水 研 機 構 研 報, 第50号, 47 − 53, 令 和 3 年
Bull. Jap. Fish. Res. Edu. Agen. No. 50, 47−53, 2021

Preliminary feasibility assessment of purple sea urchin, *Strongylocentrotus purpuratus*, roe enhancement.

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Abstract: Sea urchin barrens and can stretch over 1000s of kilometers and last decades at a time. They are characterized by a predominance of urchins and coralline algae where kelp forests once existed. In contrast to barrens kelp forests provide habitat supporting thousands of vertebrates, invertebrates and plant species. Because kelp forests are keystone hosts their presence is vital to sustaining commercial and recreational industries including fishing and tourism. However, these kelp forests can collapse and shift to alternate stable states whereby urchin barrens persist. Over the last 4 decades, transitions between kelp beds and sea urchin barrens have been widely reported along temperate coastlines globally. During a kelp forest phase, urchin predation is the primary mechanism keeping sea urchin populations in check. However, due to various factors including climate change, predator densities can be reduced leading to shifts toward urchin barrens. Development of urchin fisheries has been implicated several times in recent history as a driver to return urchin barrens to kelp forests. However, this driver most recently has not worked in California and Oregon, USA where a large barren is persisting and expanding. Both states already had established urchin fisheries but it has been uneconomical for the fisheries to operate given urchins in the barrens had little gonad development or undesirable human consumption traits necessary for commercialization. Aquaculture in the US has potential to restore kelp forests by collaborating with fisheries to harvest wild urchins from barrens and fatten them in an aquaculture setting prior to sale. Although sea urchin roe enhancement is not a novel concept there are still technical limitations to the activity, primarily being the availability of macroalgae diets given seasonality and the propensity of urchin barrens to deplete kelp forests. Development of sustainable alternative diets for urchins is necessary for future commercial urchin aquaculture. In this student lead study, a preliminary replicated diet trial was performed for enhancing roe from purple sea urchin (*Strongylocentrotus purpuratus*) collected from California barrens using 4 diet treatments including giant kelp (*Macrocystis pyrifera*), ogo (*Gracilaria pacifica*), formulated commercial diet (Urchinomics), and an unfed control. During the 10 week study duration, gonadal somatic index (GSI) was measured in a subset of urchins (5 individuals) from each replicate tank every 2 weeks. Baseline GSI at the beginning of the trial was <0.5%. A GSI of 10% was reached most rapidly in the formulated diet treatment at 6 weeks, followed by ogo and kelp at 9 and 13 weeks respectively. This study was a preliminary examination of the feasibility of urchin ranching in California, showing biological potential for alternative diets to develop urchin gonads with a view to restore kelp forests and develop a nascent aquaculture industry in California.

Key words: urchin, barren, aquaculture, kelp, restoration

2020年12月11日受理（Accepted on December 11, 2020）

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Introduction

A sea urchin barren is a benthic habitat dominated by urchins, coralline algae, rocky substrate and largely devoid of macroalgae (Filbee-Dexter and Scheibling, 2014). Such barrens have been recorded throughout the world in coastal temperate marine waters and can range in size from 10s of m^2 to 1000s of km^2. Temporarily, urchin barrens are considered an alternative stable state of a marine ecosystem persisting in timescales from one year to several decades. The alternate stable state of the urchin barren is typically a kelp forest. There are marked differences in the biodiversity and general productivity of the urchin barrens and kelp forests. While urchin barrens are characterized by a predominance of urchins and coralline algae, kelp forests provide habitat supporting thousands of vertebrates, invertebrates and plant species. It is this keystone host role of kelp that make the presence of kelp forests the desired stable state for marine resource management as it supports commercial and recreational industries including fishing and tourism.

Over the last 4 decades, transitions between kelp beds and sea urchin barrens have been widely reported along temperate coastlines globally. During a kelp forest phase, urchin predation is the primary mechanism keeping sea urchin populations in check (Filbee-Dexter and Scheibling, 2014). However, due to various factors including both natural and anthropogenic, predator densities can be reduced leading to shifts toward urchin barrens. Unlike conventional animal population theory, once sea urchin grazing consumes the surrounding macroalgal biomass, the urchin population does not collapse or relocate, but rather urchin growth rate decreases and they enter a reproductive dormancy as a result of changing their feeding activity to less nutritious encrusting algae or microalgal biofilms (Lawrence, 1982). Hence, once formed, urchin barrens are a stable state and dominant feature of rocky reefs, surviving decades with individual urchins living up to 50 years on barrens (Ling and Johnson, 2009). Additionally, modelling research suggests that in order to return an urchin barren to a kelp forest state, urchin numbers must be reduced to abundances significantly lower than the urchin population before the phase switch to the barren state (Ling *et al.*, 2015). It is characteristics such as those described above that make urchin barrens persistent and long lived resulting in marked reduction in ecosystem services and negative economic effects on associated commercial and recreational industries (Rocha *et al.*, 2015).

Many sea urchin species around the world ordinarily represent valuable fisheries based on harvesting their roe for eventual consumption in gourmet restaurants. The roe is considered a delicacy in many cultures commanding market prices up to $248/kg in Japan (Stefánsson *et al.*, 2017). Economic analyses suggest that there is an unmet demand for good quality sea urchin roe. Considering the favorable economics of sea urchin fisheries, the occurrence of an overpopulation of sea urchins as seen in urchin barrens should represent an opportunity to increase fisheries landings and profits. However, as previously stated, once urchin barrens are established in an area, the macroalage that would ordinarily sustain their roe development is largely overgrazed resulting in the sea urchins entering a metabolic dormancy during which little if any roe is produced. This characteristic coupled with sea urchins' capacity to exist in low food conditions for extended periods make the conventional fishing of the sea urchins commercially unfeasible as a solution to remove and restore urchin barrens to kelp forests.

Aquaculture offers a potential economically viable solution to aid in the removal of sea urchins from barrens. While full life-cycle aquaculture exists for some sea urchin species it's development as an industry has been hindered by relatively slow growth (5 years to reach market size) impacting its profitability (James *et al.*, 2015; Unuma *et al.*, 2015; Williamson, 2015). In contrast, gonad enhancement of wild caught sea urchins can be achieved in relatively short time periods, 6 – 12 weeks (Heflin *et al.*, 2016). Sea Urchin gonad enhancement involves the capture of urchins with inferior roe qualities and subsequent culture of the animals to actively improve their roe qualities for market. Following capture of the wild sea urchins, gonad enhancement typically involves housing the animals in an aquaculture system and fed a diet designed to rapidly increase their gonad size and improve their taste, texture and color commensurate with market expectations. With the recent increased prevalence and concern over sea urchin barrens, research efforts have increased to determine nutrition requirements and optimize culture systems for many species of sea urchins (Brown and Eddy, 2015).

Currently, a recently formed large sea urchin barren is affecting coastal kelp beds off California and Oregon, USA. It is estimated that purple sea urchin (*Strongylocentrotus purpuratus*) abundance in both states have increased 10,000% above baseline populations and have consumed over 90% of kelp coverage in northern California alone (Rogers-Bennett and Catton, 2019). This barren has attracted the attention of marine resource managers and agencies in both states looking to address its affects and restore kelp beds. The research detailed herein is a preliminary investigation into purple urchin (*S. purpuratus*) gonad enhancement using alternative feed stuffs as a potential tool to aid in the restoration of kelp beds in an economically viable manner.

Materials and Methods

The experiment was conducted at Moss Landing Marine Laboratories, California USA for a period of 10 weeks beginning in March, 2019 and concluding in May, 2019. 12 circular (1000 L) flow-through tanks (8 L/min) were used for the experiment with each one randomly assigned a diet treatment. The four diet treatments included an unfed control, a commercially formulated pellet diet (Urchinomics), giant kelp (*Macrocystis pyrifera*) and ogo (*Gracilaria pacifica*). There were three tank replicates of each treatment. The incoming seawater reflected nearshore conditions; with the temperature ranging from 10 – 17℃ and the salinity at 32 – 34 ppt. Tanks were aerated with air stones and dissolved oxygen maintained between 6 – 8 mg/L for the duration of the experiment. Each tank was initially stocked with 40 purple sea urchins, with a minimum test diameter of 39 mm. The urchins were collected from a nearby urchin barren in Monterey Bay by scuba divers, about a half a mile from shore. Before stocking the tanks, 30 urchins were randomly sampled to determine baseline values for test diameter and gonadosomatic index (GSI) (James *et al*., 2017).

$$\mathrm{GSI} = (\text{gonad wet weight}/\text{total weight}) \times 100$$

The urchins were fed to satiation with new feed added every 3 days providing ample feed within the tank to reduce searching behavior or periods where the urchins could not readily feed. Remaining uneaten feed after the 3 day period was removed from the tank and replaced with fresh feed. Mortality was negligible throughout the study and tanks were cleaned weekly to remove feces and biofouling on tank surfaces. Every 14 days, five urchins were randomly selected from each tank, dissected and GSI measured and recorded.

Changes in test diameter and GSI across time were calculated using a linear regression. In order to account for any tank effects, the average GSI from each replicate tank was used in the regression for each treatment. To predict the time it would take to raise an urchin to marketable roe content (GSI = 10%), the regression line equation was used. In order to find changes in GSI between the treatments over time while accounting for our hierarchical experimental design, a nested ANOVA using a linear mixed effects model was used. All assumptions about normality and homoscedasticity in the data and residuals were met. All statistics were conducted using R.

Results and Discussion

Expectantly, there were no changes in test diameter in any of the treatments across the course of the experiment (**Table 1**) due their reported relative slow growth. However, all of the treatments showed an increase in GSI across the course of the experiment (**Table 2**, **Fig. 1**). When accounting for the ranked experimental design, there were differences in GSI between the treatments themselves and differences across time. The effect of diet treatment on GSI also changed throughout the course of the experiment as a function of time (Nested ANOVA, Treatment: $F = 16.2$, $p = 0.0009$; Day: $F = 273.8$, $p < 0.0001$, Treatment: Day: $F = 15.9$, $p < 0.0001$). The urchins fed the formulated pellets showed the highest increases in GSI, followed

Table 1. *Change in test diameter.* The change in test diameter over time for each treatment was analyzed using linear regression

Treatment	Line Equation	df	F statistic	R^2	*p*-value
Control	y = −0.02x + 48.25	1, 14	0.296	0.005	0.589
Formulated	y = −0.04x + 48.36	1, 14	0.9	0.014	0.346
Kelp	y = 0.01x + 47.97	1, 14	0.08	0.001	0.781
Ogo	y = −0.04x + 48.74	1, 14	1.14	0.017	0.289

Table 2. *Change in GSI.* The change in GSI over time for each treatment was analyzed using linear regression

Treatment	Line Equation	df	F statistic	R^2	*p*-value
Control	y = 0.76x − 1.08	1, 14	261	0.952	5.51E-10
Formulated	y = 1.97x − 2.65	1, 14	272.63	0.954	4.20E-10
Kelp	y = 0.83x − 0.92	1, 14	46.5	0.781	1.23E-05
Ogo	y = 1.23x − 1.22	1, 14	158.77	0.924	1.16E-08

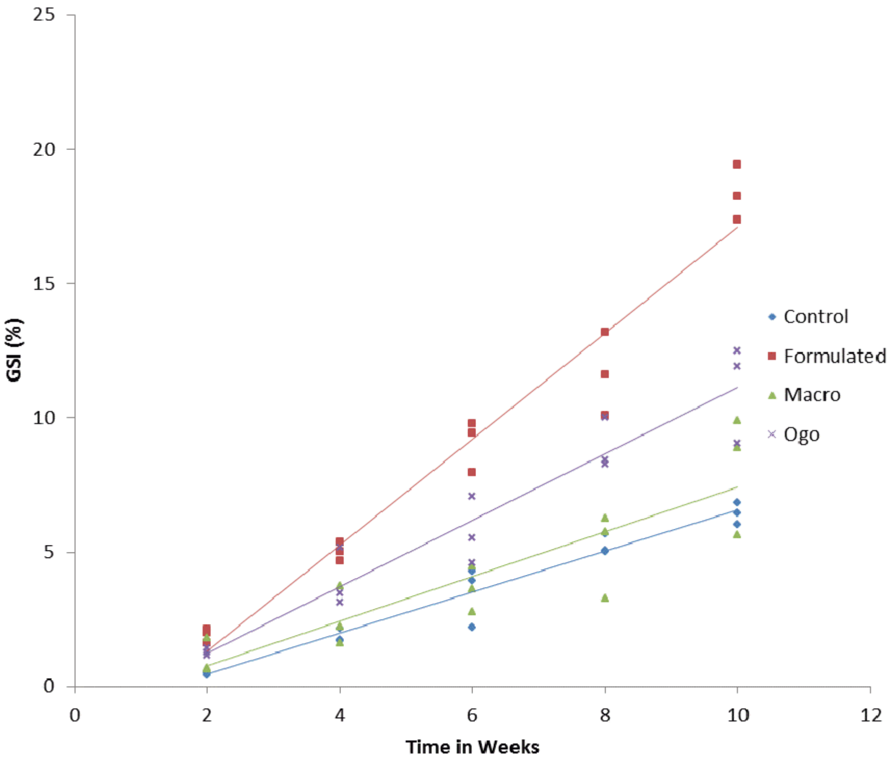

Fig. 1. Scatter plot of GSI values over time in weeks. Each data point represents a replicate tank average per treatment. Data points are distinguished to treatment level by a different color shapes/signs indicated in the figure key. Solid lines are linear regressions fitted to the respective treatment data points.

by those that were fed ogo. Interestingly, the urchins that were fed kelp showed similar growth to the control treatment wherein urchins were not provided any feed. Based on the GSI growth curves in each treatment, it would take 45 days (~6 weeks) to rear the urchins on formulated pellets to a marketable GSI of 10%. Similarly to reach 10% GSI it would take urchins 63 days (~9 weeks) fed an ogo diet, 91 days (~13 weeks) fed a kelp diet, and 101 days (~14 weeks) for urchins that are not fed but consuming tank biofilm. Note that the final two predictions are outside the range of the linear regression for this study and thus likely unreliable.

Aquaculture roe enhancement of purple urchins is a potential tool for resource managers to restore kelp forests while also creating economic opportunities for both fisheries and aquaculture sectors. This activity also avoids wanton waste of a marine resource as is typical of urchin culling activities and the antithesis of resource management. Feed type is considered the most critical aspect of sea urchin roe enhancement as it heavily influences both the rate of roe production, texture, taste and color (Heflin *et al*., 2016). All these characteristics are important to the economic feasibility of the activity. This preliminary study was conducted to examine gonad production rate for the biological feasibility of urchin roe enhancement in purple urchins collected from barrens off the California and Oregon coasts. While roe growth rate is only one aspect of roe enhancement the speed at which it develops will markedly affect profitability. The different feed types assessed for the study were selected to gauge relative growth rates on likely feed stuffs that could conceivably be used should the activity become established. They included an easily cultured seaweed (ogo) and a commercially formulated pellet diet (Urchinomics). The natural diet of kelp was also used as a type of positive control to compare their growth rates, although the authors note that due to the local presence of sea urchin barrens, kelp is not a viable feed stuff for roe enhancement. Similarly a negative control was also used, wherein no exogenous feed was supplied to the sea urchins.

Expectantly the pelleted diet treatment resulted in the most rapid roe production reaching a marketable GSI of 10% in just over 6 weeks. This is particularly noteworthy when considering the starting GSI of the sea urchins was >0.5%. Such a roe production rate is on par with some of the fastest roe enhancement rate reported elsewhere for other species (James, 2006). Also encouraging is that the ogo seaweed diet yielded relatively rapid roe production rates as well taking approximately 9 weeks to reach market size. The disparity between these two treatments is likely due to caloric density and digestibility being greater in the pellet diet compared to the seaweed diet (Cyrus *et al*., 2013) but this was not assessed for the study. While the ogo treatment yielded relatively slower roe production rates compared to the pellet diet, the finding is still encouraging as the duration is within a time period reported as acceptable for other roe enhancement research. Furthermore, the feeding of a live seaweed capable of vegetative/fragmentation growth like ogo, offers other benefits including excellent feed stability and longevity, ability to maintain continuous culture onsite and potentially extract dissolved nutrients from the water column as a result of sea urchin culture. These characteristics and general simplicity of the diet could complement the artisanal nature typical of commercial urchin fisheries, affording fishers an opportunity and low entry cost to enhance their wild caught sea urchins. In contrast the kelp positive control treatment and negative control treatment curiously yielded much slower but similar roe production rates. A potential explanation for the unfed control treatment displaying roe growth rates is a verification of sea urchins hardiness. The negative control tanks experienced observable biofouling between the weekly cleanings and thus it is theorized that the biofilm on the tank surfaces provided enough nutrition to support modest roe production. As all tanks in the experiment experienced the same biofouling activity it is unlikely to have confounded the results of the experiment overall.

In conclusion this preliminary research has indicated that rapid roe enhancement of purple urchin derived from urchin barrens in California, USA is feasible using both a formulated pellet feed and a live red seaweed species. While it was not in the scope of this preliminary experiment, future research for roe enhancement of purple urchins should include a more thorough assessment of nutrition requirements including proximate analysis of feed types, measurements of digestibility and intake rate as well as quantitatively assessing the resulting roe for important market characteristics including color, texture and taste. Based on the outcome of these and other biological considerations of purple urchin (*S. purpuratus*) roe enhancement, an economic feasibility is also required to determine the commercial potenital of this aquaculture activity for transforming urchin barrens back to kelp forests.

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This paper provides an excellent description of sea urchin barrens as to what they are comprised of, and their extent both temporally and spatially across the world. The authors examine and list the drivers of phase shifts between barrens and kelp forests.

They describe different thresholds for forward (to barrens) and reverse (to kelp beds) shifts, in accordance with alternative stable-state dynamics. They surmise that accelerating climate change and increasing anthropogenic impacts play important roles in altering alternative stable-state dynamics and triggering phase shifts.

(2) Heflin L. E., Makowsky R., Taylor J. C., Williams M. B., Lawrence A. L., and Watts S. A., 2016: Production and economic optimization of dietary protein and carbohydrate in the culture of Juvenile Sea Urchin *Lytechinus variegatus*. *Aquaculture* **463**, 51–60. (doi.org/10.1016/j.aquaculture.2016.05.023)

This manuscript is a through collection of urchin feeding experiments to understand the nutrient requirements of urchins for aquaculture. The paper creates predictive models of growth, production and efficiency outcomes and generates economic analysis models in relation to these dietary outcomes for urchins held in culture. The models compare dietary requirements and growth outcomes in relation to economic costs and provide insight for future commercialization of sea urchin aquaculture

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This is the most recent review of urchin aquaculture in Japan. Japan is the foremost consumer of urchins and sigificnfact producers of urchins both from ranching and closed life cycle aquaculture. The chapter details the history of urchin fisheries in Japan and the rise of urchin barrens and urchin aquaculture. The review discusses the diver mediated destruction of urchins to bring back kelp beds as well as reseeding efforts to restore overfished urchin grounds. The review also discusses the development of full life-cycle aquaculture to meet both reseeding and commercial production requirements. Also the movement of urchins from barrens to other kelp grounds and aquaculture facilities for commercial fattening are detailed.