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Growth of Juvenile Green Sea Urchins, Strongylocentrotus droebachiensis, Fed Formulated Feeds with Varying Protein Levels Compared with a Macroalgal Diet and a Commercial **Abalone Feed** STEPHEN D. EDDY¹, NICHOLAS P. BROWN, and ASHLEY L. KLING² Center for Cooperative Aquaculture Research, University of Maine, 33 Salmon Farm Road, Franklin, Maine 04634, USA STEPHEN A. WATTS Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA Addison Lawrence Texas AgriLIFE Research, Texas A&M System, 1300 Port Street, Port Aransas, Texas 78373, USA Abstract The effects of varying protein and carbohydrate levels in prepared diets on the somatic growth of juvenile green sea urchins, Strongylocentrotus droebachiensis, were examined. Ten diets were tested on 600 hatchery reared urchins (mean start weight = 0.11 g) for 6 mo with three replicate groups per diet. Nine of the diets were prepared specifically for urchins and varied in protein (16-40% protein) and carbohydrate (29-49% carbohydrate) levels. The other two diets consisted of a commercially available abalone diet and the kelp, Saccharina latissima. Weight measurements were carried out at 6-wk intervals, and at the end of the study urchins were individually weighed and a subsample from each treatment was analyzed for gonad weight and color. End weights after 6 mo ranged from 2.56 g for urchins fed the abalone diet to 6.11 g for urchins fed one of the prepared diets. Most of the prepared feeds outperformed kelp, and significant differences in growth were detected between some of the diets. In general, diets with lower protein levels (16-22% protein) and higher carbohydrate levels (>40% carbohydrate) produced the fastest growth. However, further diet refinement and/or use of finishing diets may be necessary to optimize gonad quality. The green sea urchin, Strongylocentrotus reduced urchin supply from capture fisheries droebachiensis, is highly valued in Japan and and their high-market value have led to efforts throughout Asia for the quality of its edible to commercially farm S. droebachiensis and gonads, known as uni. This has led to signifiother urchin species (Robinson 2004). These cant fishing effort in many regions throughout efforts include sea-based stock enhancement the circumpolar range where S. droebachienand cage methods and land-based methods sis is found. In the Gulf of Maine (USA and (Kirchhoff et al. 2008). Projects are currently Canada), overfishing and resulting ecosystem underway in Norway, Canada, and the USA to changes are considered to be major causes of develop methods for land-based echiniculture a significant decline in green sea urchin stocks of green sea urchins and to evaluate the eco-(Harris et al. 2000; Steneck et al. 2004). The nomic viability of these efforts (Kirchhoff et al. 2008; Hagen and Siikavuopio 2010; Pearce and Robinson 2010). ¹ Corresponding author.

Successful land-based echiniculture will re-² Present address: 10 John Street, Apartment 212, Dunquire the use of formulated diets. Although

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1 urchins can be grown in captivity using various 2 species of macroalgae, this approach is unlikely 3 to be environmentally sustainable or econom-4 ically viable for commercial scale production 5 (Lawrence et al. 2001). Macroalgae is relatively 6 low in protein and energy and varies season-7 ally in nutrient profiles (Larson et al. 1980; 8 Lobban and Harrison 1994; Schlosser et al. 9 2005). Formulated urchin feeds can be used 10 to maximize somatic growth during the juve-11 nile stages (McBride et al. 1998; Akiyama et al. 12 2001; Spirlet et al. 2001; Kennedy et al. 2005; 13 Kennedy et al. 2007a, 2007b) or to improve 14 gonad yield and quality during maturity (Walker 15 and Lesser 1998; Robinson et al. 2002; Pearce 16 et al. 2004). Ultimately, these diets will have 17 to produce gonads with acceptable market qual-18 ity. The use of different diets for different life 19 stages will likely be required to culture urchins 20 from hatchery to harvest (Kelly et al. 1998; 21 Lawrence et al. 2001).

22 Many of the studies to date on the nutrition 23 of urchins in culture conditions have examined 24 the role of nutrients in promoting gonad yield 25 and quality (Barker et al. 1998; Meidel and 26 Scheibling 1999; Robinson et al. 2002; Shpigel 27 et al. 2005; Siikavuopio et al. 2007). A num-28 ber of other studies have compared somatic and 29 gonad growth of urchins fed various formulated 30 feeds with macroalgal diets, or compared the 31 use of different algal species as feed (Cook 32 et al. 1998; Russell 1998; Spirlet et al. 2001; 33 Chang et al. 2005; Daggett et al. 2005; Lyons 34 and Scheibling 2007). However, the specific 35 nutrient requirements for optimal sea urchin somatic growth in aquaculture remain obscure. 36 37 Kennedy et al. (2007a) have presented evidence 38 that a lack of appropriate dietary minerals and 39 pigments is a likely factor contributing to the 40 shortcomings of prepared feeds in those cases 41 where natural kelp diets have produced bet-42 ter somatic growth than prepared diets. Other 43 recent studies have begun defining the gross 44 levels of protein and carbohydrates required 45 for somatic growth by urchins. McBride et al. 46 (1998) observed no significant differences in 47 growth of Strongylocentrotus franciscanus fed 48 prepared diets with protein levels of 30, 49 40, and 50%, but they did see a decrease

in feeding rate with increased protein levels. Fernandez and Boudouresque (2000) compared growth of Paracentrotus lividus given three feed types varying in quality ("vegetable," "mixed," and "animal"), and found that the higher protein feeds ("mixed" and "animal") with relatively lower carbohydrate levels (28.9% protein/35.3% carbohydrate and 47.2% protein/15.9% carbohydrate, respectively) gave better results than the "vegetable" type feed (12.7% protein/58.2% carbohydrate). Akiyama et al. (2001) concluded that a dietary protein level of 20% was the optimum for Pseudocentrotus depressus when casein was the sole protein source. Hammer et al. (2006) observed similar results in a feeding study with the sea urchin Lytehcinus variegatus, where they determined that a 20% protein diet was more efficient than either a 9% protein or a 31% protein diet.

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This study was conducted to determine the optimum protein level in the diet for young green sea urchins, S. droebachiensis. Given the importance of protein for growth and the expense of protein as a feed ingredient, this topic needs to be addressed to optimize the biological and economic efficiency of land-based green sea urchin culture. Eight formulated urchin diets with varying protein/carbohydrate levels were compared with one another and with the kelp, Saccharina latissima (previously Laminaria saccharina), a readily available species known to be consumed by green sea urchins (Vadas 1977; Daggett et al. 2005). A commercially available high protein abalone feed was included in the trial as a possible alternative diet for green urchins. Hatchery reared urchins with a starting test diameter (TD) of approximately 5.5 mm were used for the trial; hatchery reared urchins of this small size have rarely been used in previous formulated diet trials (Akiyama et al. 2001; Spirlet et al. 2001).

Materials and Methods

Urchins and Holding Conditions

The juvenile urchins used in the trial were selected on the basis of size from a population of approximately 10,000 9 mo post-settlement

1 hatchery urchins reared at the Center for Coop-2 erative Aquaculture Research (Franklin, ME, 3 USA), where the feed trial also took place. 4 The urchins had been reared almost exclusively 5 on the kelp, S. latissima for the 9 mo prior 6 to this study. The population as a whole var-7 ied between 3 and 15 mm TD, so to minimize 8 variation and the effect of differential growth 9 rates urchins with a TD of approximately 10 5.5 mm were selected from the population, 11 weighed using an A&D Instruments digital 12 scale to within 1 mg, and pooled to obtain 13 600 individuals with a mean start weight of 14 0.109 ± 0.011 g (CV $\leq 10.5\%$). The urchins 15 were starved for 1 wk prior to the trial. Ten 16 diets were tested, with three replicates per 17 diet and 20 urchins per replicate. Replicates 18 were segregated into slotted plastic hydro-19 ponic plant baskets $(16.5 \times 16.5 \times 12.7 \text{ cm})$ 20 deep) randomly distributed into three shallow 21 round fiberglass tanks supplied with a source 22 of flow through seawater. Tank flows were 23 equivalent to one tank turnover per 40 min to 24 maintain water quality and avoid accumula-25 tion of metabolites. Oxygen levels were mea-26 sured daily with an OxyGuard Handy Polaris 27 probe (OxyGuard International A/S, Denmark) 28 and ranged from 7.2 to 8.2 mg/L. Salinity was 29 checked weekly with a refractometer and was 30 stable throughout the trial at 30-32 ppt. Tem-31 peratures were maintained at 11.4 \pm 1.2 C and 32 the light regime was 8 L:16 D. 33

Study Diets

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36 The nine formulated diets used in this trial 37 were analyzed once for proximate composition 38 and the kelp three times to account for seasonal 39 variation. The proximate analyses were con-40 ducted by New Jersey Feed Labs, Inc. (Trenton, NJ, USA) according to AOAC methods 990.03 AQ1 (protein by combustion), 920.39 (fat by ether 42 43 extraction), 978.10 (fiber), 942.05 (ash), and 44 930.15 (moisture, using loss on drying at 135 C 45 for 2 h). Carbohydrate levels were determined 46 by subtraction from 100%. Eight of the diets 47 were prepared urchin feeds formulated and pro-48 duced at Texas A&M University (designated as 49 33, 34, 35, 36, 37, 38, 39, and 40). Protein

sources for these diets were a proprietary mix of kelp, soybean, casein, fish, and squid; and the carbohydrate sources were wheat, kelp, and soybean. Lipid levels ranged from 3.6 to 6.2%, which are within a suitable range for meeting urchin growth requirements (Kennedy et al. 2007b). Each of the eight Texas A&M diets contained up to 28% marine ingredients, 28.7% plant ingredients, 1.1% carotenoids, 0.7% vitamin premix, 24 % mineral mix, 7.2% binder, and antifungal-antioxidant. The other two diets consisted of a commercial abalone feed (designated as AN for abalone noodles) (proprietary formulation by Adam & Amos Abalone Foods Pty. Ltd., Australia), and the kelp, S. latissima (designated as K for kelp). The kelp was collected fresh from a local pier about every 2 wk throughout the course of this study and maintained between collections in a chilled seawater tank.

21 The proximate diet analyses converted to a 22 dry weight basis are summarized in Table 1 23 for all diets used in this study. The kelp 24 was sampled at the beginning, middle, and 25 near the end of the trial, corresponding to 26 winter, spring, and summer, and the varying 27 nutrient compositions likely reflect seasonal variations in growth, light, temperature, and 28 29 nutrient regimes seen in the Gulf of Maine. For 30 the purposes of analysis, a composite (average) 31 nutrient profile was used for the kelp when 32 comparing it to the formulated feeds. On a wet 33 weight basis the kelp averaged 88% moisture, 34 compared to a moisture content of 7-11.7% 35 for the formulated diets. The kelp-fed urchins 36 would thus have to consume significantly more 37 kelp to match the nutrient intake of the urchins fed formulated diets in this study. 38

39 The diets were ranked based on percent 40 protein levels (Table 1). Of the Texas A&M diets, diet 39 had the lowest protein content 41 42 (16.0% dry weight) and diet 35 had the highest 43 protein content (40.3%). The abalone feed had 44 the second highest protein content (36.3%) 45 of all the formulated feeds tested and also contained the highest levels of carbohydrates 46 47 (52.6% dry weight). The abalone diet was also 48 significantly lower in ash (7.5%) than all of 49 the other diets used in this study (25-45.3%)

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Diet	Protein	Fat	Ash	Carbohydrate	24-h stability
39	16.0	4.6	37.7	41.7	Disintegrated
40	16.4	5.0	45.3	32.9	Disintegrated
33	17.1	4.7	45.3	32.9	Disintegrated
38	22.6	5.0	25.2	47.2	Partially intac
37	23.3	4.8	30.6	41.3	Partially intact
34	24.5	4.7	36.2	34.6	Partially intac
36	32.9	5.1	25.0	37.0	Intact
35	40.3	5.3	25.3	29.1	Intact
AN (abalone feed)	36.3	3.6	7.5	52.6	Intact
Kelp (composite)	23.6	3.2	35.0	38.2	Intact
Kelp (February 19, 2008)	24.4	7.3	40.2	28.1	-
Kelp (May 6, 2008)	32.9	0.6	35.2	31.3	_
Kelp (June 25, 2008)	13.4	1.8	29.6	55.2	_

¹Texas A&M diets are ranked in order from low to high protein.

ash). In terms of average composition on a dry weight basis, kelp was most similar to the Texas A&M diet 34.

Feeding Levels

Initially, the urchins were fed the formulated feeds every 48 h, but this was increased to daily feeding after 2 wk into the trial. Feed-ing amounts were adjusted based on growth and 24-h consumption to maintain feeding at approximately 2% body weight and ad libitum (to satiation). The amount of feed added to each replicate was gradually increased through this study, but every replicate in each of the formu-lated feed treatments received the same quantity of feed at each feeding. Uneaten feed and feces was removed from all formulated feed repli-cates at each feeding and the tanks and repli-cate baskets were cleaned of biofilms twice per week. The formulated diets varied in water sta-bility so a scoring system was devised to eval-uate stability. Diets that remained completely intact after 24 h were scored as a 3, diets that had disintegrated into a powder after 24 h were scored as a 1, and diets that were partially intact (broken into intact pieces and powder) were scored as a 2. Diets were scored daily at every feeding when the uneaten feed was removed.

The kelp-fed replicates were fed on the same
schedule as the formulated feed replicates, but
any kelp left over from the previous feeding

was not removed. Instead, the amount of kelp added to the replicates was decreased or increased to maintain a constant supply of kelp in the baskets while preventing an excess from being left over. In this way, it was possible to approximate the total amount of kelp consumed by the urchins at the end of the experiment.

Urchin Measurements

Weight measurements were taken at d 0, 37, 83, 117, 142, and 216 when the trial was ended. This provided data that could be used to calculate the specific growth rates (SGRs) for five growth intervals. Survival/escapement was also recorded at these sampling intervals. Urchins were blotted dry before weighing. Mean individual wet weights were determined within replicates at d 37 and 83 by weighing all of the urchins in the replicate and dividing by the number of urchins. On all other sampling days individual urchin weights were recorded. Upon termination of this study, TDs were measured for each individual using digital calipers (model CD-6 "PMX Mitutoyo Corporation, Kawasaki, Japan), and five urchins per replicate were randomly selected and dissected to determine gonad wet weight.

Data Analysis

Whole wet weight, weight gain, SGR, and 48 end TD were calculated using averages from 49

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1 each replicate basket (n = 3). The mean urchin 2 wet weight per diet was calculated at each sam-3 pling interval using the pooled averages from 4 diet replicates (n = 3). The average weight gain 5 per urchin for each replicate between sampling 6 intervals was calculated as: weight gain (g) =7 [whole wet weight (t_2) -whole wet weight (t_1)]. 8 The mean gonad wet weight for each diet was 9 calculated at the end of this study using pooled 10 averages from the five urchins subsampled 11 from each replicate. Urchin gonadal/somatic 12 indexes (GSIs) were calculated as: GSI (%) =13 (wet gonad weight/whole wet weight) \times 100. 14 The mean GSI and mean end TD for each 15 diet were calculated using the pooled averages 16 from the diet replicates. Average SGR per ur-17 chin was determined for each replicate bas-18 ket for each sampling interval according to the 19 following equation: SGR(%) = ([ln (whole wet20 weight (t_2)] – [ln (whole wet weight (t_1))]/ 21 $[(t_2)-(t_1)]) \times 100.$ 22

Multivariate repeated measures analyses were 23 used to check for an interaction between diet 24 treatment and time for whole wet weight, 25 weight gain, and SGR. The interaction was sig-26 nificant (Wilks' λ : P < 0.05), so data from 27 each measurement day were analyzed indi-28 vidually using a one-way ANOVA. For the 29 endpoint data (TD, gonad wet weight, and 30 GSI), a one-way ANOVA was performed for 31 each response variable. Residuals from each 32 ANOVA were then analyzed for normality and 33 equal variance using the Shapiro-Wilk test for 34 normality and Levene's equal variance test, 35 respectively, and an acceptance level of P >36 0.10 was adopted for both. There were occa-37 sional violations of Levene's test for equal 38 variance, but this was disregarded when the 39 normality assumption was satisfied, because 40 differences between diets were often so great 41 that a transformation applied to all measure-42 ment days would not likely have affected the 43 results. In two cases where data were obtained 44 over time in this study (whole wet weight at 45 d 142 and weight gain from d 117 to 142), the 46 assumptions of both equal variance and nor-47 mality were not met (Levene's test: P < 0.01; 48 Shapiro–Wilk: P < 0.01). Further statistical 49 analyses in these instances were abandoned and

only means are presented. For endpoint data, if either normality or equal variance assumptions were not met the residuals and outliers were examined and transformations were applied as needed. As such, gonad wet weight data were fourth root transformed and GSI data were square root transformed prior to any further statistical analyses, but original values are presented for ease of interpretation. When normality and variance assumptions were satisfied, the Ryan–Einot–Gabriel–Welsch Q (REGWQ) post-hoc test was used to make pair-wise comparisons among treatment means with a P <0.05 level of significance.

Results

Survival and Growth

At the end of the trial, 12 animals (2%) were missing because of escapement or mortality, but losses were random across treatments and there were no significant differences in survival between treatments.

Significant differences between diets as 24 reflected in urchin whole wet weight were evi-25 dent by d 37 and continued throughout this 26 27 study (Fig. 1). By d 83, the urchins fed with the Texas A&M diets 39 and 40 surpassed all 28 others in weight, with the remaining treatments 29 30 producing weights in the following descending 31 order: 33, 38 > 37 > 34 > 36 > kelp > 35 > 32 abalone noodles (ANOVA, P < 0.05). Day 117 33 weights were highest for diet treatments 33, 38, 39, and 40, with the remaining treatments 34 35 producing weights in the following descending 36 order: 37 > 34 > 36 > kelp > 35 > abalone37 noodles (ANOVA, P < 0.05). At d 216 the end weights ranged from 2.56 g (SE = 0.120) for 38 the abalone diet to 6.11 g (SE = 0.243) for 39 40 the Texas A&M diet 38 (Fig. 1). Diets 33, 37, 38, 39, and 40 were the top performing 41 42 diets and showed statistically similar weight 43 gains (Table 2). These diets were all ranked as low to intermediate in protein levels (Table 1). 44 45 The remaining diets produced end weights 46 in the following descending order: 34 > 36 >47 kelp > 35 > abalone noodles (ANOVA, P <48 0.05). Throughout this study, the high protein diets (diets 35, 36, and AN) produced 49

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FIGURE 1. Growth of juvenile urchins fed formulated feeds or kelp. Urchins were weighed at d 0, 37, 83, 117, 142, and 216.

TABLE 2. Protein levels and the mean \pm SE weight gain, SGR (% growth/d), and gonad index at the end of the 216 d feeding trial for each of the diets.¹

Diet	Protein level (%)	Mean weight gain (g)	Mean SGR	Mean percent gonad index
39	16.0	5.81 ± 0.2^{a}	1.86 ± 0.00^{a}	$17.1 \pm 1.1^{\circ}$
40	16.4	5.66 ± 0.25^{a}	1.84 ± 0.01^{a}	17.8 ± 1.1^{b}
33	17.1	5.35 ± 0.17^{a}	1.81 ± 0.01^{b}	22.5 ± 1.5^{d}
38	22.6	6.00 ± 0.24^{a}	1.87 ± 0.02^{a}	21.1 ± 0.5^{a}
37	23.3	5.39 ± 0.21^{a}	1.81 ± 0.01^{a}	21.8 ± 1.0^{a}
34	24.5	4.9 ± 0.07^{b}	$1.78 \pm 0.01^{\circ}$	19.9 ± 0.3^{a}
36	32.9	$4.47 \pm 0.08^{\circ}$	1.72 ± 0.01^{d}	20.3 ± 0.6^{a}
35	40.2	3.47 ± 0.08^{d}	1.62 ± 0.01^{e}	20.7 ± 1.3^{a}
Abalone	36.3	2.45 ± 0.12^{e}	$1.47 \pm 0.02^{\rm f}$	$12.7 \pm 1.4^{\rm e}$
Kelp	23.6	$4.35 \pm 0.13^{\circ}$	1.71 ± 0.01^{d}	$8.2\pm0.5^{ m f}$

SGR = specific growth rate.

¹Letters associated with each value indicate statistically significant differences among diets within each parameter (Ryan–Einot–Gabriel–Welsch Q test; P < 0.05).

significantly less growth than the other diets, and the kelp treatment also produced significantly lower weight gains compared with the top performing diets. Growth of urchins fed diet 34 was statistically different from those on all other diets, and is best described as intermediate between the group containing the top five performing diets and the two lower performing groups. In terms of percent protein and carbohydrate (dry weight basis), this diet was the most similar to kelp (Table 1), although it outperformed the kelp in terms of growth (5.01 g vs. 4.47 g) (REGWQ test; P < 0.05).

48 Significant differences in end TD were also 49 found among diet treatments in this study, with TDs ranging from 18 mm (SE = 0.51) for the abalone diet to 25 mm for prepared feeds 37 (SE = 0.43) and 38 (SE = 0.58) (Fig. 1). Prepared feeds 33, 34, 37, 38, 39, and 40 all produced similarly large end TDs, with the remaining treatments producing end TDs in the following descending order: kelp > 36 > 35 > abalone noodles (ANOVA, P < 0.05).

Specific Growth Rates

There were significant differences between46the formulated diets and kelp in terms of47SGRs during four of the five sampling intervals48(Table 3). During the first interval (d 0–37),49

	0-37	37-83	83-117	117-142	142-216
33	2.70 ± 0.03^{a}	2.41 ± 0.02^{b}	1.65 ± 0.02^{a}	1.61 ± 0.01^{e}	1.16 ± 0.05
34	2.75 ± 0.02^{a}	2.00 ± 0.04^{e}	1.40 ± 0.10^{a}	2.02 ± 0.03^{a}	1.24 ± 0.03
35	2.45 ± 0.10^{b}	$1.79 \pm 0.03^{\rm f}$	1.21 ± 0.05^{b}	1.87 ± 0.07^{a}	1.20 ± 0.01
36	2.64 ± 0.05^{a}	2.06 ± 0.06^{d}	1.30 ± 0.06^{a}	1.87 ± 0.03^{a}	1.20 ± 0.01
37	2.80 ± 0.09^{a}	2.12 ± 0.01^{d}	1.56 ± 0.08^{a}	1.90 ± 0.07^{a}	1.22 ± 0.05
38	2.76 ± 0.10^{a}	2.31 ± 0.06^{b}	1.64 ± 0.17^{a}	1.97 ± 0.02^{a}	1.21 ± 0.05
39	2.73 ± 0.04^{a}	2.49 ± 0.04^{b}	1.61 ± 0.06^{a}	$1.70 \pm 0.04^{\circ}$	1.20 ± 0.04
40	2.82 ± 0.05^{a}	2.47 ± 0.05^{b}	1.50 ± 0.09^{a}	1.67 ± 0.02^{d}	1.16 ± 0.06
AN	$1.72 \pm 0.06^{\circ}$	$2.20 \pm 0.09^{\circ}$	$0.96 \pm 0.06^{\circ}$	$1.37 \pm 0.04^{\rm f}$	1.16 ± 0.04
Κ	$1.62 \pm 0.06^{\circ}$	2.70 ± 0.03^{a}	$1.50 \pm 0.09^{\circ}$	2.05 ± 0.07^{a}	1.13 ± 0.02

1 TABLE 3 Mean + SF specific growth rate (% growth/d) seen for each of the diet treatments at each sampling interval (d

¹Letters within columns indicate statistically significant differences among diets at the interval specified (Ryan-Einot-Gabriel-Welsch Q test; P < 0.05).

the urchins responded quickly to the intro-17 duction of formulated feeds and their growth 18 rates surpassed those seen in the kelp replicates 19 (ANOVA, P < 0.05). However, during the sec-20 ond interval (d 37-83), the growth rates seen 21 in the kelp replicates (2.7%/d) surpassed those 22 seen in any of the formulated feed replicates. 23 The growth rates for the kelp-fed replicates 24 remained relatively high until the fifth sam-25 pling interval (d 142-216), when they slowed to 1.13%. The average SGRs for the urchins fed 27 formulated feeds during the first two growth 28 intervals (from 0 to 83 d) exceeded 2% for 29 every diet treatment, but during the third growth 30 interval they declined to between 0.96 and 31 1.65%. The SGRs increased slightly during the 32 fourth interval (117-142 d), and then decreased 33 again during the final interval (142-216 d) to 34 the lowest rates seen in the trial, to an average 35 of 1.19%. The SGRs over the entire course of 36 the trial (d 0-216) ranged from 1.47% (SE = 37 1.83×10^{-2}) for the abalone diet to 1.87%38 $(SE = 1.60 \times 10^{-2})$ for Texas A&M diet 38, 39 and were statistically similar for diets 37, 38, 40 39, and 40 (Table 2). The high protein diets 41 and the kelp diet all showed significantly slower 42 growth rates over the course of this study than 43 those seen with the low and intermediate pro-44 tein diets. 45

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Gonadal-Somatic Index

48 Significant differences in the GSI were also 49 found at the end of this study, with GSIs ranging from 8.23% (SE = 0.506) for kelp to 21.8% (SE = 0.458) for prepared feed 37 (Table 2). Prepared feeds 34, 35, 36, 37, and 38 all produced similarly large GSIs, with the remaining treatments producing GSIs in the following descending order: 40 > 39 > 33 >abalone noodles > kelp (ANOVA, P < 0.05). However, there was no statistically significant relationship between the GSI and the protein or carbohydrate level of the diet. Diets with intermediate or high protein levels produced similar GSIs, and all of the formulated feeds with the exception of the abalone noodles had GSIs that exceeded 15%. The kelp-fed urchins had a significantly lower GSI (8.23%) than that seen in any of the formulated feeds.

Feed Efficiency

35 The sum total of feed (grams wet weight) 36 provided to each of the formulated feed 37 replicates through the course of the trial 38 was 179.5 g, whereas the kelp-fed replicates 39 received 1040 g. The total amount of kelp actu-40 ally consumed by the kelp-fed urchins could 41 be closely approximated, but this could not be 42 carried out for the formulated diets and there-43 fore the true feed conversion ratios could not be 44 calculated. However, it was possible to calcu-45 late and compare the ratio of total feed input 46 per treatment to the total biomass gain per 47 treatment as an approximate measure of feed 48 efficiency. The mean total biomass gain per 49 replicate for the top performing Texas A&M

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diet 38 was 120 g after 216 d (6 g/urchin), whereas the mean biomass gain per kelp-fed replicate was 88 g (4.4 g/urchin). Thus the ratio of feed input to biomass gain was 1.5/1 for the top performing formulated feed and 11.8/1 for the kelp. However, if the kelp is converted to a dry weight basis (average 88% moisture content), then this ratio improves to 1.4/1.

Feed Stability

The 24-h stability ratings in seawater varied between the diets but remained consistent for each diet over the course of this study. There was a clear relationship between protein levels and water stability: the low protein diets dissolved into a powder within 12–24 h, the intermediate protein diets broke up into small pieces and powder within 24 h, and the high protein diets remained entirely intact for 24 h or even longer (Table 1).

Discussion

TD has been traditionally favored as a proxy for measuring urchin growth (Swan 26 1961; Lang and Mann 1976; Raymond and Scheibling 1987; Devin et al. 2004; Pearce 28 et al. 2005). However, as Ellers and Johnson 29 (2009) point out, measuring diameter can be 30 imprecise because urchins have spines, are not 31 always exactly circular, and diameter measure-32 ments do not take into account potential height 33 variation (some urchins may be more flattened 34 than others). They recommended that weight be used in growth studies, and demonstrated 36 that a formula incorporating the cube root of the weight could be used to estimate the nomi-38 nal diameter of the urchin with six times the accuracy of a direct diameter measurement. 40 Techniques utilizing image analysis software may increase the accuracy of TD measure-42 ments (Kennedy et al. 2007a, 2007b), but they require additional investment in equipment and 44 time, and it remains to be tested whether this 45 method provides a better measure of growth 46 than weight. For these reasons, in this study weight was chosen as the primary measure of 48 growth in addition to diameter. This had the 49 further advantage of allowing for calculation

of GSIs; an important consideration for urchins reared on formulated diets. Finally, the calculation of the SGR, which is widely used in aquaculture growth and feed studies, will yield very different results in urchins if TD is used instead of weight as the defining growth number. For example, in our study when weight was used to calculate the SGR we obtained a maximum SGR of 1.87%; for the same urchin the SGR based on TD growth was 0.7%.

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11 As an urchins' growth is not linear over the 12 course of its life span (Russell 1998; Lawrence 13 2000; Ellers and Johnson 2009), it is impor-14 tant that growth comparisons between studies 15 be limited to urchins of similar size ranges. The 16 top performing diet (38) in this study resulted 17 in a net growth of 6 g (from 0.11 to 6.11 g) 18 over the course of 216 d, with a corresponding 19 SGR of 1.87%/d. In terms of TD, the urchins 20 showed a net increase in TD of 19.5 mm (from 21 5.5 to 25 mm) over 216 d; a rate of increase 22 equivalent to 2.7 mm/mo. The growth rates of the juvenile green urchins fed the formu-23 24 lated diets in our study compare favorably with 25 growth rates of similar sized S. droebachiensis 26 in the wild. Pearce et al. (2005) cite a number 27 of studies estimating growth rates of S. droebachiensis in the wild, and reported a range 28 29 of 0.2 mm to 1.2 mm/mo. Russell (2000) pro-30 jected 2-3 yr from metamorphosis for green 31 sea urchins to attain a TD of 20-25 mm in 32 the field, whereas in the current study this was 33 attained in 16.2 mo (9 mo to 5.5 mm + 216 d 34 to 25 mm). The growth rates seen in our study 35 also compare favorably with those seen in 36 studies where similar sized green sea urchins 37 were grown in controlled culture conditions. During a 490-d feeding trial with green sea 38 39 urchins, Daggett et al. (2005) reported TDs 40 of less than 20 mm and weights of less than 41 5 g at 200 d for green sea urchins grown 42 on either formulated diets or macroalgae and 43 with a starting size of about 9 mm. Kennedy 44 et al. (2007a) reported a maximum SGR of 45 0.6% (based on TD) for wild collected juve-46 nile green sea urchins fed a fortified formulated 47 diet. The maximum TD-based SGR seen in our 48 study was 0.7% (19.5 mm increase over 216 d). 49 Hagen (2004) reported near exponential growth

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of hatchery reared S. droebachiensis, with an 2 approximate "doubling time" in wet weight of 3 2.8 mo. Using his formula (doubling time = 4 [time 1-time0]/[log₂ weight₁-log₂ weight₀]), 5 we saw a doubling time of 37.2 d for the fastest 6 growing urchins in our study.

7 A strong correlation was seen between pro-8 tein levels in the formulated diets and growth 9 rates of the urchins, but there was no corre-10 lation between carbohydrate levels or carbo-11 hydrate/protein ratios and growth. The group 12 of five top performing diets showed similar 13 growth rates and they all had relatively low 14 to intermediate protein levels (16-23.3% pro-15 tein), as compared with the three high protein 16 diets (32.9-40.3% protein) that only performed 17 as well as, or even worse than, the kelp. This 18 is in general agreement with other studies indi-19 cating that protein levels of 16-25% are opti-20 mal for urchin somatic growth (Akiyama et al. 21 2001; Hammer et al. 2004, 2006; Kennedy 22 et al. 2005). It is also clear from the results 23 seen here that formulated feeds can outperform 24 kelp for urchins grown in culture. This is not 25 always the case (McBride et al. 1998; Williams 26 and Harris 1998), indicating that the nutritional 27 composition of both the formulated feeds and 28 the kelp is critical. In this study, the SGRs for 29 the kelp-fed urchins varied between sampling 30 intervals, and at the second sampling interval (d 31 37-83) they exceeded the SGRs seen for any 32 of the formulated feeds (Table 3). This interval 33 includes the period (May) when the proximate 34 analysis of the kelp showed the highest pro-35 tein levels (32.9%; dry weight basis) seen for 36 kelp during the course of this study (Table 1). 37 This shows that when seaweed is harvested at 38 peak protein levels it can be effectively used for somatic growth. However, during the last sam-40 pling interval (d 142-216, July-September), 41 the SGR for the kelp-fed urchins was 1.13%; 42 the lowest SGR seen for the kelp-fed urchins 43 during the study and ranking it at the bottom 44 of all the diets for this interval. This interval 45 coincides with reduced protein levels of 13.4% 46 observed at the June proximate analysis for 47 kelp. The correlation seen here between vari-48 able protein levels in macroalgae and urchin 49 growth rates has been observed in other studies

where macroalgae was used as an urchin feed (Vadas et al. 2000; Schlosser et al. 2005). Seasonal variation in seaweed nutritional quality (Larson et al. 1980; Lobban and Harrison 1994; Schlosser et al. 2005) underscores the need for developing formulated feeds suitable for commercial scale aquaculture.

As the trial progressed there was a general decline in the SGRs seen in all of the for-10 mulated feed treatments, beginning after d 83 11 of the trial during the third growth inter-12 val (Table 3). This decline was followed by 13 an increased SGR for all treatments during 14 the fourth growth interval (117-142 d), only 15 to be followed by a further decline during 16 the fifth growth interval (142-216 d). The 17 increased SGR seen during the fourth inter-18 val may have been due to an increase in 19 water temperature. For 30 d during the fourth 20 interval the water temperature averaged 13.6 C 21 and peaked at 15.6 C, as opposed to the 22 average temperature of 10.8 C maintained dur-23 ing growth intervals 1-3 and 11.8 C during 24 growth interval 5. The optimal temperature 25 range for somatic growth and survival of 26 early post-settled S. droebachiensis appears to 27 be 9-13 C (Pearce et al. 2005). Devin et al. (2004) reported faster growth but decreased sur-28 29 vival at 15 C for 3-5 mm TD green urchins. 30 Kennedy et al. (2005) observed an acceleration 31 in SGRs when the water temperatures increased 32 in their feeding trial (14–16 C from 12 C). In 33 the present trial, this relatively warm period of 34 30 d may have countered the overall trend of 35 declining growth; a trend that was reasserted 36 during the fifth interval once the water temper-37 ature was restored close to its former level.

The larger question is the cause of the 38 39 overall trend of declining SGRs observed with 40 the urchins fed formulated feeds after around 90 d into the trial. This phenomenon has 41 42 been documented in other feed trials as well. 43 Kennedy et al. (2007b) saw an initial increase 44 in growth rates followed by a decline after the 45 mo 5 in juvenile green urchins fed prepared 46 diets. Juvenile S. franciscanus fed formulated 47 diets also showed declining growth rates after 48 5 mo (McBride et al. 1998), and the authors 49 suggested that this may have been at least

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partially attributable to increased reproductive development.

3 Nutritive phagocytes in the gonads act as 4 a site of nutrient storage and it is well doc-5 umented in a number of species that mature 6 urchins respond to increased food availabil-7 ity or quality with increased gonad produc-8 tion (Russell 1998; Walker and Lesser 1998; 9 Lawrence 2000; Lawrence et al. 2001; Spirlet 10 et al. 2001; Schlosser et al. 2005). In the case 11 of mature urchins this is a desirable outcome, 12 as the gonads are the marketable product, but 13 for immature urchins the goal is to maxi-14 mize somatic growth. Precocious gonad growth 15 may result from a surplus of nutritional energy 16 beyond what can be effectively utilized for 17 somatic growth (Lawrence 2000). In this study, 18 all of the formulated feeds resulted in sig-19 nificantly higher GSIs than those seen in the 20 kelp-fed urchins (Table 2), and it is tempt-21 ing to hypothesize that this gonad develop-22 ment came at the expense of somatic growth, 23 resulting in declining growth rates as the feed 24 trial progressed. The large gonads observed at 25 the end of this study are indicative of pre-26 cocious gonad development for this species. 27 At the time that the decline in SGR was 28 observed, the urchins were approximately 1 yr 29 post-metamorphoses and 12 mm TD. This is 30 both younger and smaller than the 2-3 yr 31 and 25 mm observed in the field where green 32 sea urchins first reach reproductive maturity 33 and their growth rates decline (Siversten and 34 Hopkins 1995; Vadas and Beal 1999). The 35 growth curve (TD-age relationship) generated 36 by Russell (2000) for green urchins shows 37 steady growth until around 35-40 mm before 38 growth rates begin to decline. Hagen (2004) 39 observed exponential growth rates in S. droe-40 bachiensis until the urchins were at least 6-7 g, 41 and extrapolation of the curve indicated that 42 they maintain this rate until they are about 43 2-yr old.

44 Precocious gonad growth has been observed 45 with other species when they were fed formu-46 lated diets, including L. variegatus (Hammer 47 et al. 2004), Psammechinus miliaris (Kelly 48 et al. 1998), P. depressus (Akiyama et al. 2001), 49 and Loxechinus albus (Olave et al. 2001).

Hammer et al. (2004) suggested that a decrease in the rate of growth of L. variegatus fed high protein diets could have been due to the precocious gonad development they observed. Kennedy et al. (2005) saw large gonads but smaller TD in S. droebachiensis fed high energy prepared diets compared with urchins fed a lower energy kelp diet, and suggested that this was because of preferential allocation of energy into gonad production. However, Kennedy et al. (2005) note that several other nutritional factors could have also contributed to the poor somatic growth they observed. The evidence that there is a conflict between somatic growth and gonadal growth in prereproductive urchins remains inconclusive (Lawrence 2000). Both Minor and Scheibling (1997) and Meidel and Scheibling (1999) observed a parallel increase in gonadal and somatic growth in S. droebachiensis when there was an increase in diet quality or quantity. Cook et al. (1998) found that a high protein diet (salmon feed) promoted somatic and gonadal growth simultaneously in juvenile P. miliaris. Although we observed some statistically significant differences between the diets in terms of gonad index (Table 2), these differences could not be attributed to protein or carbohydrate lev-28 els. This was the case even for the two high 29 protein diets (35 and abalone feed) that performed worse than the kelp in terms of growth but produced higher GSIs than the kelp. Measurements of production efficiency and consumption rate were not utilized in this study, but have been effectively used in feed trials with other species, including S. franciscanus (McBride et al. 1998) and P. lividus (Spirlet et al. 2001). Further studies utilizing these and other tools are needed to examine the relation-40 ship between protein and energy levels in diets, 41 precocious gonad development, and somatic 42 growth in juvenile green sea urchins.

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43 Limiting nutritional factors may provide an 44 alternative explanation for the decline in SGRs 45 we saw in this study. The juvenile urchins 46 had been maintained on a diet of kelp for 47 9 mo prior to the start of this study. Kennedy 48 et al. (2007b) proposed that urchins previously fed kelp and then used in formulated feed 49

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1 trials could have stored essential nutrients, 2 such as minerals and pigments, which they 3 can then draw upon during the first period 4 of the feeding trial. Depletion of these stored 5 nutrients would then cause a subsequent decline 6 in SGRs if the diets were also lacking in those 7 nutrients. Minerals, in particular magnesium 8 and calcium, are required by urchins for test and 9 spine growth (Okazaki 1956; Chen et al. 2000), 10 and can become depleted over time. Kennedy 11 et al. (2007a) hypothesized that inadequate 12 mineral levels may have contributed to the poor 13 performance sometimes seen with formulated 14 feeds in previous studies. However, mineral 15 levels in the eight Texas A&M diets used in 16 this study were 24% dry weight, well in excess 17 of the top level of 15% that gave good results 18 for Kennedy et al. (2007a), so it is unlikely that 19 mineral depletion was the cause of the declining 20 SGRs.

21 Pigment has also been identified as an essen-22 tial nutrient for sea urchins, particularly for nor-23 mal gonad development. β-carotene is a major 24 pigment in the gonads, test, and spines, and is a 25 precursor for echinenone, which is responsible for the typical yellow to orange color of urchin 27 gonads and important for reproductive success 28 (Fox and Hopkins 1966; Griffiths and Perrott 29 1976; George et al. 2001). β-carotene appears 30 to be also required for optimal somatic growth, at least for S. droebachiensis (Kennedy et al. AQ2 2007). They saw improved somatic growth in 33 juvenile green urchins when β -carotene was 34 added to formulated diets at levels of 1.25% 35 using AlgroTM (a spray dried form of the 36 microalgae Dunaliella salina). The addition 37 of this pigment source increased the rate of 38 somatic growth even in the absence of supple-39 mental mineral premix, probably because the 40 Algro[™] also contributed 0.8% minerals to the 41 diets (Kennedy et al. 2007).

42 In this study, β -carotene was added to the 43 Texas A&M formulated diets at levels of 1.1%, 44 equivalent to the level used by Kennedy et al. 45 (2007). However, at the end of this study 46 the gonad coloration was a pale off white, as 47 opposed to the more typical orange color seen 48 in the kelp-fed urchins. This suggests that β -49 carotene levels were either inadequate or that the β -carotene source (proprietary) was somehow lacking. Pigment depletion must therefore be considered as a possible cause for the decline in SGRs seen as this study progressed. The role of pigment sources and levels in urchin nutrition, although often addressed in gonad enhancement studies (Robinson et al. 2002), remains an area for further research in somatic growth studies (Lawrence et al. 2001).

10 A potentially negative consequence of using 11 formulated diets is the effect they can have on gonad color and taste. The pale off-12 13 white gonad color we observed at the end 14 of the trial in the formulated feed urchins is 15 unacceptable for market quality, whereas the 16 kelp-fed urchins had gonads that were a more 17 suitable yellow/orange. This likely reflects an inadequacy in the pigment level or source in the 18 19 formulated diets we used. Previous studies have 20 documented the negative effects of formulated 21 feeds on gonad color and flavor, as compared 22 to the improvement in these sensory parameters when urchins are fed macroalgae. Senartna 23 24 et al. (2005) observed that the taste and smell of 25 gonads from wild collected purple sea urchins, 26 Heliocidaris erythrogramma, were better than 27 those fed formulated vegetable- or animalbased feeds. Siikavuopio et al. (2007) observed 28 29 that increased protein levels in formulated 30 diets resulted in an increased bitter taste in 31 the gonads of S. droebachiensis. Shpigel et al. 32 (2005) found that the urchin, P. lividus, fed a 33 prepared diet for 8 wk followed by 4 wk of 34 algal diet produced the optimal combination 35 of gonad color and GSI. It remains to be 36 seen whether this strategy can be used to 37 efficiently grow hatchery derived green sea urchins in culture from juveniles to market 38 acceptability, and this is currently the focus of 39 40 our research efforts. The ideal diet for urchins in culture needs to provide for fast somatic 41 42 growth without negatively affecting gonad yield 43 or quality.

The need for a readily available commercial diet to use for our sea urchin aquaculture efforts was a primary factor for the inclusion of an abalone diet in the trial. Formulated diets have been developed for abalone and there are now several commercial sources (Hahn 1989; 49

Fleming et al. 1996), whereas commercially AQ3 available urchin feeds remain in short supply 3 (Lawrence et al. 2001). Abalone is similar 4 to sea urchins in that both feed primarily 5 on macroalgae, and like urchins the energy 6 metabolism of abalone is carbohydrate and protein-based rather than lipid-based (Fleming 1995; Bautista-Teruel and Millamena 1999). It thus seemed possible that formulated abalone 10 diets could meet the nutritional requirements 11 of sea urchins and might prove to be a 12 convenient feed source until more urchin diets 13 became available. In addition, abalone diets 14 are typically high in protein and carbohydrate 15 (Fleming et al. 1996), which allowed us to 16 include a diet in the trial that had combined 17 protein and carbohydrate levels higher than 18 those found in any of the formulated Texas 19 A&M diets (Table 1).

20 However, the abalone diet used here resulted 21 in poor growth, underperforming the kelp diet 22 and all of the Texas A&M diets, and it is 23 therefore not a suitable feed for juvenile green 24 sea urchins. As only a proximate analysis is 25 available for the abalone feed we used, we 26 have limited information on which to base 27 an analysis of its poor performance. The low 28 mineral levels seen in this diet, as reflected in 29 an ash content of only 7.5%, indicates that there 30 may have been inadequate levels of calcium 31 and magnesium capable of supporting urchin 32 test growth (Kennedy et al. 2007). In addition, 33 the abalone diet may have also had inadequate 34 levels of β -carotene to support somatic growth 35 of urchins. Although algae is often incorporated into abalone diets as a binder or feed attractant, 36 37 thus contributing some level of carotenoids, 38 dietary pigment levels do not appear to be 39 an essential concern to the industry, and 40 abalone diets are not typically supplemented 41 with additional carotenoids (Fleming et al. 42 1996; Bautista-Teruel and Millamena 1999). 43 In addition, other factors such as palatability, 44 digestibility, and protein and lipid sources may 45 have played a role. In particular, the abalone 46 diet was notable for its extreme hardness and water stability, and it did not appear to be 47 48 consumed by the urchins as readily as the other 49 diets.

Despite the poor performance of the abalone diet seen in this trial, it might be premature to dismiss the use of abalone feed as an urchin feed. Formulations differ between manufacturers and for different life stages (Fleming et al. 1996; Bautista-Teruel and Millamena 1999), and a different abalone diet could possibly provide better results with urchins. There appears to be little if any previously published research carried out on this topic. Certainly, the history of the development of commercial abalone feeds after an initial industry reliance on seaweed provides a model for the further development of feeds for urchin aquaculture.

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In this study, we observed a direct relationship between the protein level in the diet and the 24-h water stability, with stability increasing along with protein content. This complicated efforts to make a definitive recommendation regarding protein levels in green sea urchin feeds. The low and intermediate protein diets, which gave the best growth performance, also disintegrated more readily. The high protein diets (including the abalone feed) gave relatively poor growth performance but were highly stable, remaining intact after 24 h. There was also a noticeable (although unquantified) difference in texture between the low stability and high stability diets; the high stability diets were "harder" and more brittle than the low stability diets. This difference in texture and water stability may have affected the availability, palatability, or digestibility of the diet to the juvenile urchins.

Typically, a high degree of water stability is desirable for aquaculture feeds. As feeds disintegrate, they leach nutrients, become unavailable to the animal, and compromise water quality, particularly in closed recircu-40 lating systems. Pearce et al. (2002) looked at the effects of binder type and concentration in 41 42 prepared diets on the gonad yield and quality 43 in S. droebachiensis, and observed that more 44 stable feeds increased gonad yields, possibly 45 due to the longer period of time that the feed 46 remained available to the urchins. They rec-47 ommended gelatin as the optimum binder, at 48 levels of 3–5%. However, Pearce et al. (2002) were working with mature adult urchins. Small 49

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juveniles, which at 5 mm have only recently 2 switched from grazing on diatom films to feed-3 ing on macroalgae (Raymond and Scheibling 4 1987; Sakai et al. 2004; Pearce et al. 2005), 5 may prefer or be better able to graze on disin-6 tegrated or softer diets versus intact and harder 7 diets. Klinger (1982) did not find any difference 8 in consumption rates in the urchin, L. variega-9 tus, fed "soft" versus "hard" extruded feeds, but 10 was working with larger individuals than those 11 used in this study. The issues of appropriate 12 texture, shape, palatability, and water stability 13 in diets formulated for somatic growth of small 14 juvenile urchins are all topics in need of further 15 study. 16

Conclusion

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19 The results from this study indicate that protein levels of 16-23% in formulated diets 20 can support good somatic growth of small 21 juvenile green sea urchins, and that formulated 22 23 diets can outperform the kelp, S. latissima, as 24 a primary diet. Kelp protein levels fluctuated 25 seasonally, with the best growth of kelp-fed urchins seen when the kelp was at its highest 26 27 protein level of 32.9%. Protein levels in the 28 formulated diets in excess of 23% were of 29 no benefit and indeed resulted in less growth. 30 However, the variable water stability of the 31 diets created some ambiguity in interpreting 32 the results, and more work needs to be carried 33 out to determine if urchins at this small size 34 (5.5–25 mm) might benefit from softer or less 35 water stable feeds. A commercially available 36 abalone diet fed to urchins resulted in poor 37 growth, but there are opportunities for further 38 research regarding the use of abalone diets for urchins. All of the formulated diets resulted in 40 precocious gonad growth, and the gonads had 41 a pale off-white color that would make them 42 unsuitable for market. Gonads of urchins fed 43 kelp had a normal yellow/orange color. It may 44 be the case that at least two diet formulations 45 are required to grow green sea urchins in culture 46 from settlement to harvest: a diet that promotes 47 fast somatic growth during the juvenile stages, 48 and a finishing diet to enhance gonad quality 49 prior to harvest.

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