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Experimental field tests of natural algal diets on gonad index and quality in the green sea urchin, *Strongylocentrotus droebachiensis*: a case for rapid summer production in post-spawned animals

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Abstract

We tested whether the roe (gonads) of “post-spawned”, green sea urchins, *Strongylocentrotus droebachiensis*, from barren grounds could be enhanced in the field during summer. Experiments were initiated using a low roe-yielding (barren ground) population, which also served as a field control. Specifically, we determined the ability of naturally occurring macroalgae to increase roe yield and enhance roe color relative to field controls. Sixty experimental cages with algae and five test animals per cage (mean test diameter = 50.0 mm) were suspended along the seaward end of a commercial lobster impoundment on Beals Island, Maine, on 18 June 1996. Urchins were fed ad libitum all (mixed diet) or one of four species of macroalgae (*Palmaria palmata*, *Alaria esculenta*, *Laminaria saccharina* and *Ulva lactuca*). Four replicate cages of each algal treatment and 20 individuals from the control population were sampled (without replacement) every 3 weeks until 20 August. This design permitted the use of orthogonal contrasts in both one- and two-factor ANOVAs. These analyses revealed significant enhancement, relative to controls, of both color and roe yield (gonad index doubled or tripled within 2 months). Algal-fed animals attained a mean gonad index greater than 10%, the minimum commercial standard in Maine, while field populations ranged from 4% to 6%. These analyses revealed differential roe enhancement among palatable seaweeds. The red alga, *P. palmata*, induced the quickest and highest response, singly, among the four algae tested. Roe production on *P. palmata* was generally higher, but similar to

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that of the mixed diet. The two kelp species, *A. esculenta* and *L. saccharina*, consistently produced the lowest yields. Roe yields were correlated with protein levels in the algae. Our study also provides some insight into seasonal allocation of energy and nutrients into gonadal tissue. These data show that off-season allocation to gonadal tissue is biologically feasible in the absence of photoperiodic manipulation and that summer enhancement could be used to meet the off-season (August) market demand for roe in Asia. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Green sea urchin; *Strongylocentrotus droebachiensis*; Gonad; Macroalgae; Roe yield; Roe color

1. Introduction

Sea urchins are widely distributed throughout the world's oceans and often play major roles in controlling macroalgal populations and organizing the structure of shallow subtidal communities (Harrold and Reed, 1985). The diets of these echinoderms consist largely of seaweeds (Lawrence, 1975; Vadas, 1990), but they also feed on microalgae, detritus, a variety of drift items (terrestrial and marine) and small invertebrates (Himmelman and Steele, 1971; Chapman, 1981; Harrold and Reed, 1985; Sebens, 1986; Vadas et al., 1986; Witman, 1987; Briscoe and Sebens, 1988). In many regions, intensive grazing and behavioral activities create halos (Ogden et al., 1973; Andrew, 1994; Andrew and Choat, 1985), barren grounds or coralline pavements (Breen and Mann, 1976; Ayling, 1981; Himmelman and Lavergne, 1985). At high densities, grazing becomes intense and food availability becomes limiting, thereby reducing energy potential for growth and reproduction (Keats et al., 1984). On barren grounds, urchins produce significantly less roe than conspecifics in areas dominated by macrophytes (Lang and Mann, 1976; Vadas, 1977). Although the term roe is generally reserved for egg bearing ovaries, in the urchin industry roe is synonymous with gonad and roe is used in this sense here.

The ecological roles of sea urchins worldwide are changing due to large-scale commercial harvests which reduce average body size and population density. For example, the intensive commercial exploitation of green sea urchins in Maine (USA) during the past decade has altered the dynamics of macroalgal and urchin assemblages (Steneck et al., 1994; Vadas and Steneck, 1995). Exploitation of sea urchins began in Maine as early as the 1950s. Mean annual landings between 1950 and 1984 was 31.2 metric tons (Fig. 1). A large Asian market for Maine sea urchins was created in 1987, and annual landings have increased dramatically (Fig. 1). Landings peaked in 1993 at 18,880 t (NMFS, 1999), but have declined by ca. 40% during the last 3–4 years. These data and the reduced catch per effort by divers (T. Creaser, Maine Dept. Mar. Res., pers. comm., 1997) have led to concerns of overharvesting. Some of the issues involved in the commercial urchin fisheries are: length of season, discontinuous market demands, off-season harvests, roe quality and quantity, and long-term sustainability.

Historically, the dynamics of commercial markets have not been in-phase with the life cycle of the green sea urchin in Maine. Only one of three Asian holidays (January) coincides with naturally high roe yields. The other two (mid-May and early August) occur when roe yields are decreasing or naturally low across the state (Vadas et al.,

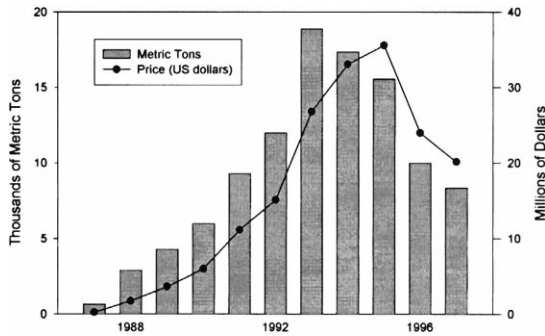


Fig. 1. Annual landings and economic value of green sea urchins in Maine (NMFS, 1999). (Mean [in metric tons] from 1950–1984 per year = 31.2 ± 1 SE = 2.9 MT; $n = 33$; no data available for 1982–1983 and 1985–1986).

1997). One method of addressing the discordance between gonadal cycles and market demand is to enhance artificially roe. Gonad enhancement can be done in the laboratory or in the field using artificial or natural diets (Tegner, 1989). Laboratory studies indicate that roe can be enhanced using various diets and manipulating photoperiod (Fuji, 1967; Vadas, 1977; Larson et al., 1980; Keats et al., 1984; Lawrence et al., 1992; Minor and Scheibling, 1997; Walker and Lesser, 1997). It is clear from laboratory studies that roe can be enhanced over longer (3–5 months) periods prior to spawning, when roe are naturally increasing. Recent work suggests that the roe of post-spawned urchins may also be enhanced in the laboratory (Klinger et al., 1997). It is not clear whether the roe of immediately post-spawned urchins can be enhanced in shorter time frames and under field conditions. We are unaware of published attempts to enhance roe in field enclosures except for Keats et al. (1983) and Lemire and Himmelman (1996). Lastly, the relationship between roe enhancement and photoperiod is unclear, possibly because of the requirement of short day lengths for gametogenesis (Pearse et al., 1986; Hagen, 1997; Walker and Lesser, 1997).

Here, we utilize animals from a barren habitat to provide a value-added product and an alternative strategy for the urchin industry. Specifically, we test the ability of various naturally abundant macroalgae to increase the roe yield of *Strongylocentrotus droebachiensis* in the field. Urchins in Maine spawn during late winter and early spring (Cocanour and Allen, 1967; Vadas et al., 1997). Our efforts to enhance roe yields are somewhat unique because they are directed at summer (off-season) post-spawned urchins to provide roe for an August market in Asia. Utilizing ranched urchins would obviate the need to harvest urchins prematurely during August when average roe yields are low and many urchins would be required to satisfy market demands. We also test the effects of varying macroalgal diets on the color of summer induced roe. The relationship between the diversity of macroalgae assayed, their nutritional (e.g., protein) content, and roe enhancement is analyzed. Our results contribute to ongoing discussions on the enhancement of roe, the seasonal allocation of nutrients and energy to roe yields, and the relationship of day length to roe enhancement.

2. Materials and methods

2.1. Experimental cages and diets

This study was conducted in an unused commercial lobster impoundment (Bayer, 1984) on Beals Island adjacent to Mooseabec Reach near Jonesport, Maine, USA (44°31'N; 67°36'W). The lobster pound was influenced by semi-diurnal tides having a mean amplitude of 3.5 m. The lower (seaward) portion of the pound was bulkheaded and approximately 1.75 m of water was retained within the pound at low tide. Sixty experimental cages with algae and test animals were randomly arranged in a long line (1 × 60 array) and suspended along the seaward end of the pound.

Cages (30 cm × 30 cm × 30 cm) were constructed from 12-gauge vinyl-coated lobster trap wire with a mesh size of either 12.7 mm or 25.4 mm. The two mesh sizes were randomly assigned macroalgal treatments. The top of each cage was removable and made from lobster trap wire or extruded plastic netting (aperture = 12.7 mm). The inside bottom of each cage was covered with flexible plastic netting (aperture = 3.2 mm) to permit better attachment of tube feet.

On 18 June 1996, 320 post-spawned green sea urchins (Vadas et al., 1997) were collected by divers from a subtidal (6 m), non-commercial site near Johnson Bay, Lubec, Maine (44°51'N; 66°59'W). The shoreline was covered by a steep, artificial enhancement of boulders and granite blocks sloping to a sandy sediment bottom. Water currents were strong on the ebbing tide (ca. 2 knots) but were relatively weak during the flood tide. The site was a barren environment with few attached macroalgae except *Agarum clathratum* and coralline algae on rock outcrops. Drift *Laminaria* spp. were scattered across the bottom on the first and subsequent dives at this site and large numbers of moderately sized urchins (< 55 mm test diameter) were usually aggregated on it. Animals were immediately transported to Beals Island in coolers and five urchins selected randomly (mean test diameter = 50.0 mm; range = 47 to 54 mm) were added to each cage. The cages were suspended 30 to 50 cm above the bottom of the pound to minimize fouling and possible anoxia. All cages were fully submerged at low tide and were shaded by the bulkhead of the lobster impoundment. A random sample of 20 urchins from the original collection provided an initial baseline for measurements of test diameter, body weight, gonad color and gonad weight.

Gonad indices for each 3-week phase were calculated as (gonad weight ÷ total weight) × 100 and given as a percent. Total urchin weight was determined by shaking off excess water and then weighing the individual. Gonads were removed from the test and blotted dry on a paper towel prior to weighing. Roe color was determined qualitatively for each urchin on each sampling date by two independent samplers (R. Vadas and T. Dowling). Roe color was evaluated using color standards (Eiseman and Herbert, 1990). Gonads were further delineated into market grades: A = yellow, yellow-orange, orange; B = light-brown, orange brown; C = rust, dark brown (Table 1).

Caged urchins were fed ad libitum with one of four species of macroalgae (*Palmaria palmata* [Rhodophyta], *Alaria esculenta* [Phaeophyta], *Laminaria saccharina* [Phaeophyta] or *Ulva lactuca* [Chlorophyta]) and an even mixture (by weight) of the four species. The macroalgae utilized were based on the potential to enhance gonad produc-

Table 1
Relationship between roe color of *S. droebachiensis* and color standards

Color category ^{a,c}	Number of color standard and description ^b	
(1) Dark Brown	18-1425 (Mahogeny)	18-1415 (Marron)
(2) Rust	17-1353 (Apricot Orange)	16-1448 (Burnt Orange)
(3) Orange Brown	16-1257 (Sun Orange)	16-1253 (Orange Ochre)
(4) Light Brown	12-0817 (Cream)	12-0911 (Nude)
(5) Orange	16-1359 (Orange Peel)	16-1462 (Golden Poppy)
(6) Yellow/Orange	12-0736 (Lemon Drop)	13-0840 (Snapdragon)
(7) Yellow	11-0616 (Pastel Yellow)	12-0727 (Sunshine)

^aColor standards and rank numbers used in our analyses of roe color.

^bColor standards after Eiseman and Herbert (1990).

^cGrade A = 5, 6, 7; Grade B = 3, 4; Grade C = 1, 2.

tion, color and quality (Larson et al., 1980). Also, *Palmaria* and *Ulva* were relatively abundant in the mid to low intertidal, and both kelps (*Alaria*, *Laminaria*) were easily obtained from the shallow subtidal. Algal diets were assigned randomly to each of the sixty cages. Fresh algal collections were made weekly from mid-June to mid-August as tides permitted. *A. esculenta* and *L. saccharina* were collected from Black Duck Cove on Great Wass Island (44°28'N; 67°36'W). *P. palmata* and *U. lactuca* were collected from Bell Farm Cove in Whiting Bay (44°49'N; 67°09'W). Urchins fed on the various macroalgal diet treatments, which were contained within two nylon lobster bait bags (2 cm mesh size) used to constrain the algae (125 g/bag) within each cage. Since the food was retained within the bait bags, cage mesh size was not a factor in constraining the algae. Bags were tied securely to the opposite sides of each cage (off the bottom of the cage) to provide urchins ready access to the algae through the bait bag. This arrangement also prevented the food bags from moving and disturbing the feeding urchins. At weekly intervals (5–7 days) all cages were removed from the water for inspection and for adding fresh algae to the bait bags. Fresh algal samples were taken from the collection sites on four occasions during the experimental period for tissue analysis (nutrient, protein and lipid content). In addition, samples of tissue remaining in the bait bags after 1 week were also collected at the end of each 3-week phase. Both types of algal samples were gently washed in freshwater prior to analysis to remove sediment and grazing macrofauna such as amphipods, isopods and littorinids.

On 9 July, 30 July, and 20 August, 20 cages (four cages of each of the five algal treatments) were removed from the pound and the urchins measured and dissected to provide temporal data on gonad and color indices. To compare summer gonad index and roe enhancement with a natural population, 20 urchins (controls) were sampled haphazardly using scuba from the field collection site (Johnson Bay, Lubec) every 3 weeks on the same date experimental urchins were sampled from cages.

2.2. Statistics

To test the effects of the five experimental algal diets (and field control) and sampling periods on roe enhancement, we performed both one- and two-way model I

analyses of variance (SAS Institute, 1989) on mean gonad index per cage ($n = 4$). A two-way model I ANOVA on the ranked color data (Quade, 1966) was used to test for diet and sampling date effects. To balance the experimental design and allow us to test for enhancement effects relative to field controls, we used mean gonad index of the field collection (control) of twenty animals (grouped into four random lots of five animals each) in our analysis of treatment effects. Gonad indices of experimental and control animals were arcsine-transformed to meet assumptions of variance homogeneity (Cochrans Test; Winer et al., 1991) and normality (Shapiro–Wilk’s Test; SAS Institute, 1989).

We used several pre-planned comparisons of treatment means to test a priori hypotheses (Winer et al., 1991). The following is an incomplete set of mutually orthogonal, single degree-of-freedom contrasts and their rationale that relates to both main effects.

Diets: (1) Field control vs. five experimental diets — tests whether the algal diets result in an enhancement of gonad index and/or roe color compared to field controls.

(2) Mixture vs. four single diets — tests if there are components of a mixed (balanced) diet that result in greater roe yield or better roe color than the mean of all single diets.

(3) *Palmaria* vs. other three single diets — tests the possibility that the bright red alga, *P. palmata*, which is nitrogen-saturated (due to presence of phycobilin pigments, Lobban and Harrison, 1994) and, therefore, protein-rich, is conducive to enhancing roe yield and/or color.

(4) *Alaria* vs. *Laminaria* — compares two kelps with different life history strategies; *Alaria* tends to be short-lived (*r*-selected) whereas *Laminaria* tends to be longer-lived (more *K*-selected).

Dates: (1) 9 July vs. (30 July/20 August)/2 — tests if enhancement of roe yield and color differs between the first 3 weeks and the averaged, cumulative effects of the last 6 weeks.

(2) 30 July vs. 20 August — tests whether the cumulative treatment effects on roe yield and color differ between weeks 6 and 9.

The interactions between diet and sampling date for mean gonad index were significant ($P < 0.05$) making interpretations of main effects difficult. To facilitate interpretation of these interactions, the influence of diet on gonad index was examined separately using one-way ANOVAs for each sampling date. To control for potential excessive type I errors, we followed the suggestion of Winer et al. (1991) and used a conservative ($\alpha = 0.0167$) error rate based on the number of one-way analyses (3) to make decisions about significance.

2.3. Ancillary measurements

Surface temperatures in the lobster pound were taken weekly in the vicinity of the cages to provide background data on summer patterns. Temperature was determined with calibrated thermometers. Salinity was not determined, but generally ranges from 28‰ to 30‰ in adjacent Mooseabec Reach during this time of year (B. Beal, pers. obs.).

Nutrient and lipid analyses were conducted on algal tissues provided as diet items during each of the three phases of the experiment. Standard plant nutrient composition was analyzed by Plasma Emission Analyses. Nitrogen was determined by the total Kjeldahl method (TKN) (protein content = $6.25 \times \text{TKN}$). Percent lipid was analyzed by the soxhlet lipid extraction method (AOAC, 1997). Dry weight was determined by drying to a constant weight at 70°C . On two or three occasions it was not possible to collect fresh specimens of all algae, especially sublittoral forms (e.g., *Alaria* and *Laminaria*), and the material collected for the previous week was utilized. Because of the possibility of nutrient changes with time in these tissues, analyses were also run periodically on the older algal tissues.

3. Results

3.1. Gonad index

Green sea urchins fed the five experimental algal diets showed significant enhancement of roe (gonad index) compared to the field (control) population (Fig. 2; Tables 2 and 3). It is apparent that gonadal indices increased steadily throughout the experimental period but that the rate of change varied among algal species (Diet \times Date interaction, $P = 0.003$, Table 2). Normally, if interactions are present and significant, main factors cannot be fully evaluated. Our use of *a priori* contrasts, however, permits us to examine two of the four planned diet comparisons (Mixture vs. four single diets, and *Alaria* vs. *Laminaria*). This is possible because neither contained significant variation within interaction contrasts (Table 2, contrast #s 8, 10, 12, 14). Overall, the mixed algal diet, compared to the four single diets pooled over all three sampling dates, resulted in significantly greater mean gonad index ($\bar{x}_{\text{mixed}} = 10.50 \pm 0.83\% \text{ [SE]}$, $n = 12$ vs. $\bar{x}_{\text{single diets}} = 8.34 \pm 0.41\%$, $n = 48$). Conversely, there were no significant differences in mean gonad index between the two kelp species ($\bar{x}_{\text{Alaria}} = 6.71 \pm 0.59\%$, $n = 12$ vs. $\bar{x}_{\text{Laminaria}} = 7.69 \pm 0.69\%$, $n = 12$). Similarly, urchins fed a diet of *U. lactuca* differed little in their mean gonad index from those of either kelp treatment (Fig. 2).

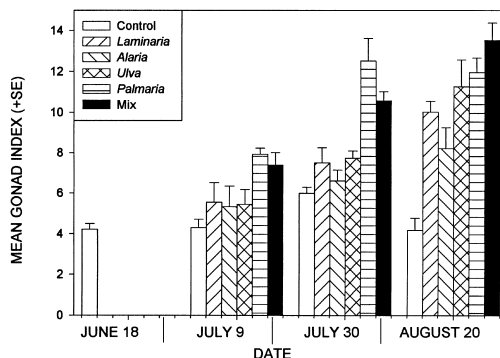


Fig. 2. Biomass of roe during the summer, post-spawning phase in *S. droebachiensis* fed five natural macroalgal diets and a field control (expressed as gonad index).

Table 2

Two-factor ANOVA (model I) on the influence of algal diet and sampling date (3-week phase) on arcsine-transformed mean gonad index in *S. droebachiensis*. Field control animals were grouped into four random lots of five animals each to balance the analysis. *A priori*, orthogonal contrasts^a appear below each main factor and the interaction term. ($n = 4$; $\alpha = 0.05$)

Source of variation	df	SS	MS	F	Pr > F
DIET	5	359.052	71.810	28.09	0.0001
(1) Control vs. Rest	1	198.774	198.774	77.75	0.0001
(2) Mixed vs. Other algae	1	44.130	44.130	17.26	0.0001
(3) <i>Palmaria</i> vs. Nonmixed algae	1	100.076	100.076	39.15	0.0001
(4) <i>Alaria</i> vs. <i>Laminaria</i>	1	7.270	7.270	2.84	0.0975
DATE	2	213.730	106.865	41.80	0.0001
(5) 9 July vs. (30 July and 20 Aug)/2	1	196.179	196.179	76.74	0.0001
(6) 30 July vs. 20 Aug	1	17.551	17.551	6.87	0.0114
DIET × DATE ^b	10	79.818	7.982	3.12	0.0033
(7) Cntrl vs. Rest × 9 JI vs. (30 JI and 20 A)/2	1	22.785	22.785	8.91	0.0042
(8) Mix vs. Oth × 9 JI vs. (30 JI and 20 A)/2	1	2.242	2.242	0.88	0.3532
(9) <i>Pal</i> vs. <i>Nomix</i> × 9 JI vs. (30 JI and 20 A)/2	1	0.597	0.597	0.23	0.6307
(10) <i>Ala</i> vs. <i>Lam</i> × 9 JI vs. (30 JI and 20 A)/2	1	1.637	1.637	0.64	0.4271
(11) Control vs. Rest × 30 July vs. 20 Aug	1	32.404	32.404	12.68	0.0008
(12) Mix vs. Other × 30 July vs. 20 Aug	1	0.686	0.686	0.27	0.6067
(13) <i>Pal</i> vs. <i>Nomix</i> × 30 July vs. 20 Aug	1	14.185	14.185	5.55	0.0222
(14) <i>Ala</i> vs. <i>Lam</i> × 30 July vs. 20 Aug	1	0.658	0.658	0.26	0.6140
Error	54	138.050	2.557		
Total	71	790.650			

^aNot all of the possible orthogonal contrasts were used under the DIET and DIET × DATE sources of variation. Therefore, the sum of the individual contrasts for these sources does not add up to the sum of squares for DIET and DIET × DATE.

^bAbbreviations for terms listed under DIET and DATE.

A priori, orthogonal comparisons demonstrated that two of the three significant interaction contrasts involved the relationship between the field control vs. the algal diets from one sampling date to another. In the first instance (Table 2, contrast #7), the difference in mean gonadal index between the field control and the five algal diets on 9 July was 1.95% ($\bar{x}_{\text{control}} = 4.3 \pm 0.41\%$, $n = 4$ vs. $\bar{x}_{\text{diets}} = 6.25 \pm 0.35\%$, $n = 20$) whereas this difference was 4.90% ($\bar{x}_{\text{control}} = 5.12 \pm 0.41\%$, $n = 8$ vs. $\bar{x}_{\text{diets}} = 10.02 \pm 0.26\%$, $n = 40$) when the average of the second two sampling dates is considered. This indicates that the rate of enhancement increased significantly during the experiment, but, primarily during the later 6 weeks. The other significant interaction involving the field control and other algal diets (Table 2, contrast #11) again demonstrates that the rate of enhancement was not static, but increased significantly from the second to the third sampling date. For example, the difference in mean gonad index between field control and the five algal diets on 30 July was 3% ($\bar{x}_{\text{control}} = 5.99 \pm 0.29\%$, $n = 4$ vs. $\bar{x}_{\text{diets}} = 8.99 \pm 0.58\%$, $n = 20$), whereas this difference was 6.8% by 20 August ($\bar{x}_{\text{control}} = 4.17 \pm 0.60\%$, $n = 4$ vs. $\bar{x}_{\text{diets}} = 11.00 \pm 0.55\%$, $n = 20$). The remaining significant interaction (Table 2, contrast #13) involved the relationship between mean gonad index of urchins fed *Palmaria* vs. the mean of those fed single diets of *Alaria*, *Laminaria*, and

Table 3

Single-factor ANOVA (model I) of the influence of algal diet on the arcsine-transformed mean gonad index in *S. droebachiensis* at 3, 6, and 9 weeks. Field control animals were grouped into four random lots of five animals each to balance the analysis. Orthogonal contrasts are utilized to test specific a priori hypotheses. ($n = 4$; $\alpha = 0.0167$)

Source of variation	df	SS	MS	F	Pr > F
<i>(A) 12 June–9 July 1996</i>					
DIET	5	37.93	7.59	3.59	0.0199
(1) Control vs. Rest	1	13.64	13.64	6.46	0.0205
(2) Mixed vs. Other algae	1	5.63	5.63	2.67	0.1198
(3) <i>Palmaria</i> vs. Nonmixed algae	1	18.57	18.57	8.79	0.0083
(4) <i>Alaria</i> vs. <i>Laminaria</i>	1	0.09	0.09	0.04	0.8354
Error	18	38.01	2.11		
Total	23	75.94			
<i>(B) 12 June–30 July 1996</i>					
DIET	5	127.40	25.48	15.28	0.0001
(1) Control vs. Rest	1	29.90	29.90	17.93	0.0005
(2) Mixed vs. Other algae	1	12.65	12.65	7.59	0.0131
(3) <i>Palmaria</i> vs. Nonmixed algae	1	82.01	82.01	49.18	0.0001
(4) <i>Alaria</i> vs. <i>Laminaria</i>	1	1.63	1.63	0.98	0.3359
Error	18	30.01	1.67		
Total	23	157.41			
<i>(C) 12 June–20 August 1996</i>					
DIET	5	219.93	43.99	14.16	0.0001
(1) Control vs. Rest	1	155.61	155.61	50.10	0.0001
(2) Mixed vs. Other algae	1	32.06	32.06	10.32	0.0048
(3) <i>Palmaria</i> vs. Nonmixed algae	1	13.43	13.43	4.32	0.0522
(4) <i>Alaria</i> vs. <i>Laminaria</i>	1	6.54	6.54	2.11	0.1640
Error	18	55.01	3.11		
Total	23	275.84			

Ulva during the final two sampling dates. After 6 weeks (30 July), urchins fed *Palmaria* had significantly higher roe yields than those fed the three single species diets (3SSD) (Fig. 2; $\bar{x}_{Palmaria} = 12.51 \pm 1.11\%$, $n = 4$ vs. $\bar{x}_{3SSD} = 7.28 \pm 0.33\%$, $n = 12$). By 20 August, however, the relationship between the enhancement effects of these single algal diets vs. *Palmaria* had changed ($\bar{x}_{Palmaria} = 11.9 \pm 0.71\%$, $n = 4$ vs. $\bar{x}_{3SSD} = 9.84 \pm 0.65\%$, $n = 12$). The significant interaction resulted from both a leveling off of gonad production in urchins fed *Palmaria* and an apparent compensatory increase in gonad production in urchins fed *Ulva*, *Laminaria*, and *Alaria* (Fig. 2).

Single-factor ANOVAs for each sampling date are shown to facilitate interpretation of main effects (Fig. 2; Table 3A,B,C). After 6 (30 July) and 9 weeks (20 August) there was a significant ($P = 0.0005$ and 0.0001 , respectively) enhancement of roe (Table 3B and C, contrast #1). Urchins fed a diet of *Palmaria*, however, exhibited the greatest increase in mean gonad index among the four single diets after 3 and 6 weeks ($P = 0.008$ and 0.0001 , respectively, Fig. 2; Table 3A,B). By the final sampling date

(20 August), however, there was no significant difference in roe enhancement between diets of *Palmaria* and the mean of the other three single algal diets ($11.96 \pm 0.71\%$, $n = 4$ vs. $9.84 \pm 0.65\%$, $n = 12$; $P = 0.052$; Table 3C). Similarly, urchins fed a diet of *U. lactuca* differed little in their mean gonad index from those of either kelp treatment (Fig. 2).

3.2. Color index

We performed a two-way ANOVA on the ranked color index data (Quade, 1966) to test hypotheses concerning the main and interactive effects of diets and sampling date (Table 4; Fig. 3). The data were normal ($P > 0.05$, Shapiro–Wilk test) and variances were homogeneous ($P > 0.05$, Cochran’s test). The ANOVA indicated that neither main (diet and date) nor interactive factors were significant ($P > 0.05$). Since the use of a priori contrasts does not depend on the significance of the overall F-test, we can examine the individual planned comparisons (Winer et al., 1991). These revealed that both the single and mixed diets enhanced the quality of roe color relative to the field control. Roe color changed from an intermediate quality (i.e., orange brown/light brown [#s 3,4; Table 1]) to a higher quality (i.e., orange, yellow/orange, yellow [#s 5,6,7; Table 1]) (Fig. 3). Color enhancement in roe did not occur immediately; significant changes in mean color index took place between the third and the sixth week (Table 4; $P = 0.0272$; contrast #5). There was no significant change in color between the final two sampling dates ($P = 0.3299$; Table 4).

The change in roe color is also reflected in the broader market grading categories. Frequencies of the three grades of roe color (A, B, C; see Section 2) for each diet and sampling date are shown in Fig. 4. The frequency of “Grade A” urchins from the control (field) population varied little throughout the experimental interval (range = 0.35 to 0.55). The highest proportion of urchins exhibiting Grade A roe occurred during the

Table 4

Two-factor ANOVA (model I) on the influence of algal diet and sampling date (3-week phase) on the untransformed mean color index in *S. droebachiensis*. Field control animals were grouped into four random lots of five animals each to balance the analysis. A priori, orthogonal contrasts appear below each main factor. ($n = 4$; $\alpha = 0.05$)

Source of variation	df	SS	MS	F	Pr > F
DIET	5	6.90	1.38	2.19	0.0682
(1) Control vs. Rest	1	5.40	5.40	8.59	0.0049
(2) Mixed vs. Other algae	1	0.01	0.01	0.01	0.9935
(3) <i>Palmaria</i> vs. Nonmixed algae	1	0.15	0.15	0.24	0.6233
(4) <i>Alaria</i> vs. <i>Laminaria</i>	1	0.17	0.17	0.27	0.6087
DATE	2	3.85	1.92	3.06	0.0551
(5) 9 July vs. (30 July and 20 Aug)/2	1	3.24	3.24	5.15	0.0272
(6) 30 July vs. 20 Aug	1	0.61	0.61	0.97	0.3299
DIET × DATE	10	7.43	0.74	1.18	0.3232
Error	54	33.94	0.63		
Total	71	52.12			

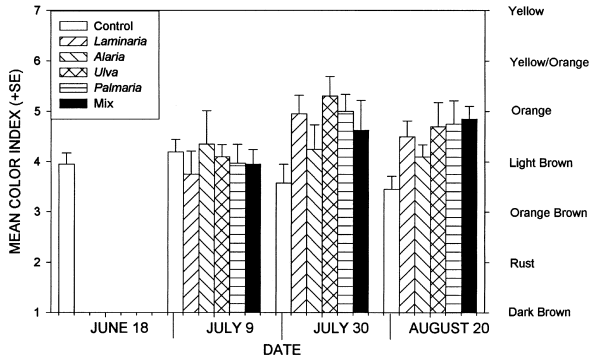


Fig. 3. Color of roe during the summer, post-spawning phase in *S. droebachiensis* fed five natural macroalgal diets and a field control (see Table 1 for equivalent color standards).

second and third sampling dates (30 July and 20 August). By the end of the experiment, all algal diets, except *Alaria*, yielded nearly twice as many high quality urchins as the field control (Fig. 4).

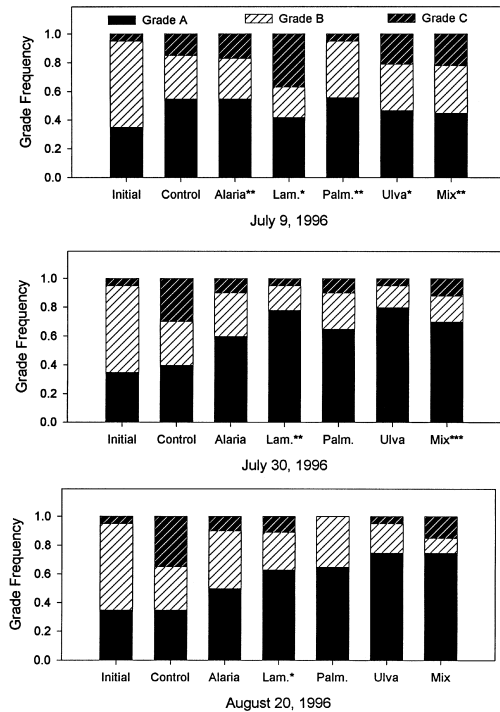


Fig. 4. Market quality of summer roe in *S. droebachiensis* fed five natural macroalgal diets and a field control on three sampling dates (based on market grades: A = yellow, yellow-orange, orange; B = light brown, orange-brown; C = rust, dark-brown); ($n = 20$, * $n = 19$, ** $n = 18$, *** $n = 17$).

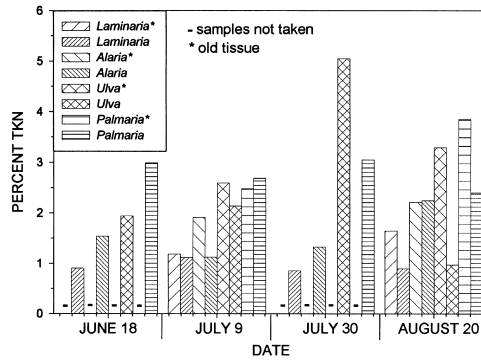


Fig. 5. Summer protein levels (% TKN) in the algal diets used for gonad enhancement in *S. droebachiensis*.

3.3. Nutrient and other measures

The nutritional content of the fresh algal diets varied between species but in a relatively consistent manner (Fig. 5). Until the final sampling date, fresh collections of *Laminaria* and *Alaria* consistently exhibited the lowest protein levels (0.85–2.24%). Protein levels generally were highest in fresh *Palmaria* and *Ulva*. Similar trends were observed in the old tissue samples (Fig. 5) although there appeared to be a secondary enhancement of nitrogen (e.g., *Alaria* and *Ulva* on 9 July, *Laminaria*, *Palmaria* and *Ulva* on 20 August). Lipid levels generally declined through time (Fig. 6), but showed a pattern similar to that of protein with slightly higher values in both *Ulva* and *Palmaria* (Fig. 6). Lipid levels in fresh *Laminaria*, *Alaria* and *Palmaria* declined steadily between June and August, whereas levels in *Ulva* did not decline until August. Lipid levels ranged from 0.10% to 0.39% in fresh *Laminaria* and *Alaria*. In *Ulva* and *Palmaria*, levels ranged from 0.12% to 0.63% (but more commonly 0.35%–0.40%). Percent algal dry weights during this period were: 5.9%–11.1% for *Ulva*, 9.3%–14% for *Palmaria*, 11.0%–13.8% for *Alaria* and 11%–17.2% for *Laminaria*.

Seawater temperatures during the study were relatively uniform: 11°C on 18 June, 15°C on 30 July, and 14°C on August 20.

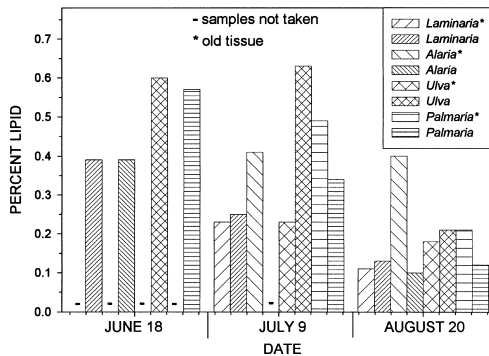


Fig. 6. Summer lipid levels in the algal diets used for gonad enhancement in *S. droebachiensis*.

4. Discussion

4.1. Gonad enhancement

Our primary objectives were to determine if, and how quickly, the roe of post-spawned green sea urchins could be enhanced under field conditions. Also of interest was the nutritional value of the various macroalgal diets and how the different diets affected both roe quantity and quality. These data show that green sea urchins can double or triple their roe content in controlled field conditions in approximately 2 months under normal summer day lengths. Further, these urchins attained (within 6 weeks) roe yields greater than 10%, which is the minimum standard used by commercial dealers in Maine. Interestingly, our data show differential roe enhancement among seaweeds that are all highly palatable to these urchins. The red alga, *P. palmata*, induced the quickest and highest mean response of the four individual algae tested. Roe production on *Palmaria* was higher on two of the three sample dates but similar to that of the mixed diet (Fig. 2). The two kelp species, *A. esculenta* and *L. saccharina* consistently produced equal, but lowest, amounts of roe. This is despite *Laminaria* spp. generally being considered to be preferred by *S. droebachiensis* (Larson et al., 1980; Lemire and Himmelman, 1996). By the end of 6 weeks, we observed a significant enhancement of roe in experimental vs. natural, field controls. This result is likely due to the differential effects of the *Palmaria* and mixed diets (Fig. 2). Our data also show that the four seaweeds enhanced the quality of roe color (Fig. 3). There were no differences in roe color among urchins fed the four algal and mixed diets over the 9 weeks. The mixed diet and the four single species diets, however, produced significantly higher color grade roe than the field control.

Our experimental design included the use of planned and independent comparisons that enabled us to partition the total sums of squares for the two main effects (DIET and DATE) from the one- and two-way ANOVAs (Tables 2–4) into several single-degree-of-freedom contrasts. Our choice of comparisons were mutually orthogonal and represent only a small subset of possible contrasts (Sokal and Rohlf, 1981; Winer et al., 1991). Similarly, the interaction sums of squares can be partitioned into a series of single-degree-of-freedom comparisons that can lead to a clearer understanding of the interactive effects. Collectively, these contrasts indicated that: (1) roe enhancement occurred after the first 3 weeks (field control vs. experimental animals); (2) rate of roe enhancement increased through time; (3) roe enhancement varied with algal species; (4) animals offered a mixed diet consistently produced higher mean gonad indices than three of the four single species diets; and (5) roe color and grade were higher on the four algal foods, especially the mixed diet.

Our results raise some interesting questions regarding gonad production in these urchins. If roe enhancement is possible in post-spawned animals, what prevents it from occurring naturally during the early-summer recovery phase? One possibility is the lack of high quality algae in the subtidal during summer. Macroalgae in subtidal areas are often overgrazed whereas *Ulva* and *Palmaria* have a refuge in the low intertidal and subtidal fringe and are largely unavailable to urchins. A related concern is the nature of our control (field) population. Similar controls have been employed in other enhance-

ment studies (e.g., McBride et al., 1997; Walker and Lesser, 1997). In our study, we compared experimental data with a barren-ground population which may have increased the observed differences between diet treatments and the control. Ideally, a second field control site in areas containing greater amounts of algae also might have been employed.

To test the possibility that our field control under-represented natural rates of gonad development during summer, we analyzed the net changes in summer gonad indices from independently collected data. Previously (1986–1987), we sampled nine widely separated urchin populations along the coast of Maine (Vadas et al., 1989, 1997). The sites were both barren and quasi-kelp habitats and most areas were overgrazed. Table 5 shows that in many populations in Maine, little variation in mean gonad index occurs during summer months, especially in barren ground habitats. It is common knowledge in the urchin industry that roe indices during the summer are well below 10%, and, therefore, not commercially acceptable. In part, the impetus for our study was based on this information. The mean change in gonad index between June and August, the months comparable to our study, ranged from -2.3% to 5.4% for the various field sites (Table 5). The overall mean change for these sites was 1.61% , whereas the mean difference in our 1996 field control was -0.05% (Table 6). Conversely, in the present study, gonad indices increased by an average 6.78% (range = 4.00% to 9.32%) on the five experimental diets (Table 6). In addition, the data in Table 5 also show that gonad index begins recovery ($P < 0.05$) in some (four) populations in summer. One possible reason for this might be the periodic availability of drift macroalgae (see also Minor and Scheibling, 1997).

Another concern was our choice not to employ an unfed treatment within the tidal impoundment. This treatment could have controlled for potential contributions to roe yields due to fouling. However, fouling was not observed during the course of our study.

Table 5

Comparison of mean gonad indices of *S. droebachiensis* in Maine from June to August. (Data are from Vadas et al., 1997)

Mean increase in gonad index = 1.61% .

Site ^a	Mean gonad index ^b		<i>t</i>	Pr > <i>T</i>	Net change	Average test diameter ^d
	June	August				
Bailey Isl.	11.2	11.7	0.77	0.4504	0.5	67.8
Five Islands	8.3	12.1	3.57	0.0013	3.8	61.0
Boothbay Hbr.	9.3	13.1	4.07	0.0003	3.8	64.2
Owls Head	9.1	7.0 ^c	-1.92	0.0654	-2.1	63.2
Stonington	6.1	3.8	-3.35	0.0024	-2.3	59.7
Lamoine Beach	3.4	4.1	0.83	0.4134	0.7	47.9
Schoodic Pt.	5.8	8.9	3.14	0.0039	3.1	67.5
Jonesport	7.2	12.6	3.73	0.0009	5.4	66.8
Lubec	10.2	11.8	1.83	0.0786	1.6	66.1

^aSites ordered from southwest to northeast.

^b*n* = 15 unless otherwise noted.

^c*n* = 14.

^dBased on animals used for gonad indices.

Table 6

Comparison of mean gonad indices of green sea urchins fed five experimental and one control diets
 Mean increase in gonad index = 6.78%.^a

Diet	Mean gonad index		Net change
	June	August	
Control	4.22	4.17	-0.05
<i>Alaria</i>	4.22	8.22	4.00
<i>Laminaria</i>	4.22	10.03	5.81
<i>Ulva</i>	4.22	11.27	7.05
<i>Palmaria</i>	4.22	11.96	7.74
Mixture	4.22	13.54	9.32

^aBased on four algal and the mixture diets.

All cages were systematically removed from the water every 5–7 days during which time food was changed and cages and urchins inspected. Since all cages were subjected to similar environmental and handling conditions, effects due to fouling would have been equal across all treatments. Further, had fouling been important, our ability to detect treatment (algal diet) differences in gonad index or color would have been minimized. As noted above, we found highly significant dietary differences during each 3-week interval. This suggests that fouling was not a problem, presumably due to the short duration of the study, regular inspection of cages, and shading from the bulkhead of the tidal impoundment.

Most enhancement efforts have been directed toward the pre-spawning period when roe are produced naturally (McClintock et al., 1982; Byrne, 1990; Klinger et al., 1995). With few exceptions (Fuji, 1962, 1967), early studies on roe production focused on the effects of habitat and food quality on fitness rather than on roe enhancement per se (Vadas, 1985). In general, roe production in urchins was highest on the most preferred algal foods, usually kelps (Vadas, 1977; Larson et al., 1980; Keats et al., 1984; Andrew, 1986; Lemire and Himmelman, 1996; Minor and Scheibling, 1997). Also, other macrophytes have been shown to enhance roe production or fitness in urchins: red algae (Leighton, 1966; Anderson and Velimirov, 1982); seagrasses (Fernandez and Caltagirone, 1994); and species in all three macroalgal divisions (Lemire and Himmelman, 1996). Laboratory studies show that roe yields of green (Vadas, 1977; Larson et al., 1980; Minor and Scheibling, 1997) and red (McBride et al., 1997) sea urchins can be increased significantly in 3 to 5 months. Recent efforts, however, indicate that roe enhancement in green (Russell, 1998) and red (McBride, 1997) urchins can occur in as short as 3 to 8 weeks (McBride's work with kelp indicates that with aggressive feeding, gonad indices can increase 1% per week).

Although not always specified as "post-spawned experiments," several studies have evaluated diet and temperature effects on urchins when roe are at their lowest annual levels. For example, Russell (1998) fed frozen algae to green urchins from food-limited populations during June and observed significant increases in gonad wet weight after 3 and 6 weeks. Lawrence et al. (1997) fed post-spawned *Loxechinus albus* natural and prepared diets over a 3-month period and observed significant increases in gonad indices

with both treatments relative to starved controls. Walker and Lesser (1997) manipulated photoperiod while feeding green urchins an artificial diet and observed significant enhancement of gonad index after 4 months. Although they conducted experiments between March and June, the animals at the initiation of their experiment appear (Walker and Lesser, 1997; Fig. 2) to have gonad indices of 18%, which are well above post-spawned levels.

4.2. Components of diets

Our data suggest that roe yields are related to protein levels in seaweeds. Urchins fed *Palmaria*, which usually contained the highest protein levels during our study (Fig. 5), produced higher gonad indices during the first 6 weeks. After 9 weeks, however, all four of the single algal diets had enhanced roe yields relative to controls (Fig. 2). Concomitantly, there was a general increase in the protein levels of the older (1–2 weeks) algal foods in the cages. This may have resulted from a secondary enhancement of nitrogen by microbes (Fenchel, 1970). The cumulative effects of these nitrogen-enriched foods may have contributed to the higher roe yields observed on the final sampling date for urchins fed the brown algal diets. Recent studies utilizing artificial diets support the hypothesis that protein levels in seaweeds control roe yields in urchins. The strategy for developing artificial foods for urchins is to formulate diets that induce rapid growth of tests and gonads (Klinger et al., 1988, 1995; de Jong-Westman et al., 1995; Fernandez and Caltagirone, 1994; Lawrence et al., 1997; Dowling, 1998). Most feeding trials have used formulations with protein levels between 15% and 20%, which are considerably higher than that found in most seaweeds (2%–10%). Urchins fed high protein diets consistently produced higher roe yields than urchins fed low protein or algal diets (Lawrence et al., 1992, 1997; de Jong-Westman et al., 1995; McBride et al., 1997; Dowling, 1998). Other properties of the food, such as physical and chemical characteristics, that affect availability and palatability, can confound interpretations of nutrient or energy relationships (Nagai and Kaneko, 1975).

In general, differences in lipid levels in the four algal diets (Fig. 6) parallel the variation in protein values. *Palmaria* and *Ulva* generally contained higher lipid levels than the two brown algae suggesting that the higher energy content of these two species may have contributed to the enhancement of roe. However, de Jong-Westman et al. (1995) demonstrated that protein concentrations were more important than energy levels. Interestingly, Floreto et al. (1996) observed lowest lipid levels in the red alga, *Gloiopeltis furcata*, compared to the green alga, *Ulva pertusa*, and the kelp, *Undaria pinnatifida*. Growth rates in the white urchin, *Tripneustes gratilla*, were lowest on the red alga. Also, it is well recognized that the energy content (caloric value) of most seaweeds is relatively low. Among the macroalgae tested by Paine and Vadas (1969) and Larson et al. (1980), brown algae (Phaeophyta) were usually the lowest (3.6–5.3 kcal), red algae (Rhodophyta) were intermediate (3.3–5.4 kcal) and green algae (Chlorophyta) were highest (4.5–5.2 kcal). Attempts to correlate gonad production directly with the caloric values of seaweeds, however, have not been successful (Vadas, 1977; Larson et al., 1980). McClintock (1986) indicates that separating the soluble (digestible) from the insoluble carbohydrate (non-digestible) component of diets will reduce the energetic

value of food. Algae with higher levels of soluble carbohydrates do not necessarily induce higher feeding or growth rates in urchins (e.g., Floreto et al., 1996). Further studies, perhaps with artificial diets, are required to delineate the potential contribution of natural energy levels in seaweeds to roe enhancement (Lawrence et al., 1997).

4.3. Environmental cues

Our studies contribute to discussions on allocation of energy to test vs. gonadal growth and the role of day length in roe enhancement. Our data for post-spawned urchins suggest that energy may have been shunted into roe rather than, or in addition to, test growth. We made no attempt to measure changes in test diameter because of the short interval of the experiment. Our data and previous work on natural populations (Table 5) shows that roe enhancement or recovery can occur in the absence of a short-day signal. Enhancement of roe during summer suggests that the switch from test growth to roe production is not under strict genetic or photoperiodic control. Allocation of resources to growth or reproductive effort is usually viewed as a competitive process with test growth dominating during non-gametogenic phases (Thompson, 1982; Pearse et al., 1986; Lawrence and McClintock, 1994; Russell, 1998). Day length has long been implicated in gametogenesis in sea urchins (Giese, 1959). The cues regulating growth and gametogenesis are usually thought to be under photoperiodic control (Thompson, 1982; Pearse et al., 1986; Walker and Lesser, 1997). The short days of late summer are thought to induce the switch from test growth to gametogenic processes in temperate/boreal forms (Pearse et al., 1986). Recent work shows that the gametogenic cycle in urchins can be manipulated with photoperiod and artificial diets (Walker and Lesser, 1997; McClintock and Watts, 1990). The enhancement of green urchin roe in the absence of short-day cues suggests that photoperiodic induction is superfluous for roe enhancement during summer, but not necessarily for gametogenesis. It is possible that nutritive, rather than gametogenic cells, contributed to the observed enhancement (cf. McBride et al., 1997). This supports the idea that production of roe is, at least in part, independent of gametogenesis.

4.4. Industry application

Management of the urchin fishery is a contentious issue in Maine caused by the asynchrony between market demands and urchin growth and reproductive cycles. Rejection of urchins by the industry is related to the size of animals and the quantity and quality of roe. If barren ground animals could be harvested, a higher quality roe could be produced during the off-season. Post-spawned enhancement of roe, as shown here, provides the industry and managers with another option for expanding the fishery. Currently, managers have two options for improving roe yields: shorten the fishing season and wait until roe yields are high or enhance the roe of wild stocks. A number of methods have been employed to enhance the roe of field populations (Tegner, 1989). Typically, excess food is required regardless of whether the urchins are held captive or transplanted (Lemire and Himmelman, 1996; Mottet, 1976). The purpose of our study was to provide basic data on commonly available forms of macroalgae for short-term

summer (off-season) enhancement. Whether land- or sea-based commercial enhancement can be made cost effective, however, remains to be determined.

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