Hindawi
Aquaculture Research
Volume 2023, Article ID 6360865, 10 pages
https://doi.org/10.1155/2023/6360865

Research Article

Effect of Season and Increased Temperature on Survival, Roe Enhancement, and Reproductive Cycle of the Green Sea Urchin (*Strongylocentrotus droebachiensis*) Collected from Four Relatively Close Sites in Northern Norway

Philip James, Tor Evensen, and Gunhild Johansson

Nofima, 9-13 Muninbakken, Tromsø 9291, Norway

Correspondence should be addressed to Philip James; philip.james@nofima.no

Received 1 December 2022; Revised 15 March 2023; Accepted 17 March 2023; Published 17 April 2023

Academic Editor: Carlos Rosas

A series of three trials were conducted in the North of Norway at different times of the year. The trials consisted of harvesting sea urchins from four sites, situated within 5 km of each other and then holding them in land-based enhancement facilities. They were held at ambient seawater temperatures as well as ambient plus 3.0°C. Samples were taken from the wild at the time of collection and at the end of the enhancement period as well as from the enhanced sea urchins from each site. Results showed that utilising the sea urchins from a variety of sites, even from areas where there is an abundance of sea urchins (sea urchin barrens) and the Gonad Index (GI) is almost always low, is viable in terms of the final, post roe enhancement GI. The results of the study indicate that differences in initial and final GI and the increase in GI between the sites, despite the relatively close proximity, show site selection is most likely not as important as ease of harvesting and sea urchin availability and the positive environmental impact from removal of sea urchins and these are the factors that harvesting strategies should focus on. The study has shown that it is possible to have a relatively consistent roe enhancement from *S. droebachiensis* from the North of Norway regardless of seasonality (time of year), site selection, and initial GI. Relatively small changes (up to 3°C) in the seawater temperature appear to have a slightly negative impact on enhancement efforts. However, this may vary when larger differences in temperature are experienced.

1. Introduction

There is significant interest in sea urchin roe enhancement around the world [1]. This involves harvesting mature sea urchins of market size, often from areas that have an abundance of sea urchins (often referred to as "sea urchin barrens"). In the barrens, the sea urchins mostly have very poor roe quality as there is very little available food (macroalgae) present in these areas for the excessive number of sea urchins. Sea urchin roe enhancement allows these sea urchins to be harvested from these barrens and turned into a valuable seafood product within 10–12 week period. This process involves transporting the sea urchins to holding facilities at sea or on land and feeding them formulated aquafeeds designed to produce large, tasty, high-quality roe. A secondary advantage of this process is that when the sea urchin's density is lowered at the harvest site, it is possible to revert the sea urchin barrens to productive macroalgae forests with an increased biodiversity, productivity, and increased ecological benefits [2]. This has led to the development of a number of commercial enterprises based on sea urchin roe enhancement in Norway and around Europe. Currently, the most developed and widespread (globally) is Urchinomics which has activities in Japan, North America, Europe, and Australasia (https://www.urchinomics.com). In addition, there are numerous startup companies in a variety of countries around the world. For example, in Norway, there are three startup companies working on developing sea urchin roe enhancement activities at the time of this manuscript being written.

Sea urchin roe enhancement relies entirely on increasing the size and improving the quality of the gonad (commonly referred to as roe). Sea urchin gonads are unique in that they have both reproductive and storage/phagocytic cells (nutritive phagocytes) in the gonad. The relative amount of these cells changes naturally throughout the annual reproductive cycle, which is well described for this species [3]. It normally consists of a spawning event in May/April, followed by a period where the sea urchins have small GI values and are normally at reproductive stages 1-2 [3]. This is followed by a period of gonad growth (stages 2-3) from June to October and then a final period from October to Feb when the gonads produce reproductive cells in the gonad in readiness for the next spawning event [4]. In addition, there is considerable variation between sea urchins found at different sites and even within a single site in the GI values and the reproductive stage at any given time [3].

When sea urchins are harvested for enhancement, there are additional physiological changes (to those that occur naturally) occurring in the gonad of sea urchins. Enhanced sea urchins tend to have a much larger percentage of nutritive phagocytes than wild sea urchins, even those at the same reproductive stage [5]. This information is critical when planning harvesting and enhancement strategies for a possible future sea urchin industry. In addition to the reproductive stage, there are additional factors that may affect sea urchin roe enhancement. One of these is temperature, which is known to impact a range of physiological process in both lower vertebrates [6] and invertebrates [7]. Previous studies have investigated the effects of seawater temperatures on somatic growth and survival of a variety of different sea urchin sizes and from a variety of species. Examples include the study by Zhan et al. [8], which investigated the impact of chronic heat stress growth, survival, feeding, and differential gene expression in the sea urchin *Strongylocentrotus intermedius.* Leach et al. [9] also looked at the impact of marine heatwaves on the fertilisation success of the purple sea urchin (*Strongylocentrotus purpuratus*). The impact of temperature on feeding and roe enhancement in the same species (*Strongylocentrotus purpuratus*) was studied by Azed et al. [10]. A study by James et al. [11] looked at the impact of temperature and season on the temperate species *Evechinus chloroticus* in New Zealand, and Gouda and Agatsuma [12] studied the effect of high temperature on gametogenesis of the sea urchin *Strongylocentrotus intermedius* in the Sea of Japan, northern Hokkaido, Japan. There has been one study on *Strongylocentrotus droebachiensis* to assess the effects of both temperature and food ration on the gonad growth and oogenesis from a single population sampled in winter and summer [5]. They summarised that conditions of high food availability in the summer and low temperature in the winter would be the most favorable for reproductive output of this species.

There is a considerable interest in utilising land-based recirculating aquaculture systems (RASs) for sea urchin roe enhancement (Brian Takeda, Urchinomics, *pers com*). This will offer the potential to increase water temperatures in enhancement facilities, and the impact this might have on roe enhancement is unclear. In addition, rising seawater temperatures resulting from global warming could also impact on the gonad development of wild sea urchins. To date, there are no similar studies on *Strongylocentrotus droebachiensis*, one of the most widely spread and commercially exploited sea urchin species in the Northern and Arctic regions of the Northern Hemisphere.

The aim of the current study is to identify whether there are differences in the ability of sea urchins to enhance the gonad (GI) of sea urchins from four different sites (within 5 km of each other) and whether these differences change throughout a 12-month period. Also, the study will investigate the additional impact of increasing seawater temperature on gonad enhancement and the reproductive cycle of enhanced sea urchins (*Strongylocentrotus droebachiensis*).

2. Methodology

2.1. Collection Sites. Sea urchins were collected at regular intervals from four different collection sites within 5 km of each other in a sound system (Kvalsund) in Northern Norway (Figure 1). The four sites were as follows:

Site one (referred to as "tunnel"): It consists of flat rocky hard substrate interspersed with sand that gently shelves from 1 m to 4-5 m. The currents at this site are very strong and collections can only be made at slack high or low tides. There are macroalgae (mixed species) growing on all structures and hard surfaces close to the water surface and previous surveys have shown that at depths greater than 5 m, there are significant macroalgae (kelp) beds. Sea urchins are found at relatively high density at the site.

Site two (referred to as "harbour"): It consists of a soft substrate with occasional rocks and hard patches. It is formed by a man-made rock boulder wall to protect boats in the harbor. It is shallow throughout with a maximum depth of 2-3 m. There are some drift macroalgae present at the site, but they mostly consist of *Ascophyllum nodosum*. Sea urchins are found at relatively medium density at this site.

Site three (referred to as "Kårvika"): It consists of a hard substrate mixed with sandy areas that shelves rapidly from 1 m to 7-8 m. There is macroalgae present in very small quantities and occasionally as drift algae. Sea urchins are found at relatively high density at the site, but most are relatively small. Larger individuals (>50 mm test diameter) are sparse.

Site four (referred to as "Ytre Kårvika"): This is a shallow reef consisting of hard flat rock areas and scattered boulder areas. On the seaward side, it becomes sand bottom at approximately 2-3 m depth. Sea urchins are found at relatively high density at the site. There is macroalgae present in very sparse amounts at this site.

2.2. Harvesting. Sea urchins were collected by free divers at each site into catch bags before being transferred into plastic mesh bags for transport. They were held in water until immediately prior to transport back to the land-based

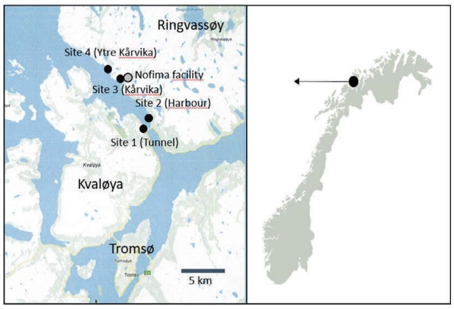

Figure 1: The location of the four collection sites and the Nofima facility in Kvalsund between the islands of Kvaløya and Ringvassøy in Northern Norway.

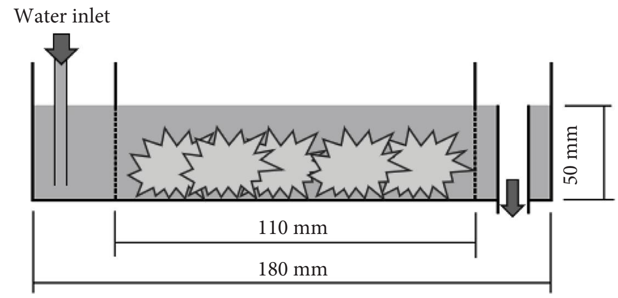

Figure 2: The tank dimensions (tank width = 100 mm) and water inlet systems used in the tanks to enhance adult *Strongylocentrotus droebachiensis*.

enhancement facility. They were transported in the mesh sacks out of water in insulated and sealed containers with moist sacks covering the bags to maintain a moist atmosphere. The transport time was a maximum of 10 minutes' drive from the collection site to the enhancement facility.

2.3. Trial Timing. Three trials were conducted over a 12-month period. Trial one started on 3rd November 2020 and finished on 21st February 2021. Trial two started on 29th March 2021 and finished on 1st June 2021. Trial three started on the 15th July 2021 and was completed on the 29th September 2021. The start date of the trial was the date that the sea urchins first received feed in the experimental tanks.

2.4. Holding Systems and Enhancement Protocols. For each trial, a total of 320 sea urchins, 80 from each collection site were harvested. Sea urchins from each site were divided into groups of eight (per tank, density 72 sea urchins m^{-2}). The ten tanks of sea urchins from each site were haphazardly assigned to one of the 40 experimental tanks (divided evenly between the two temperature treatments, five tanks per site, per temperature treatment). Tanks 1–20 were supplied with ambient seawater + 3°C (AMB + 3) and tanks 21–40 were supplied with ambient seawater (AMB). Each site had five replicate tanks per site and per water temperature treatment. The dimensions of the experimental tanks are shown in Figure 2.

Each tank was supplied with filtered (nominal 50 μm) ambient seawater via a supply manifold situated above the tanks (0.25 l/min). To achieve the AMB + 3 treatment, heated seawater was mixed to increase the water temperature approximately 3°C. The tanks were exposed to continuous light (24 L) with a light intensity of 50 lx at the water level. Water temperature was recorded every 6 hours in the inflow water of each of the temperature treatments 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 sea 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 sub-licensed 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 Mitsubishi Corporation. 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.

2.5. Sampling Protocols. Each of the three trials ran for a period of 9 weeks. At the beginning of each trial and at the conclusion of each trial, a sample of 30 wild sea urchins from each site were collected and measured. Throughout the experiment, mortality was recorded, and at the conclusion, the percentage survival was calculated (number of mortalities divided by initial number × 100). The reproductive stage of the sea urchins was determined at the conclusion of the trials by taking histology samples from a single gonad taken from each of 15 urchins. These were selected randomly from each site and temperature treatment for histological analysis. These were collected from enhanced sea urchins as well as from the wild sample collected at the conclusion of the trial (note: a wild sample was not taken at the conclusion of trial 1). To take the histology samples, a thin section (1-2 mm) was removed from approximately the middle of each gonad and stored in 10% buffered formalin before being fixed and stained using standard Harris's haematoxylin stain and counterstained with eosin [13]. The reproductive condition was assessed using the four reproductive stages described in Walker et al. [4]. At the final census of the trials and for the wild samples, the live WW (nearest 0.1 g) and test diameter (TD) (nearest 0.1 mm) of each sea urchin were recorded. Each sea urchin was then opened and drained, and the gonad was removed from each animal and weighed (G). These data were used to calculate the gonad index (GI) = $G/\mathrm{WW} \times 100$. The increase in GI was calculated using the following formula GI*inc* = Final experimental GI (site 1, site 2, site 3, and site 4, respectively) – Final wild GI (site 1, site 2, site 3, and site 4, respectively).

2.6. Statistical Analysis. A three-way analysis of variance was used to compare the "final GI" and "increase in GI" between trial (1, 2, and 3), temperature treatments (Amb, Amb + 3), and the four sites, taking into account all possible two-and three-way interactions. These analyses allowed the description of the main effects of each factor and identified interactions of potential importance. Homogeneity of variances was tested using Modified Levene's test.

A one-way ANOVA was used to test for differences in the percentage increase in GI between the sea urchins exposed to the temperature treatments from the different sites for each trial. Homogeneity of variances was tested using Modified Levene's test. Tukey–Kramer post hoc comparison tests were used to identify the experimental treatment means, which differed significantly.

Statistical analyses were conducted using NCSS 2000 (Number Crunching Statistical Systems, Kaysville, Utah, USA). Statistical tests were considered significant if $P < 0.05$. Errors are expressed as ± one standard error.

3. Results

3.1. Temperature Results. The seawater temperatures in the AMB treatment followed the natural variation experienced in the Tromsø area with a low of 4.2°C in April (in trial 2) and a high of 9.9°C in August (in trial 3). The AMB + 3 treatment reflected the natural changes in the ambient treatment with an approximate 3.0°C difference in trials two and three, which remained relatively constant throughout the trial. In trial one, the AMB + 3 treatment started approximately 2.0°C warmer and at the conclusion of the trial was approximately 4.0°C warmer. In trial one, the average temperature in the ambient treatment was 6.6°C (SE ± 0.04) and in the AMB + 3 treatment was 9.9°C (SE ± 0.02). In trial two, the average temperature in the ambient treatment was 4.7°C (SE ± 0.03) and in the AMB + 3 treatment was 7.8°C (SE ± 0.03). In trial three, the average temperature in the ambient treatment was 9.1°C (SE ± 0.01) and in the AMB + 3 treatment was 12.2°C (SE ± 0.03) (Figure 3).

3.2. Mortality. The mortality recorded in sea urchins held in each of the trials (1–3), from the two temperature treatments as well as the total mortality from sea urchins from each site are shown in Table 1. Higher mortality (10.0% or above) was experienced in the tunnel/AMB (17.5%) and harbour/AMB + 3 (10.0%) in trial 1 and in the Kårvika/AMB + 3 (15.0%) in trial 3.

3.3. Natural Variation in GI in Wild Sea Urchins. The GI of the sea urchins collected from the four sites throughout the 12-month period that the trials were running varied according to the natural reproductive cycle of the sea urchins. The GI was the highest for all sites in January and dropped between January and March as spawning occurred. Gonad Index values were the lowest in May for all sites, and then this was followed by a gradual increase in the GI from all sites through to September. This rise in GI was the most pronounced for the Tunnel site (Figure 4).

3.4. Final GI in Enhanced Sea Urchins. The final GI of the sea urchins collected from the four sites and held at either AMB or AMB + 3 temperature treatments in the three trial is shown in Figure 5. Included in this figure is the final wild GI of sea urchins collected from each site at the conclusion of the respective trials.

A three-way ANOVA showed there were significant differences in the final GI of sea urchins enhanced in the three trials over a 12-month period (Table 2). A Tukey–Kramer post hoc test showed the final GIs in trial two were significantly lower compared to trial one and three (ANOVA: $F = 18.48$, d$f = 2$, $P < 0.001$). There was a significant difference in GI between sea urchins enhanced in the two temperature treatments with those enhanced at ambient temperatures having a significantly higher final GI (Table 2). There were significant differences in the final GI of the sea urchins collected from the four sites (Table 2). A one-way ANOVA followed by a Tukey–Kramer post hoc test showed the sea urchins from the Ytre Kårvika site had a significantly higher GI than those from both the harbour and tunnel sites. There were no significant differences between the Kårvika site and any other site (ANOVA: $F = 5.55$, d$f = 3$, $P = 0.001$). There were no significant interactions between any of the factors (trial, site, or temperature) (Table 2).

3.5. Increase in GI in Enhanced Sea Urchins. The increase in GI of the sea urchins collected from the four sites and held at either AMB or AMB + 3 temperature treatments in the three trials is shown in Figure 6. A three-way ANOVA showed there were significant differences in the "increase in GI" of sea urchins enhanced in the three trials over a 12-month period (Table 2). A Tukey–Kramer post hoc test showed the increase was significantly higher in trial two than trial one, and there was no significant difference between the increase in trials two and three (ANOVA: $F = 17.70$, d$f = 2$, $P < 0.001$). As with the final GI values, there was a significant difference in GI between sea urchins enhanced in the two temperature treatments with those enhanced at ambient temperatures having a significantly higher "increase in GI" (Table 2). There was a significant difference in the "increase in GI" of the sea urchins collected from the four sites. A one-way ANOVA followed by Tukey–Kramer post hoc test showed the Kårvika site had a significantly higher increase than all other sites. There were no differences between the Ytre Kårvika and harbour sites, and these were significantly higher than sea urchins from the tunnel site (ANOVA: $F = 13.57$, d$f = 3$, $P = 0.001$). There was a significant interaction between the trial and site factors (Table 2).

3.6. Histology in Enhanced Sea Urchins Compared to Wild Sea Urchins. The reproductive stages of the enhanced and wild sea urchins at the conclusion of each trial are shown in Figure 7 (note: there were no wild samples taken in trial 1). They follow the same general trend that has been documented for this species in this location in the North of Norway [3]. This involves a progression from stages 3-4 in the period from November to January, followed by a progression through the spawning period (April-May) from stages 3-4 to stages 1-2. In July to September, the sea urchins progress through to stages 2-3. The results show a wide

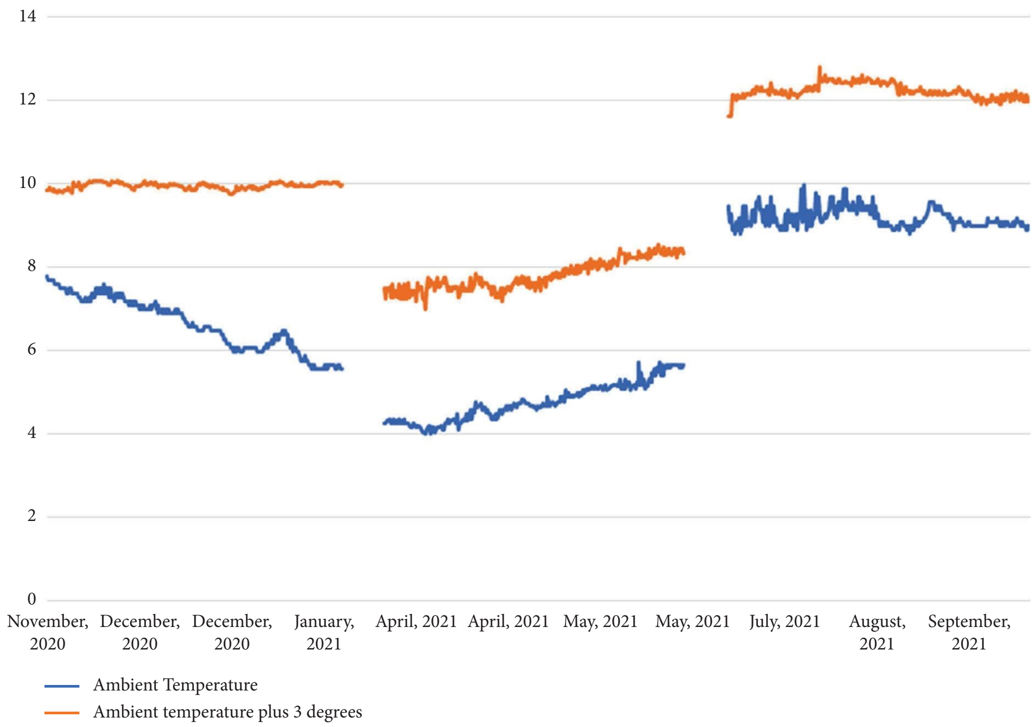

Figure 3: The temperatures in the experimental system during the enhancement trials.

Table 1: The percentage mortality recorded in sea urchins held in each of the trials (1–3) and from each of the sites (Tunnel, Harbour, Kårvika, and Ytre Kårvika) and from the two temperature treatments (ambient and ambient + 3.0°C) and the combined percent mortality for each site.

Trial	Population	Ambient	Ambient + 3°C	Total
1	Tunnel	17.5 (7)	2.5 (1)	20.0 (8)
	Harbour	2.5 (1)	10.0 (4)	12.5 (5)
	Kårvika	0 (0)	5.0 (2)	5.0 (2)
	Ytre Kårvika	0 (0)	2.5 (1)	2.5 (1)
2	Tunnel	0 (0)	2.5 (1)	2.5 (1)
	Harbour	0 (0)	0 (0)	0 (0)
	Kårvika	0 (0)	0 (0)	0 (0)
	Ytre Kårvika	0 (0)	0 (0)	0 (0)
3	Tunnel	2.5 (1)	7.5 (3)	10.0 (4)
	Harbour	5 (2)	5.0 (2)	10.0 (4)
	Kårvika	2.5 (1)	15.0 (6)	17.5 (7)
	Ytre Kårvika	2.5 (1)	5.0 (2)	7.5 (3)

variability between sites in each trial (for both wild and enhanced sea urchins). In general, the reproductive stages in the enhanced sea urchins tended to lag behind the wild sea urchins collected from the same site. This is particularly obvious in trial two, which was run during the spawning period, and it appears that the sea urchins that were collected and enhanced slowed the progression of the reproductive stages compared to those that were left in the wild (Figure 6). In general, it appears that the enhancement of the sea urchins meant the sea urchin reproductive stage was more advanced of the wild samples, and in general, the sea urchins held at 3.0°C higher temperatures had a more advanced reproductive stage.

4. Discussion

For sea urchin roe enhancement to develop as an industry, it is necessary to have a thorough understanding of holding systems and seawater parameters as well as harvesting strategies that will optimise roe production. Temperature, season, and diet are known to influence somatic growth in sea urchins [1, 14, 15]. The current study investigates the enhancement of roe from sea urchins collected from a variety of sites that are relatively close together (within 5 km of each other) over three separate trials conducted over a 12-month period. The urchins collected from each site have variable initial GI, and the study investigates whether their roe enhancement is affected by their initial GI, seasonality, and also by an increase in ambient temperature of 3.0°C during the enhancement period.

A previous study on this *S. droebachiensis* looked at the impact of seawater temperatures on roe enhancement and the reproductive cycle [5]. They found that food availability is the most important factor regulating energy storage and the relative size of the gonads. This has also been shown for other species such as *Evechinus chloroticus* [16]. Garrido and Barber [5] also showed that temperature affected the rate of growth and the reproductive cycle of this species. In their study, sea urchins exposed to 12.0°C in both summer and winter trials had a higher final GI than those exposed to 3.0°C. This is a significantly higher temperature difference than tested in the current trial, where the temperature treatments were ambient seawater compared to ambient seawater plus approximately 3.0°C. Another study to look at the impact of temperature on the feed intake, gonad growth, and oxygen consumption on *S. droebachiensis* was by

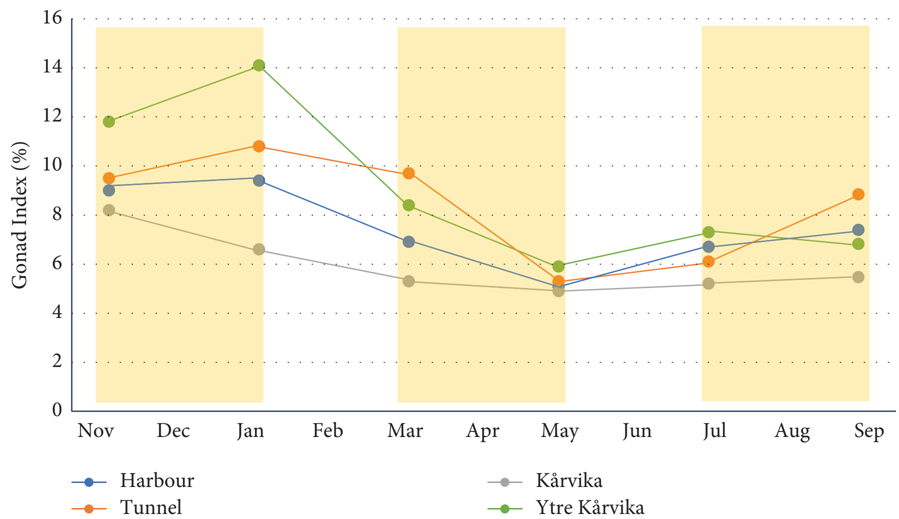

Figure 4: The GI of the sea urchins collected from the four sites throughout the 12-month period that the trials were running. The shaded areas indicate when the three trials were run.

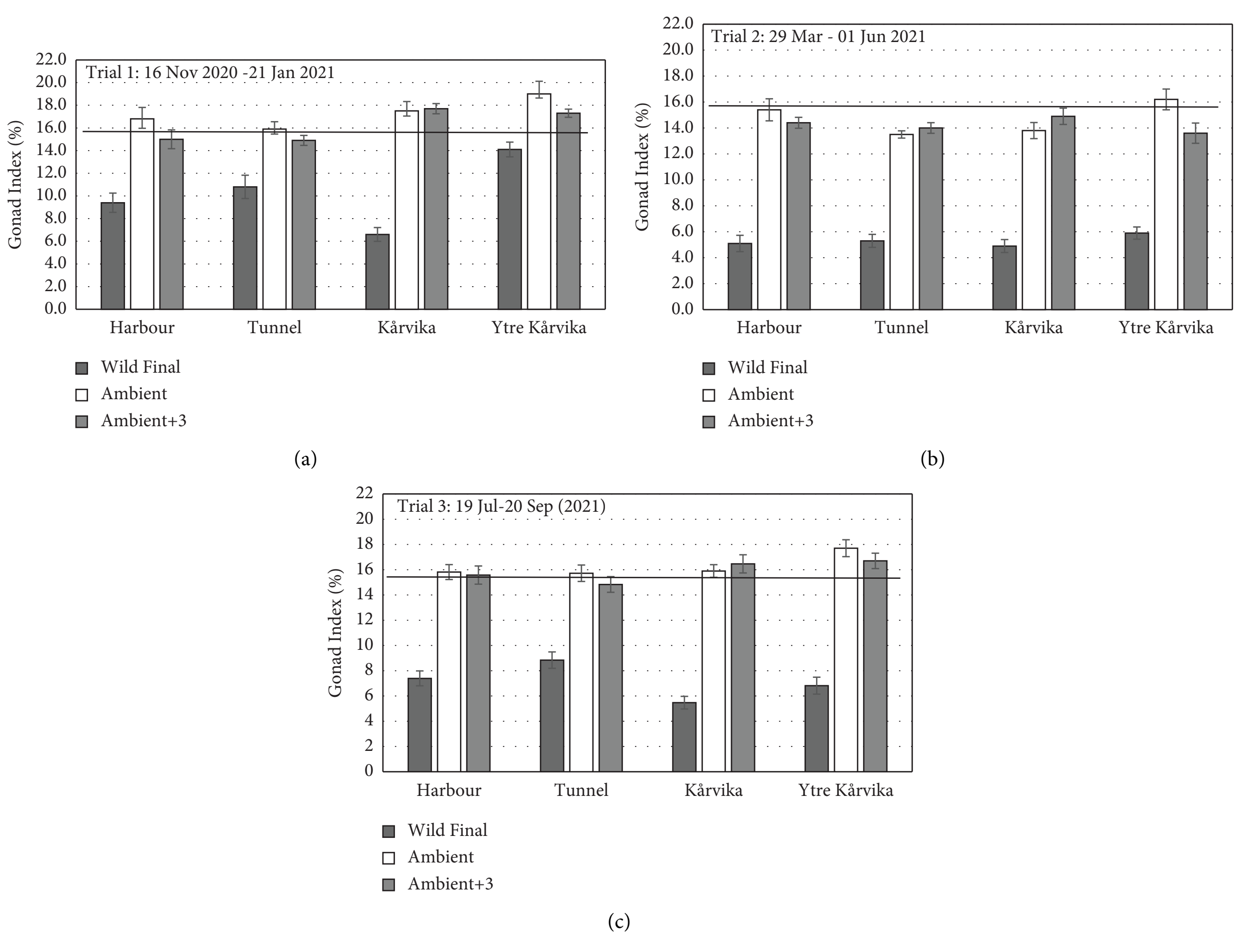

Figure 5: The final wild GI values (±SE) from sea urchins collected at the four sites, final GI values for sea urchins enhanced at ambient seawater temperatures and final GI values for sea urchins enhanced at ambient + 3°C seawater temperatures in trial 1 (a), trial 2 (b), and trial 3 (c).

Table 2: Significance tests and related statistics (degrees of freedom, mean square, F ratio, P value) for the ANOVA analysis of the "final GI" and "increase in GI" from the enhancement trials.

Effect	df	Mean square	F ratio	P
Final GI				
Trial (1, 2, 3)	2	66.43	28.63	<0.001*
Temperature (Amb, Amb + 3)	1	9.52	4.10	0.045*
Trial × temperature	2	1.61	0.69	0.503
Site	3	19.39	8.36	<0.001*
Trial × site	6	2.30	0.99	0.435
Temperature × site	3	3.68	1.59	0.198
Trial × temperature × site	6	1.96	0.85	0.320
Error	96	2.32	—	—
Increase in GI				
Trial (1, 2, 3)	2	91.40	39.39	<0.001*
Temperature (Amb, Amb + 3)	1	11.12	4.79	0.030*
Trial × temperature	2	2.74	1.18	0.310
Site	3	73.61	31.72	<0.001*
Trial × site	6	34.39	14.82	<0.001*
Temperature × site	3	3.82	1.65	0.183
Trial × temperature × site	6	3.08	1.33	0.251
Error	96	2.32	—	—

*indicates significant differences.

Siikavuopio et al. [14]. In this study, sea urchins collected from a single site, of different sizes, were exposed to increasing temperatures from 4.0°C to 14.0°C. The study showed that feed intake and oxygen consumption continued to increase in linear fashion with increasing seawater temperatures. Gonad index also increased with increasing temperatures, but there were no significant differences in seasonal increase in gonad index between sea urchins held between 4.0°C and 8.0°C. In the current trial, there were relatively small temperature differences between the AMB or AMB + 3 treatments, but sea urchins held at ambient had a higher increase in GI than those held at 3.0°C higher temperatures.

The results of the current study indicate that it would not be advantageous to increase the holding temperatures by 3.0°C in sea urchin enhancement systems. In recirculating aquaculture systems, the temperature is often increased due to the mechanical pumping and resulting frictional heat in manifolds and delivery lines. In this case, the results of this study indicate that a slightly lower increase in GI can be expected than when sea urchins are held at ambient seawater temperatures (relative to where they are collected). However, the differences are unlikely to influence the overall annual farm production.

As a number of studies on other species have shown, as well as previous studies on *S. droebachiensis,* an abundance of food results in larger roe and an acceleration of the reproductive cycle [1]. This is supported by the current study where the enhanced sea urchins tended to be slightly ahead of the reproductive stage found in the sea urchins collected from the wild at the conclusion of the enhancement period. Again, these changes are not large and would unlikely have any impact on the annual production from a sea urchin enhancement facility. What is a surprising but positive result in terms of annual production of enhanced sea urchins is that the results from trial two (29th March to 01st June) showed the best overall and most consistent increase in GI of all three trials (Figure 6). The spawning season in this area of North Norway normally occurs between March and April and is when the GI of wild sea urchins is at its lowest (Figure 3). The results from this study show it will be possible to produce roe all year round from this species in this locality, despite the natural spawning season and associated decrease in wild GI. These results must be followed up by future studies looking at the quality of the roe during this period. These should also investigate the relationship between the presence of macroalgae at the collection sites and a possible link to improved enhancement.

The overall GI results must be considered together with the total increase in GI when planning optimal harvesting strategies for sea urchin roe enhancement. The results of the current study showing the total GI after each trial from each of the three trials (Figure 5) are remarkably consistent across trials (season) with an average overall final GI across all trials and sites of 15.79% (±SE 0.17). Sea urchins in trial 2 had lower final GI results than trials 1 and 3. The lower GIs in trial 2 are due to the lower starting GI from each site. These results differ from the increase in GI which are largest and most consistent for trial 2 when the start GIs were lowest. The average overall increase in GI was 8.13% (±SE 0.25) and the sea urchins from the Kårvika site had by far the largest and most consistent increase in GI over the three trials (Figure 6). The results indicate that harvest sites with lower initial GI tend to enhance more than those with higher initial GI. This can be taken into consideration when planning harvesting strategies from a wide geographic area with diverse sites as most likely the final results will be quite consistent. Sea urchins that have a lower initial GI enhance more and tend to have a similar final GI to those from sites with a higher initial GI. This is particularly useful when planning harvest strategies around sea urchin barrens, where the sea urchins are abundant but usually have a low GI. Fishing these sites is often easier (because of the high abundance of sea urchins) and a number of companies investigating sea urchin enhancement are linking this to removal of the sea urchins from sea urchin barrens and subsequent reforestation of macroalgae forests [17, 18]. Harvesting sea urchins from the barrens where the initial GI is low, but the sea urchins are likely to have an enhanced ability to increase their GI would be an ideal harvesting strategy for roe enhancement.

The results of this study show that utilising the sea urchins from a variety of sites, even from the sea urchin barrens, where the GI is almost always low is viable in terms of the final, post roe enhancement GI. The results of the current study indicate that differences in initial and final GI and the increase in GI between the sites, despite the relatively close proximity, show site selection is most likely not as important as ease of harvesting and sea urchin availability, and the positive environmental impact from removal of sea urchins and these are the factors that harvesting strategies should focus on.

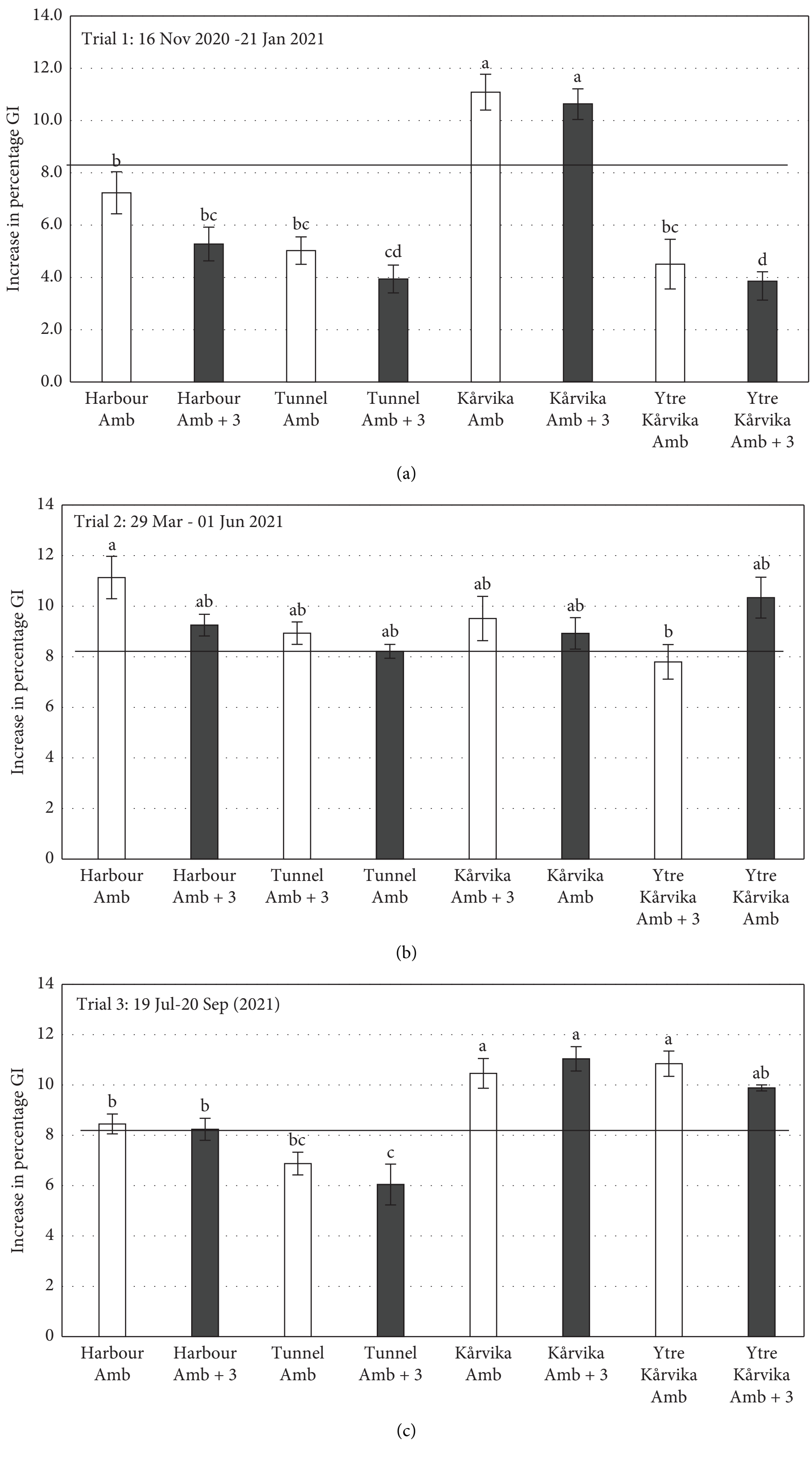

Figure 6: The final increase in GI values (±SE) from sea urchins collected at the four sites, final GI values for sea urchins enhanced at ambient seawater temperatures, and final GI values for sea urchins enhanced at ambient + 3.0°C seawater temperatures in trial 1 (a), trial 2 (b), and trial 3 (c) Letters indicate significant differences between treatments.

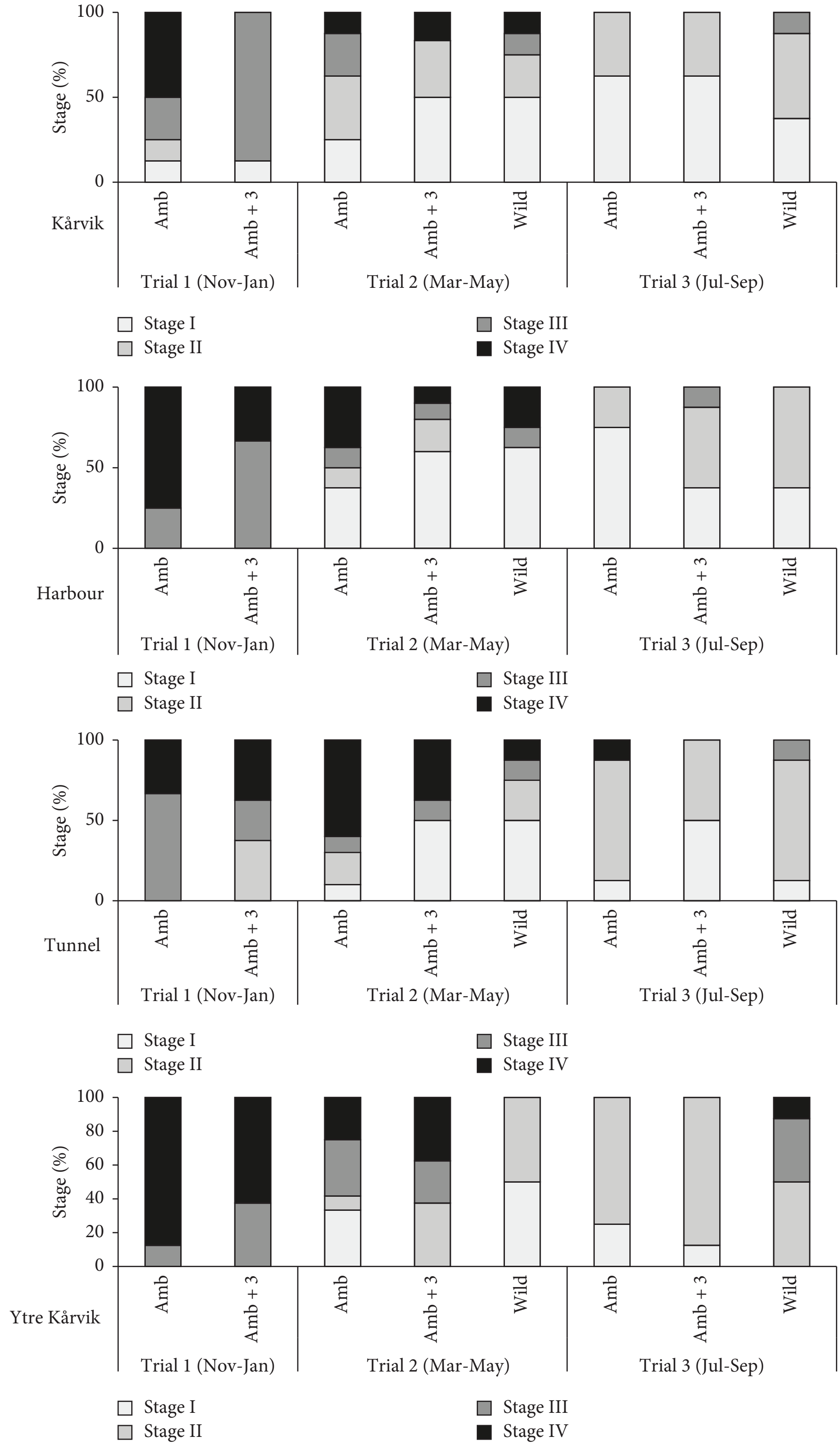

Figure 7: The histology results from the sea urchins collected from the four sites at the conclusion of the trials as well as from the final wild sample.

As has been seen in previous studies, a low initial GI appears to stimulate a higher increase in GI during the enhancement period. Even though the sea urchins are not reaching the maximum GI capable for this species, this study has shown that it is possible to have a relatively consistent roe enhancement from *S. droebachiensis* from the North of Norway regardless of seasonality (time of year), site selection, and initial GI. Relatively small changes (up to 3.0°C) in the seawater temperature appear to have a slightly negative impact on enhancement efforts. However, this may vary when larger differences in temperature are experienced.

Data Availability

The data used to support the findings from these trials are available at Zenodo 10.5281/zenodo.7376916.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors gratefully acknowledge funding from EU H2020 Blue Bio, project number: 311736.

References

[1] L. G. Harris and S. D. Eddy, "sea urchin ecology and biology," *Echinoderm Aquaculture*, pp. 3–24, John Wiley & Sons, Inc, Hoboken, New Jersey, 2015.

[2] A. M. Eger, E. M. Marzinelli, H. Christie et al., "Global kelp forest restoration: past lessons, present status, and future directions," *Biological Reviews*, vol. 97, no. 4, pp. 1449–1475, 2022.

[3] P. James, S. Siikavuopio, and G. Johansson, *A Guide to the Sea Urchin Reproductive Cycle and Staging Sea Urchin Samples*, Nofima AS, Tromsø, Norway, 2 edition, 2018.

[4] C. W. Walker, T. Unuma, and M. P. Lesser, "Gametogenesis and reproduction of sea urchins," *Evelopments in Aquaculture and Fisheries Science*, vol. 37, pp. 11–33, Elsevier, Amsterdam, Netherlands, 2007.

[5] C. L. Garrido and B. J. Barber, "Effects of temperature and food ration on gonad growth and oogenesis of the green sea urchin, *Strongylocentrotus droebachiensis*," *Marine Biology*, vol. 138, no. 3, pp. 447–456, 2001.

[6] A. F. Bennett, "Activity metabolism of the lower vertebrates," *Annual Review of Physiology*, vol. 40, no. 1, pp. 447–469, 1978.

[7] R. C. Newell and G. M. Branch, "The influence of temperature on the naintenance of metabolic energy balance in marine invertebrates," *Advances in Marine Biology*, vol. 17, pp. 329–396, 1980.

[8] Y. Zhan, J. Li, J. Sun et al., "The impact of chronic heat stress on the growth, survival, feeding, and differential gene expression in the Sea urchin Strongylocentrotus intermedius," *Frontiers in Genetics*, vol. 10, p. 301, 2019.

[9] T. Leach, B. BuyanUrt, and G. Hofmann, "Exploring impacts of marine heatwaves: paternal heat exposure diminishes fertilization success in the purple sea urchin (*Strongylocentrotus purpuratus*)," *Marine Biology*, vol. 168, no. 7, p. 103, 2021.

[10] A. K. Azad, C. M. Pearce, and R. S. McKinley, "Effects of diet and temperature on ingestion, absorption, assimilation, gonad yield, and gonad quality of the purple sea urchin (*Strongylocentrotus purpuratus*)," *Aquaculture*, vol. 317, no. 1-4, pp. 187–196, 2011.

[11] P. James, P. Heath, and M. Unwin, "The effects of seasonality, temperature and initial gonad condition on roe enhancement of the sea urchin *Evechinus chloroticus*," *Aquaculture*, vol. 270, no. 1-4, pp. 115–131, 2007.

[12] H. Gouda and Y. Agatsuma, "Effect of high temperature on gametogenesis of the sea urchin *Strongylocentrotus intermedius* in the Sea of Japan, northern Hokkaido, Japan," *Journal of Experimental Marine Biology and Ecology*, vol. 525, Article ID 151324, 2020.

[13] J. Bancroft and A. Stevens, *Theory and Practise of Histological Techniques*, Elsevier Health Sciences, Amsterdam, Netherlands, 1990.

[14] S. I. Siikavuopio, A. Mortensen, and J. S. Christiansen, "Effects of body weight and temperature on feed intake, gonad growth and oxygen consumption in green sea urchin, *Strongylocentrotus droebachiensis*," *Aquaculture*, vol. 281, no. 1-4, pp. 77–82, 2008.

[15] S. I. Siikavuopio, *Green sea urchin (Strongylocentrotus droebachiensis, Müller) in aquaculture: the effects of environmental factors on gonad growth*, University of Tromsø, Tromsø, Norway, 2009.

[16] P. James and P. Heath, "Long term enhancement of *Evechinus chloroticus*," *Aquaculture*, vol. 278, no. 1-4, pp. 89–96, 2008.

[17] K. M. Norderhaug and H. C. Christie, "Sea urchin grazing and kelp re-vegetation in the NE Atlantic," *Marine Biology Research*, vol. 5, no. 6, pp. 515–528, 2009.

[18] Urchinomics, "Restoring Ocean habitats to feed the world," https://static1.squarespace.com/static/5a9e21884eddecded2210b22/t/5bab2d71419202c59851cf4b/1537944946188/Urchinomics_Press_Info.pdf.

[19] S. I. Siikavuopio, J. S. Christiansen, and T. Dale, "Effects of temperature and season on gonad growth and feed intake in the green sea urchin (*Strongylocentrotus droebachiensis*)," *Aquaculture*, vol. 255, no. 1-4, pp. 389–394, 2006.

[20] S. I. Siikavuopio, J. S. Christiansen, B. S. Sæther, and T. Dale, "Seasonal variation in feed intake under constant temperature and natural photoperiod in the green sea urchin (*Strongylocentrotus droebachiensis*)," *Aquaculture*, vol. 272, no. 1-4, pp. 328–334, 2007.