary is taken from the Nofima Internal report 7/2017 (James *et al*., 2017).

4.3.1 Methodology

The sea urchin consignment was delivered to Tromsø airport following packing of the animals directly from the sea-based holding system at Kårvika, Tromsø (3 hrs packing and transport to airport in total). The consignment was delivered to Tromsø airport at 4:30am and the flight to Oslo departed at 5:30am. The total flight time from Tromsø to Narita, Tokyo was 26 hrs. Following arrival at Narita the urchins went through the following monitoring process (the results are shown in Table 7):

Time record of sea urchins consignment after arrival at Narita, Tokyo (Date 14/Sept/2016)

- 12:20 SAS flight arrived at Narita airport
- 14:30 Customs Clearance
- 15:10 Cargo released to our forwarder
- 16:45 Received from forwarder Flight check-in
- 17:25 Departure Local flight to Chitose, Hokkaido
- 19:20 Arrival Chitose airport
- 19:45 Departed Chitose by car
- 22:40 Arrived at the Processor (Thyms and Co Ltd, Hokkaido) where the packs were opened and sea urchins placed in a chiller (lids were put back on due to the low temperature in the chiller) overnight until processing the following morning.

(Total 10 hrs 20min in Japan prior to opening of packs).

Total time-frame for travel from Tromsø to Japanese processor:

Packing and transport to airport	3 hrs
Flight time	26 hrs
Time travelling to and at processor	10 hrs
TOTAL transport time:	**39 hrs**

4.3.2 Reults

Report from Japanese processor on condition of sea urchin an arrival in Japan:

- On arrival at the Processor, the inside temperature of each carton was measured without opening the lids. The average inside temperature was 14.3℃.
- On arrival, a visual inspection showed the urchins were in good condition with almost 70-80 % of urchins still alive and their spines moved slowly when touched. Almost all spines remain standing upright (See Figure 17 A).
- Due to the late arrival time at the processor the sea urchins were kept in cartons in a chilled room (-1.6℃) overnight. Due to the low temperature in the chilling-room the lids were kept on.
- On 15th September at 9:00am, the inside temperature in the cartons was checked again (this had fallen to 11.8℃). Each individual carton was weighed (net weight). The results are displayed in Table 7. Approximately 8 % drip (loss of sea urchin wet weight in the form of water loss into the packaging container) was measured and almost all sea urchins were dead by this time.
- The sea urchins from number 4 carton were selected for test processing on the same day.
  Total gonad weight was 900g exactly from 100 individual sea urchins = 4,708g
  (Origin weight) or 4,360g (arrival weight).
  Yield of gonad was 19.1 % (from Origin weight)
- The sea urchins were then packed in 100g small boxes which are a standard for packing sea urchin roe in Japan (Figure 17).

Figure 17 (from left to right) Sea urchins on arrival at the processing plant in Hokkaido, being opened, cleaned and sorted, and packed in traditional trays for sale.

Table 7 The temperatures, weight and weight loss of each carton during transport to Japan (recorded by Japanese processors).

Carton No.	Inside Temp at arrival (°C)	Weight (g) at Origin	Weight (g) at Processor	Difference (g) (Drip)	Lost Drip (%)	Temp (°C) Next morning
1	14.2	5 167	4 740	427	8.26	12.0
2	14.9	5 336	4 920	416	7.80	10.2
3	14.5	5 088	4 660	428	8.41	12.3
4	14.0	4 708	4 360	348	7.39	13.3
5	13.9	5 204	4 780	424	8.15	11.1
Total	**71.5**	**25 503**	**23 460**	**2 043**		**58.9**
Ave.	**14.3**	**5 101**	**4 692**	**409**	**8.01**	**11.8**

5 Summary

5.1 Road/Sea transport

For short time periods (14hrs to 44 hrs depending on transport temperatures) there are simple and effective methods of transporting live sea urchins (outlined in Section 3.1). Transporting sea urchins for longer periods requires more sophisticated transport techniques. There are transport systems available for live seafood species that are also effective for transporting sea urchins. The trials conducted as part of the URCHIN project indicate that simple chilled, aerated seawater systems would be capable of transporting live sea urchins for considerable periods (at least 22 days) without affecting sea urchin mortality. Such systems would enable live sea urchins to be transported from peripheral areas of the NPA (such as Greenland) to international markets by sea.

5.2 Air transport

The methodology and protocols for air transport are outlined in Section 4.1 of this report. A study conducted as part of the URCHIN project showed that there was a 34 hour window for transporting sea urchins if they are to go back into seawater post transport. When transport was extended to 44 hrs the mortality rate increased. However, in terms of getting sea urchin to market for sale or processing then 44 hrs would still be viable as the sea urchins were still alive and in relatively good condition when unpacked after 44 hrs, regardless of the quantity of sea urchins and gel ice.

5.3 General

The ability to transport sea urchin without mortality or loss of quality is an important factor when developing markets in peripheral areas such as the NPA. The methodologies and protocols outlined in this report give sufficient details to transport live sea urchins by air packed in polystyrene boxes (up to 44hrs) and by road by simply covering and protecting them (up to 44hrs depending on temperature). For transport by road or sea for longer periods transport systems that immerse the sea urchins in chilled and aerated seawater are required. The results of the study in this report indicate that live sea urchins can be transported for periods of at least 22 days with chilling and aeration. The results also indicated that sea urchins could be transported in immersion systems for longer periods if denitrifying equipment is installed as part of the transport system. Systems are available that are effective at transporting live sea urchins but issues such as back freight of empty containers, logistical requirements such as power supply and other factors also play a role in developing suitable transport systems and these must be taken into account when considering live transport of sea urchins over significant distances for extended periods.

It is also important to note that the transport limits defined in this report are for sea urchins that are not exposed to direct sunlight, excessive heat, wind or air movement of any type (e.g. when travelling on a boat) and excessive cold at any stage of the collection and transport process. If this occurs then the sea urchins may die immediately or sometime after transport and it will not be clear when the stress event occurred unless they all die immediately from a major stress event. Care must be taken during every step of collection (e.g. rough handling should be avoided), moving the sea urchins from one form of transport to another (e.g. boat to trailer) and during the transport itself.

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Appendix I

Sea Urchin transportation techniques
(Activity A6.4.1 of the NPA URCHIN project)

Activity 6.4	*Activity title:* Transport system for live sea urchins	*Start month:* 01.2016	*End month:* 03.2017
Description: Develop a suitable system for transporting live sea urchins to markets			Deliverables: Design and protocols for holding and transport systems for live sea urchins
6.4.1	***Deliverable:*** **Report on design and protocols**	***Target value*** **Delivered to a minimum of 10 SME's in NPA**	*Delivery month* 03.2017

Appendix II

Royal Greenland Aqualife testing

Royal Greenland undertook a number of trials using the AquaLife system as part of the URCHIN project. Initially, simulated trials (Aqualife system held in land-based facilities) were conducted to estimate whether the system could sustain live sea urchins for transport from Greenland (Nuuk) to Copenhagen (Denmark) (a period of approximately 10 days by sea). A preliminary trial conducted by Royal Greenland indicated that sea urchins would survive these transport periods (details of this trial are not available).

Royal Greenland commercial scale sea transport trial

In 2016 Royal Greenland attempted a final commercial scale trial to test transportation of sea urchins in the AquaLife system from Greenland (Nuuk) to Denmark (Copenhagen) (see Figure below). Unfortunately, the chilled container was not connected to a power supply when the vessel departed from Nuuk. This was only discovered 4 days later and it was found that all the sea urchin had subsequently died. It was not possible to repeat this trial. Subsequent trial run by Nofima investigating transporting sea urchin in chilled, aerated, static seawater holding systems (described in this report) have shown that this type of transport system is effective for at least 22 days.

The capture and loading of sea urchins in the AquaLife system in Nuuk (Greenland) clockwise from top left: Sea urchins after capture by dredge; sea urchin loaded in the AquaLife baskets; The stack of baskets being lowered into the Aqualife system container (x 2); the reefer containing the Aqualife system awaiting loading.

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