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ORIGINAL ARTICLE

Live transport of the green sea urchin (*Strongylocentrotus droebachiensis*) in air and immersed in seawater and the impact on subsequent roe enhancement after in-water transport

Philip James | Tor Evensen

Nofima, Tromsø, Norway

Correspondence
Philip James, Nofima, 9-13 Muninnbaken, Tromsø 9291, Norway.
Email: philip.james@nofima.no

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Abstract

The roe enhancement of sea urchins from barrens requires suitable transport techniques to enable transport of live sea urchins to enhancement facilities. For the green sea urchin *Strongylocentrotus droebachiensis*, the maximum period that the 'out of water' techniques described in this manuscript could be used is temperature dependent. When transported at 3.0°C, this should be no longer than 44-hours, 36-hours at 5.0°C and 14-hours at an average temperature of 14.3°C. The survival results from the 'in water' transport system described in this manuscript indicate that this type of transport method will be suitable for transporting sea urchins for periods up to 22-days and possibly longer if ammonia-stripping (denitrifying) capacity is included in the transport system. The results of the post transport enhancement trial show that it is feasible to hold sea urchins at the densities tested for periods up to 14-days and then to re-immerse them in seawater holding systems and enhance the roe of the sea urchins successfully. There was a significant increase in gonad index in the sea urchins held in all the transport treatments compared to the initial wild GI, except in the sea urchins held at high density for 7 days. However, higher mortalities occurred in all transported sea urchins, and these were higher in sea urchins held at higher densities and for longer periods. The authors recommend the development of stress and welfare indicators for captive sea urchins to enable the 'fitness' of sea urchins during transport and captivity.

KEYWORDS
mortality, roe enhancement, sea urchin, transport

1 | INTRODUCTION

There is a paucity of published information regarding transportation techniques and protocols for sea urchins. The lack of available protocols is surprising given that there are an estimated 70,000 t of sea urchins consumed per annum in Japan alone and a high percentage of these sea urchins are imported from other countries (Chile, Russia and Europe), sometimes as fresh whole, live sea urchins (Stefánsson et al., 2017).

There has been a substantial increase in interest and activity related to sea urchin roe enhancement in the past decade (Stefánsson et al., 2017). This involves the collection/harvesting of mature sea urchins from areas where the roe content may be very low or, too poor of quality for commercial processing (these areas are referred to as sea urchin barrens). The harvested sea urchins are then transferred and held in land or sea-based holding systems for periods between 8–12 weeks and fed formulated feeds to enhance the quantity and quality of the roe (Warren & Pearce, 2020). This creates a valuable, much sought after and lucrative product from a previously valueless resource in a relatively short period of time. In addition, there are a range of environmental benefits and ecosystem services regarding the habitat restoration and reforestation of macroalgae where the sea urchins are removed (Carlsson & Christie, 2019; Leinaas & Christie, 1996). This significant growth in interest in sea urchin roe enhancement has highlighted the need for adequate transport techniques to move sea urchins from the harvest site to aquaculture holding facilities. This can be a matter of hours or days depending on the relative locations of the harvesting and enhancement sites. It is important to define the meaning of 'live' sea urchins. The authors are unaware of any available protocols or quality ranking system for market acceptability of sea urchin and, to their knowledge, there is no clear definition of a 'live' sea urchin. What has been clearly documented is that it is possible that a sea urchin exposed to adverse conditions will suffer delayed mortality for up to 2 weeks (James et al., 2017) (Pers. Obvs. Algafres SLR, AquaVitae project). Many of the transport techniques currently used for wild harvested sea urchins that are processed and sold immediately only focused on keeping the sea urchins in good enough saleable condition for processing. In the case of sea urchins sold alive at market, these animals also only need to arrive at the market alive and in reasonable saleable condition. However, to transport harvested sea urchins and then return them to live holding facilities for subsequent roe enhancement requires more rigorous transport techniques and protocols. It also requires some knowledge regarding the impact on pre-enhancement transport on subsequent survival and the roe yield and quality of sea urchins that are enhanced post-harvest transport.

Sea urchins are relatively robust animals and if handled correctly can spend significant periods out of water without suffering from high levels of mortality after re-immersion in seawater (James & Evensen, 2018). 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. In general, most sea urchin fisheries rely on transportation methods that minimize exposure to excess high temperatures, direct sunlight, wind and prolonged air exposure. As with most marine animals transported out of water sea urchins are prone to drying out and these factors exacerbate this process. This is generally avoided by keeping sea urchins in cool, well-oxygenated running seawater (this avoids the issue of drying out completely) or if removed from water transporting them in a completely covered and protected chilled environment. Preferably with a moist lining to maintain a moist atmosphere during transport and some form of chilling. For road transport, this can vary but a simple and effective technique is covering the sea urchins with moist cloth and tarpaulins to create a moist, protected environment.

Best practices for handling and transportation of live mussels are relatively simple and consist of simply holding the mussels in mesh bags on ice (Barrento et al., 2013). These 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 & Siikavuopio, 2005). There are relatively few other guidelines and/or studies for handling and transport of shellfish and especially echinoderms. A study on handling and transport of sea urchins investigated the effects of rough handling during transport on subsequent roe enhancement (Dale & Siikavuopio, 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. A study by Warren and Pearce (2020) studied the effects of transport on the roe enhancement of the Pacific red sea urchin *Mesocentrotus franciscanus*. The study examined survivorship, gonad yield, and gonad quality of red sea urchins 2-weeks after exposure to 2 hours of either 'wet' or 'dry' transport methods. While no gonadal parameters significantly differed between transportation methods, the percentage of survivorship for the wet treatments (sea urchins in water) was 100%, while for the dry treatment (in a sealed moist environment) was 58.3% after 2 weeks, indicating that wet transport should be preferred over dry transport for Pacific red sea urchins. This study did not investigate the impact of subsequent transport on this sea urchin species and similar studies have not been published on *S. droebachiensis*.

In the current study, a variety of experiments were conducted on the green sea urchin *Strongylocentrotus droebachiensis* which is the focus of roe enhancement efforts in Norway as well as a number of other Northern latitude countries (e.g. Canada). The aim of this study was, first to investigate the impact on the survival of sea urchins held out of water but in a moist environment for periods of 12–52 hours (referred to as 'dry transport' for the remainder of this manuscript). This would be for small scale transfers of sea urchin and due to the high cost of air transport is unlikely to be used for commercial scale sea urchin roe enhancement activities. Second, to investigate the impact on water quality when sea urchins are held in static seawater transport containers at three densities at a constant 5-6°C for 22 days. This in-water method of transport is the most likely scenario for the transport of sea urchins on a large commercial scale. Finally, the impact of length of transport and transport density on subsequent sea urchin enhancement using static seawater transport systems. The results are discussed in relation to the development of commercial scale sea urchin roe enhancement for this and other species of sea urchin.

2 | METHODOLOGY

2.1 | Dry transport trials

Sea urchins were collected by freediving at a site (1-3 m deep) close to Tromsø (average test diameter = 42.7 mm) and transported to a live holding system and held in an ambient, oxygenated seawater until the beginning of the experiment (approximately six hours).

They were then randomly allocated to the simulated transport system at one of two transport temperature regimes (Trial 1: ambient temperatures and an average temperature of 4.3°C and Trial 2: average temperatures of 3.1 and 5.0°C). The simulated transport systems in both trials consisted of an insulated plastic container (90 × 51 × 52 cm; L × W × D) (Sæplast Tub 220). Inside the container, the sea urchins were covered with hessian sacks that had been soaked in seawater. The container was covered with a fitted lid and a tarpaulin to avoid any air movement. This method has been used extensively by Nofima to transport sea urchins in vehicles, trailers as it provides and maintains a moist, protected environment.

2.1.1 | Dry transport trial 1

The sea urchins remained in the simulated transport systems (at the two experimental temperatures, monitored using EBI- 125A, WINLOG 2000-S temperature loggers) for 12 hours, after which samples (ambient n = 20; cold n = 20) were removed every second hour (up until 28 hours post removal from water) and returned to a seawater holding systems (see description of holding system in the *In water transport and subsequent sea urchin roe enhancement*). The sea urchins were then held for a further 2 weeks in the seawater holding system to monitor post transport mortality.

2.1.2 | Dry transport trial 2

The sea urchins remained in these conditions for 24 hours (at two experimental temperatures, monitored using EBI- 125A, WINLOG 2000-S temperature loggers), after which samples (3.1°C n = 20; 5.0°C n = 20) were removed every fourth hour (up until 52 hours) and returned to the seawater holding systems. The sea urchins were then held for a further 2 weeks in the seawater holding system to monitor post transport mortality.

2.2 | Long term in water transport trial

Sea urchins were collected by free divers from a site (1-3 m deep) close to Tromsø and 20 min' drive from the live holding facility (average test diameter = 42.8 mm, average wet weight = 46.9 g). They were collected and transferred to the experimental tanks within 2 hours of collection.

A series of nine tanks were used (90 × 51 × 52 cm; L × W x D) (Sæplast Tub 220). Each tank held 100 L of seawater and was aerated with 10 cm of 'leaky hose' connected to an electric air pump. A ventilated plastic crate (60 × 40 × 20 mm) was placed in each tank. The crate sat on two upstands that raised it 4 cm from the bottom of the tank, allowing water circulation around the crate. The nine tanks were randomly allocated one of three density treatments:

- 50 urchins/crate (0.64 m^2 surface area per crate/total wet weight per crate 1.5 kg).
- 100 urchins/crate (0.64 m^2 surface area per crate/total wet weight per crate 2.9 kg).
- 150 urchins/crate (0.64 m^2 surface area per crate/total wet weight per crate 4.5 kg).

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 unionized ammonia (the most toxic form of ammonia, compared to unionized ammoina) was measured using analysis kits and a WTW portable colorimeter (pHotoFlex STD). The condition of the urchins was monitored throughout, and any dead sea urchins removed. After 22 days a sub-sample of sea urchins from each crate (n = 20) were randomly selected and transferred to sea-based live holding systems where they were held for 2 weeks to monitor post transport survival and condition.

A modified-Levene test was used to verify equal variance and a one-way ANOVA was performed to compare the accumulated total Unionized Ammonia (UiA) readings in the three densities after 22 days. A Tukey–Kramer multiple comparison test was used to identify which treatments differed.

2.3 | In water transport and subsequent sea urchin roe enhancement

Sea urchins were collected by free divers from a site (1-3 m deep) close to Tromsø and 20-min' drive from the live holding facility (average test diameter = 49.7 mm, average wet weight = 46.9 g). They were collected and transferred to the experimental facility within 2 hours of collection. The simulated transport trial consisted of the following Treatments:

- 7 days transport (0.64 m^2 surface area per crate/total wet weight per crate 4.0 kg).
- 7 days transport (0.64 m^2 surface area per crate/total wet weight per crate 8.0 kg).
- 14 days transport (0.64 m^2 surface area per crate/total wet weight per crate 4.0 kg).
- 14 days transport (0.64 m^2 surface area per crate/total wet weight per crate 8.0 kg).

An additional control treatment consisted of sea urchins collected from the same site as previous collections but at the conclusion of the simulated transports.

- 0 Days transport (control wild collection, no transport).

The sea urchins were collected and held for either 7 or 14-days at the two densities (three replicates of each treatment) at 2°C in simulated static seawater transport systems. This consisted of insulated containers (90 × 51 × 52 cm; L × W × D) (Sæplast Tub 220). Each tank held 100 L of seawater with ventilated plastic crates

(60×40×20mm) that held one of two densities of sea urchins (4 or 8 kg) and were held in a chilled room (2°C). Each container had an air stone to provide aeration in the tank. The high-density treatment was designed to simulate the most likely conditions in a commercial transport system design (80kg sea urchins per m^3 of seawater in a chilled container) (Pers. com., Harm Kamp, URCHINOMICS). The sea urchins were not fed during the transport trial.

Following the simulated transport, the sea urchins were transferred to four standard experimental raceways at the Nofima research station at Kårvika, Tromsø. The sea urchins were held in individual compartments (64 per raceway) consisting of ventilated plastic which allowed water to flow through the compartments and waste feed and faeces to drop through the bottom of the compartments into the raceway. Each raceway was supplied with filtered (nominal 50µm) ambient seawater via a supply manifold situated above the tanks (1.5L/min). The tanks were exposed to continuous light (24L) with a light intensity of 50Lx at the water level. Water temperature was recorded every 6hours in the inflow water using EBI- 125A, WINLOG 2000-S temperature loggers. Oxygen levels were measured weekly with Handy Delta logger (OxyGuard) in the outflow water and were above 98% dissolved oxygen throughout the experiment. The sea urchins were fed Urchinomics sea urchin roe enhancement formulated feed pellet at a rate of one pellet (approximately 4 g) per urchin per feed. The feed was developed by Nofima over the last two decades and has been successful in several feeding trials. It is now licensed to Urchinomics who have sublicensed it to Mitsubishi in Japan for larger-scale commercial production. It is now referred to as the Urchinomics Urchin Feed V10.1.10, made by Nosan Corporation. The proximate analysis of this diet is as follows: Moisture 11.23% (±0.14), Ash 22.79% (±0.07), Protein 18.21% (±0.06), Fat 1.08 (±0.01), Carbohydrate 46.69% (±0.07). Further information on this diet can be found in Angwin et al. (2022).

All the tanks were cleaned once a week by removing the outlet upstand and flushing the bottom and sides of the tank after the uneaten feed was collected.

Twelve sea urchins from each treatment were then held in each of four raceways ($n = 4$) and fed twice per week. The raceways were cleaned twice per week. Seawater was supplied at ambient temperatures. The enhancement trial ran for 10-weeks.

At the conclusion of the experiment, the GI and survival data were collected for sea urchins from each of the treatments and compared. A modified-Levene test was used to verify equal variance and a one-way ANOVA was performed to compare gonad index results from the sea urchins exposed to the various transport treatments. A Tukey–Kramer multiple comparison test was used to identify which treatments differed.

3 | RESULTS

3.1 | Dry transport trials

The average temperature in the dry holding systems during the trials is shown in Figure 1. The survival results from Trial 1 show that for the sea urchins exposed to a dry transport, followed by a 14-day period where the sea urchins are re-immersed in seawater are shown

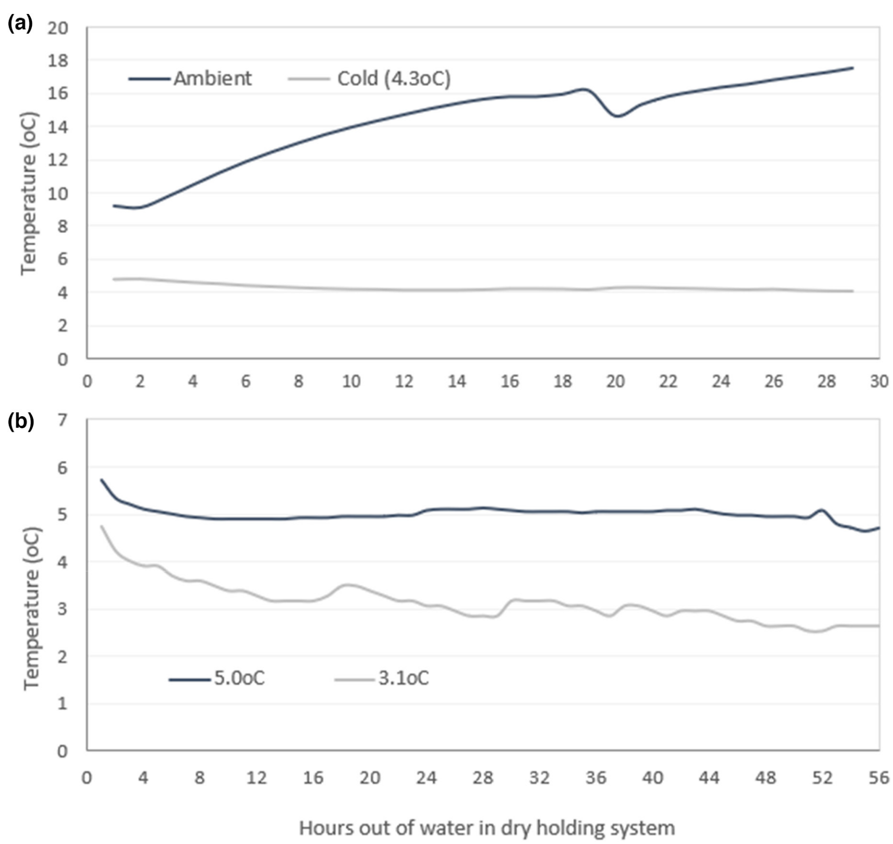

FIGURE 1 The temperatures in the holding containers in dry transport trials: (a) Dry transport trial 1 and (b) Dry transport trial 2.

in Table 1. The cutoff point of ≥90% survival was used to indicate the maximum acceptable mortality in sea urchins re-immersed in seawater after 14 days. This cutoff point was reached by sea urchins that were transported for 14-hours (or longer) at ambient temperatures (varied from 9.1 to 17.5°C with an average of 14.3°C) but was not exceeded by sea urchins transported up to 28-hours at 4°C (the longest sea urchins were held out of water in this trial).

The survival results from Trial two of sea urchins exposed to a dry transport trial followed by a 14-day period where the sea urchins are re-immersed in seawater are shown in Table 1. The ≥90% survival cutoff point after 14-days re-immersion in seawater was reached by sea urchins that were transported for 36-hours at 5°C and 44-hours at 5°C.

3.2 | Long term in water transport trial

The survival of the sea urchins held in the experimental holding systems at the various density treatments after 22-days were 98.3 ±(1.6) (150 sea urchin density), 98.3 ±(1.6) (100 sea urchin density) and 100% (50 sea urchin density). After being re-immersed in seawater for 14 days in a flow through sea-based holding system the mortalities were 98.3 ±(1.6) (150 sea urchin density), 98.3 ±(1.6) (100 sea urchin density) and 100% (50 sea urchin density). The water quality parameters in the holding tanks throughout the 22-day simulated transport are shown in Table 2. The average accumulated total Unionized Ammonia (UiA) readings (mg/L^{-1}) recorded in the three density treatments over the experimental period (22-days) are shown in Figure 2.

A one-way ANOVA revealed that there was a statistically significant difference in the accumulated total UiA readings in the three densities at 22 days ($F_{2,6} = 15.78$, $p = 0.004$). A Tukey–Kramer multiple comparison test found that the mean value of accumulated total UiA readings was significantly different between the sea urchins held at 150 sea urchins per crate compared to those held at 50 and 100 sea urchins per crate. There were no significant differences between the latter.

TABLE 1 Temperature and survival results for dry transport trials 1 and 2

Trial 1

Hours 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

Hours 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**

Note: Results in bold text indicate they are at or below the survival threshold of 90%.

TABLE 2 Water quality in the three density treatments throughout the 22-day trial period

	150/basket				100/basket				50/basket			
Day	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	349 ±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

3.3 | In water transport and subsequent sea urchin roe enhancement

The temperature in the simulated transport tanks was on average of 2.3°C (minimum = 1.6°C, maximum 3.4°C) throughout the simulated transports. The temperature in the roe enhancement system that the sea urchins were transferred to after the simulated transport was an average of 6.6°C (minimum = 5.5°C, maximum 7.7°C) throughout the 10-week enhancement.

The mortality measured during the 10-week post transport enhancement trial in sea urchins exposed to different transport treatments was 2.0% for 0 days, 10.4% for 7-days/4 kg, 8.3% for 7-days/8 kg, 14.5% for 14-days/4 kg and 10.4% for 14-days/8 kg. The initial GI and final GI of the sea urchins in the post transport sea urchin roe enhancement trial are shown in Figure 3.

A one-way ANOVA revealed that there was a statistically significant differences between the initial wild GI and the final GI from the sea urchins exposed to the different transport treatments ($F_{5,18}$ =4.23, p = 0.01). A Tukey–Kramer multiple comparison test found that the GI of the initial wild sample was significantly lower than sea urchins exposed to the control (no transport treatment), 4 kg density treatment for either 7 or 14 days and 8 kg density treatment for 14 days. There were no significant differences between sea urchins exposed to any of four the transport treatments and the control.

4 | DISCUSSION

4.1 | Dry transport trials

The Trial 1 survival results (14-days after re-immersion in seawater) show that the capacity for sea urchins to survive when transported out of water and then re-immersed in seawater is highly temperature dependent. When held at ambient temperatures (varied from 9.1 to 17.5°C with an average of 14.3°C) the 7-day mortality increased after sea urchins were held 2-hours out of water and the mortality rate increased rapidly for exposure periods longer than this. In contrast, there were no mortalities in sea urchins held at 4°C for periods up to 28-hours and then re-immersed in seawater.

In Trial 2, the results were similar with sea urchins being held out of water at 3°C having higher survival rates when re-immersed in seawater than those held at 5°C. However, the results indicate that when held in suitable transport systems they can be transported for extended periods of time out of water and still have good (≥ 90% survival) rates when re-immersed in seawater.

An increase in mortality as a response to increased transport temperatures is not surprising as this has also shown to have an impact on feed intake, gonad growth and oxygen consumption in the *S. droebachiensis* (Siikavuopio et al., 2008).

There were multiple observations of sea urchins spawning during the air exposure period in Trials One and Two and this would

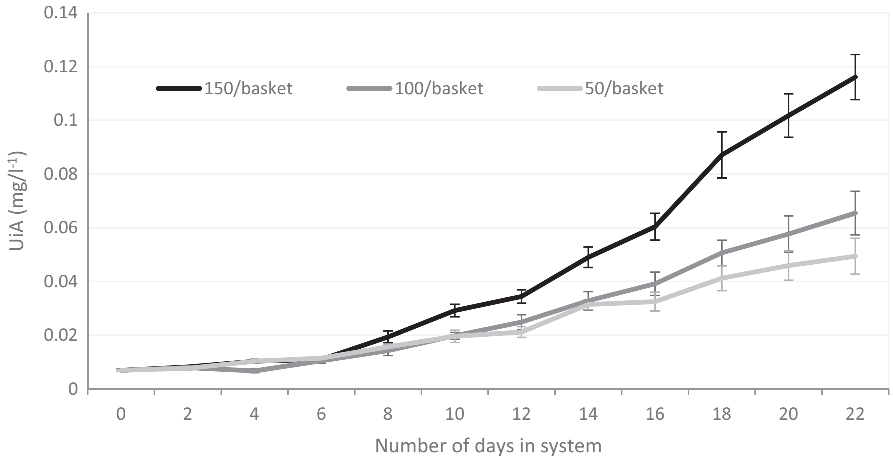

FIGURE 2 The average accumulated total unionized Ammonia (UiA) readings (mg/L^{-1}) recorded in the three density treatments over the experimental period (22 days).

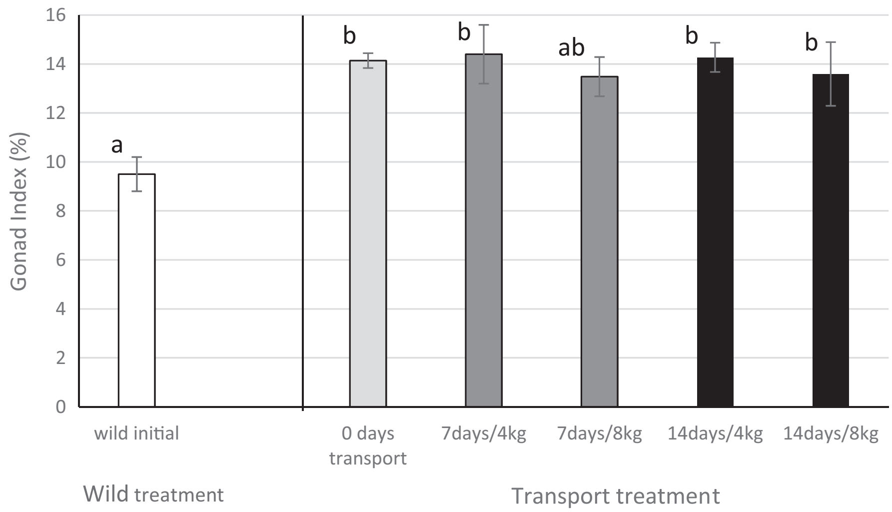

FIGURE 3 The initial wild GI and the final GI of sea urchins exposed to various transport treatments followed by a 10-week enhancement period. The letters indicate treatments that differ significantly.

certainly have had an impact on roe quantity and quality. Spawning events resulting from stress events such as dry transportation are well documented for sea urchins and the reproductive cycle of the sea urchins should obviously be considered when using this transport technique. The authors recommend that for *S. droebachiensis* the maximum period that this transport technique could be used is temperature dependent and when transported at 3.0°C should be no longer than 44-hours, at 5°C no longer than 36-hours and at an average temperature of 14.3°C no longer than 14-hours.

4.2 | Long term in water transport trial

There was very little difference in most of the water parameters experienced between the three density treatments in this trial with minor variations in temperature and little differences in the dissolved oxygen levels in the three experimental treatments. The aeration provided in the experimental tanks was sufficient that the lowest dissolved oxygen recorded in any of the tanks was 97.8% during the experimental period.

The ammonia results show a gradual buildup in the experimental system over time. The levels of UiA exceeded the long term (42 days) threshold limit for reduced growth (0.016 mg UIA L^{-1}) observed in the study by Siikavuopio et al. (2004) in each of the density treatment by day 9 of the trial period. Despite this there is little or no difference in the mortality of the sea urchins from the three density treatments over the limited period (22-days) that the experiment was conducted, or in the 14-day period the sea urchins were held in the holding systems after the transport treatments. These results can be used as a guide 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 they can be used as a guide to calculate the quantity of ammonia required to be removed by any given amount of sea urchins. This 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°C) this would need to be taken into consideration. It is also important to note that any future comparisons that are made are done using unionized ammonia as in the current study.

The survival results from the trial indicate that this type of transport method will be suitable for sea urchins for periods up to 22-days and possible longer. If ammonia-stripping (denitrifying) capacity is included into the transport system, transportations times may be significantly longer still. This would enable transportation from the likes of Norway to European markets in relatively simple transport systems. Although the sea urchins appeared in very good condition at the conclusion of the experimental period this study did not investigate the roe quality of sea urchins transported in this manner and this would also need to be considered when using these techniques.

4.3 | In water transport and subsequent sea urchin roe enhancement

The results of this trial show that it is feasible to hold sea urchins at the densities tested for periods up to 14-days and then transfer them to seawater holding systems and enhance the roe of the sea urchins successfully. There was a significant increase in GI in the sea urchins held in all the transport treatments compared to the initial wild GI, except in the sea urchins held at high density for 7 days. There were no significant differences between the GI of roe from sea urchins in any of the transport treatments at the conclusion of the subsequent roe enhancement trial, nor was there any difference between these and the control which was not exposed to any transport treatment prior to enhancement. However, the sea urchins that were transported had mortalities from 8.3% to 14.5% compared to 2.0% in those that experienced no transport treatment. This would indicate that although the sea urchins are able to enhance the amount of roe after the transport treatments experienced in this trial, they are obviously under some stress, which results in higher mortality rates when compared to sea urchins that are not transported. The results would also indicate that the stress increases with higher density but not necessarily for periods of 7 or 14 days when transported in water.

These results may be useful in areas where there are significant transport times between harvesting and enhancement facilities (for example, at this time in Norway, there are enhancement facilities in Stavanger in the South that are enhancing sea urchins harvested from Tromsø in the North). Alternatively, they could be used to establish the transport of sea urchins to enhancement facilities closer to markets (e.g. harvested in Norway and transported to enhancement facilities in central Europe).

There is considerable understanding of the factors that cause stress and their physiological impact on marine vertebrate species, but in comparison, little is understood about the mechanisms of stress in invertebrate species such as sea urchins (Bose et al., 2019). It is known that stress events such as handling and temperature changes induce a stress response in behaviour (Bose et al., 2019), and in the cell population dynamics of the coelomic fluid (Shannon & Mustafa, 2015) of specific sea urchin species. Shannon and Mustafa (2015) showed that changes in the cell structure found within the coelomic fluid of *Strongylocentrotus purpuratus* had an impact on sea urchin mortality and hypothesized this reflected a reduced immune capacity. Bose et al. (2019) showed that handling stress was sufficient to significantly change the behaviour of wild caught *Mesocentrotus franciscanus*. Neither study postulated on the physiological changes that may be occurring and the results of these studies as well as the current study highlight that in addition to the ability to enhance (increase) the size of the roe it would be very beneficial to be able to measure the stress in captive sea urchins and set benchmarks for levels that result in higher mortalities or lower quantities and/or quality of roe. In addition, the development and use of welfare indicators, incorporating individual, group and operational indicators can and should be established for this purpose.

AUTHOR CONTRIBUTIONS

Philip James: Conceptualisation; Methodology; Investigation; Wrtiting-reviewing and Editing. **Tor Evensen:** Methodology; Investigation; Data collection and Writing.

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CONFLICT OF INTEREST

The authors confirm there are no conflicts of interest associated with this research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo 10.5281/Zenodo.6244243.

ETHICS STATEMENT

Ethics approvals are not required for the research in this manuscript.

ORCID

Philip James https://orcid.org/0000-0003-0825-3435

REFERENCES

Angwin, R. E., Hentschel, B. T., & Anderson, T. W. (2022). Gonad enhancement of the purple sea urchin, *Strongylocentrotus purpuratus*, collected from barren grounds and fed prepared diets and kelp. *Aquaculture International*, *30*, 1353–1367.

Barrento, S., Lupatsch, I., Keay, I., & Shields, S. (2013). *Protocol on best practice guide on holding and conditioning mussels* (Technical Report supported by Project No. 243452). FP7-SME Mussels Alive.

Bose, A. P. H., Zayonc, D., Avrantinis, N., Ficzycz, N., Fischer-Rush, J., Francis, F. T., Gray, S., Manning, F., Robb, H., Schmidt, C., Spice, C., Umedaly, A., Warden, J., & Côté, I. M. (2019). Effects of handling and short-term captivity: a multi-behaviour approach using red sea urchins, *Mesocentrotus franciscanus*. *PeerJ*, *7*, e6556. https://doi.org/10.7717/peerj.6556

Carlsson, P. M., & Christie, H. C. (2019). *Regrowth of kelp after removal of sea urchins (Strongylocentrotus droebachiensis)* (Report 7431-2019). NIVA.

Dale, T., & Siikavuopio, S. I. (2005). Roe enhancement in sea urchin: effects of handling during harvest and transport on mortality and gonad growth in *Strongylocentrotus droebachiensis*. *Journal of Shellfish Research*, *24*, 1235–1239.

James, P., & Evensen, T. (2018). *Sea urchin transportation systems for land, sea and air* (Report 12/2018). Nofima.

James, P., Evensen, T., & Samuelsen, A. (2017). *Commercial scale sea urchin roe enhancement in Norway: Enhancement, transport and market assessment* (Report 7/2017). Nofima.

Leinaas, H. P., & Christie, H. (1996). Effects of removing sea urchins (*Strongylocentrotus droebachiensis*): Stability of the barren state and succession of kelp forest recovery in the east Atlantic. *Oecologia*, *105*, 524–536.

Shannon, R., & Mustafa, A. (2015). A comparison of stress susceptibility of sea urchins and sea cucumbers in aquaculture conditions. *Bioengineering and Biosciences*, *6*, 100–107.

Siikavuopio, S. I., Mortensen, A., & Christiansen, J. S. (2008). Effects of body weight and temperature on feed intake, gonad growth and oxygen consumption in green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture*, *281*, 77–82.

Siikavuopio, S. I., Mortensen, A., & Dale, T. (2004). Effects of chronic ammonia exposure on gonad growth and survival in green sea urchin *Strongylocentrotus droebachiensis*. *Aquaculture*, *242*, 313–320.

Stefánsson, G., Kristinsson, H., Ziemer, N., Hannon, C., James, P., (2017) *Markets for sea urchins: A review of global supply and markets* (Internal Matis Report). Skýrsla Matís, October 10–17, 2017. ISSN 1670–7192.

Warren, E. M., & Pearce, C. M. (2020). Effect of transport method on subsequent survivorship and gonad yield/quality in the red sea urchin *Mesocentrotus franciscanus*. *North American Journal of Aquaculture*, *82*, 371–376.

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