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Protein and energy digestibility and gonad development of the European sea urchin *Paracentrotus lividus* (Lamarck) fed algal and prepared diets during spring and fall

Susan C Schlosser[1], Ingrid Lupatsch[2], John M Lawrence[3], Addison L Lawrence[4] & Muki Shpigel[2]

[1]California Sea Grant Extension Program, Eureka, CA, USA

[2]National Center for Mariculture, Israel Oceanographic and Limnological Research, Eilat, Israel

[3]Department of Biology, University of South Florida, Tampa, FL, USA

[4]Texas A&M Shrimp Mariculture Project, Port Aransas, TX, USA

Correspondence: S C Schlosser, California Sea Grant Extension Program, 2 Commercial St., Ste. 4, Eureka, CA 95501, USA. E-mail: scschlosser@ucdavis.edu

Abstract

Protein and energy are two of the main limiting factors for sea urchin growth. However, the requirement of daily protein and energy to maximize gonadal production is still unknown. *Paracentrotus lividus* were fed three experimental diets: *Ulva lactuca, Gracilaria conferta* and a prepared diet for 2 months in the fall of 1999 and spring of 2000. Sea urchins from a laboratory-cultured population of equal age, weight and test diameter were used. Apparent digestibility coefficients (ADC%) for protein and energy, using acid-insoluble ash as a marker, were measured for all experimental diets. Apparent digestibility coefficients for protein was high ($>75\%$) for all diets. Energy digestibility varied among the diets and was lowest for *G. conferta* (50–62%). The three diets contained varying digestible protein (DP) to digestible energy (DE) ratios of 25, 26 and 12 mg kJ^{-1} for *U. lactuca, G. conferta* and the prepared diet respectively. Digestible protein intake was similar for all treatments, but DE intake was greater for sea urchins fed the prepared diet in both seasons. As a result, the gonad production was significantly higher for urchins fed the prepared diet, suggesting that energy was limiting in the algal diets. *Paracentrotus lividus* spawned during the spring experiment, resulting in protein loss in all treatments. Protein loss was lowest in the sea urchins fed the prepared diet. Enhanced gonadal growth and gamete development of *P. lividus* resulted from the higher dietary energy content of the prepared diet.

Key words: *Paracentrotus lividus*, sea urchin, gonad, protein, energy, diet

Introduction

Aquaculture of sea urchins requires understanding the quantity and quality of algal and prepared feeds for successful gonad production. *Paracentrotus lividus* (Lamarck) is a commercially important species in the Mediterranean region (Boudouresque & Verlaque 2001). Many aspects of *P. lividus* nutrition have been studied including feed type (Fernandez & Boudouresque 1997; Ferenandez & Pergent 1998); comparison of different algal diets (Frantzis & Grémare 1992) and effects of prepared diets (Fernandez & Boudouresque 2000; Spirlet, Grosjean & Jangoux 2001). The advantage of prepared diets over natural algal diets on sea urchin gonad growth is known for some species (Lawrence, Olave, Otaiza, Lawrence & Bustos 1997; Barker, Keogh, Lawrence & Lawrence 1998; Cook, Kelly & McKenzie 1998) but has not been tested for *P. lividus*. The cause of greater gonad growth with prepared diets is not clear.

Protein, as a main factor in sustaining gonadal growth has been examined. Varying dietary content

and using plant or animal protein sources affects biochemical composition of gonads (Fernandez 1997) and gonad production (Lawrence, Fenaux, Corre & Lawrence 1991). However, little is known about the effect of dietary energy and especially the balance between protein and energy in the feeds. Most sea urchin prepared diets contain 20–40% protein and digestibility is generally greater than 60% (Frantzis & Grémare 1992; Klinger, Lawrence & Lawrence 1998; McBride, Lawrence, Lawrence & Mulligan 1998; Akiyama, Unuma & Yamamoto 2001), but information about energy content or digestibility is generally lacking. The total energy or protein of an algal or prepared diet may not represent the quantity of these nutrients that are available to *P. lividus*. As in ecological studies, digestibility values of protein and energy of the food consumed are necessary to determine the amount of each nutrient utilized by *P. lividus* (McClintock 1986).

Gonads of sea urchins vary in size and gametogenic state during the year. Gonad production is greater in the post-spawning season than in the spawning season (Lozano, Galera, Lopez, Turon, Palacin & Morera 1995; Unuma, Kooichi, Furuita, Yamamoto & Akiyama 1996; Lawrence *et al.* 1997; Klinger *et al.* 1998). Protein and energy are allocated to increases in body size or to gonadal production, depending on the animals' reproductive condition (Edwards & Ebert 1991; Pearse & Cameron 1991; Fernandez & Boudouresque 2000).

The purpose of this study is to compare the effects of protein and energy on gonad production and body composition in adult *P. lividus* fed algal and prepared diets during and after the spawning season. Application of this information will help improve and optimize diet formulations for the culture of sea urchins.

Materials and methods

Two identical, 60-day studies were conducted during fall (October–December 1999) and spring (March–May 2000). All methods apply to both studies unless otherwise stated. All reported values are mean ± SD.

Experimental conditions

Seawater flow from a common manifold was 0.5 L min^{-1} to each aquarium with temperature recorded twice daily. Dissolved oxygen, pH and salinity were measured weekly. Mean seawater temperature was 24.2 ± 0.9 °C ($n = 120$, two measure-

ments day^{-1}) in the fall and 21.4 ± 0.7 °C ($n = 120$) in the spring. Dissolved oxygen was 90–100% saturation and mean pH was 8.17 ± 0.05 ($n = 12$ per experiment, one sample week^{-1} for dissolved oxygen, pH and salinity). Salinity was constant at 41 ppt. The seawater system supplied a continuous flow of seawater filtered to 100 μm to the experimental aquaria. Aquaria dimensions were 20 × 35 × 15 cm. Each aquarium was vigorously aerated and contained 10.5 L of seawater. Sea urchins were always in close proximity to their food and were checked every morning and evening when temperatures were recorded.

Adult *P. lividus* were selected from a cohort produced by spawning individuals in the laboratory in December 1997. Animals were starved for 1 week prior to starting both studies to ensure similar nutritional condition for each individual. Sea urchins were measured (horizontal test diameter, HD) to the nearest 0.01 mm, weighed (whole wet weight, ± 0.01 g) and placed in replicate, randomly arranged, glass aquaria ($n = 3$ aquaria diet^{-1}, 10 urchins aquaria^{-1}). Initial mean test diameter and whole animal wet weight were 30.7 ± 1.5 mm and 13.1 ± 1.5 g in the fall and 34.3 ± 1.4 mm and 18.7 ± 1.7 g in the spring.

An initial sample ($n = 20$) of *P. lividus*, and all animals at the conclusion of both studies, were dissected. Wet body compartments (gonad, gut, lantern, test) were weighed to the nearest 0.01 g. The body compartments were dried at 105 °C for 24 h and reweighed. Interstitial water and coelmic fluid lost during dissection and desiccation is not relevant to protein and energy intake, efficiency or gonad production. Whole animal dry weight in this study is the sum of the dry body compartments. Gonad index (%) was calculated as (dry gonad weight (g)/dry body weight (g) × 100) and as (wet gonad weight (g)/ whole animal weight (g) × 100). Dry body compartment indices are given for gut, lantern and test.

One gonad section from each animal in the initial sample ($n = 20$) and a sub-sample from each treatment ($n = 10$ per diet treatment, five females, five males) were preserved in neutral-buffered formalin to determine the reproductive state according to Byrne (1990). These were: (1) recovering stage with small previtellongenic oocytes or primary spermatocytes; (2) growing stage with many large nutritive phagocytes; (3) mature stage with no or few nutritive phagocytes around oocytes or spermatozoa; (4) partly spawned stage with some void spaces containing few nutritive phagocytes and loosely packed gametes and (5) spent stage with gonads appearing empty.

Feeding study

The diets fed *P. lividus* were fresh, cultured *Ulva lactuca* and *Gracilaria conferta* (Cohen & Neori 1991) and an extruded moist pellet, hereafter referred to as the prepared diet. The prepared diet was manufactured by Wenger International (Kansas, MO, USA). Qualitative differences between the three diets were not considered in this study.

Sea urchins were fed every 3 or 4 days. At each feeding, uneaten food was removed and fresh algae or prepared diet added. Each week, feed intake was measured during one 3-day interval. To measure feed intake, uneaten food was siphoned through a 1 mm mesh to remove feces, pieces of spines and detritus. Mean daily food ingested per urchin was calculated as the difference between the feed introduced and that removed. Excess moisture was removed from the algae by squeezing it in a mesh net and blotting on paper towels before weighing. The percent moisture of each feed was determined by drying samples to constant weight at 60 °C. Feed intake is given as milligrams of dry food consumed animal^{-1} day^{-1}. Food was available in excess at all times. Four individuals died. In both fall and spring, one mortality occurred in the *U. lactuca* and one in the prepared diet treatments. Feed ingestion calculations were adjusted for mortalities.

Dry matter, protein, ash and energy of the algae, *U. lactuca* and *G. conferta*, were determined at weekly intervals to determine the composition. Average values during the 2-month trials were used to calculate diet composition (Table 1).

For the algal diets, control aquaria (*n* = 2 per species) without *P. lividus* were used to measure algal growth or degradation. There was no significant change in algal weight during the 3-day feeding intervals. Changes in algal biomass were 0.016 ± 0.013% and 0.019 ± 0.018% for *U. lactuca* and *G. conferta*, respectively, during both seasons. The protein of the algae did not change in 3 days (Shpigel, Ragg, Lupatsch & Neori 1999).

Digestibility

Nine separate groups of *P. lividus* from the same laboratory cohort were fed *U. lactuca*, *G. conferta* or the prepared diet to obtain feces samples (*n* = 10 animals aquaria^{-1}, three replicates diet^{-1}). Incoming sea water for feces collection was filtered to 1 μm. Feed was removed in the evening. Feces were collected 10 h later by siphoning onto a 60 μm mesh. The feces

Table 1 Composition of algae and prepared diet fed to *Paracentrotus lividus* in the fall and spring experiments (per g dry matter)

	Ulva lactuca	Gracilaria conferta	Prepared diet
Fall			
Dry matter (%)	11.0	12.0	90.0
Total protein (mg)	374	152	234
Ash (mg)	174	397	94
Lipid (mg)	28	12	70
Total energy (kJ)	15.65	11.52	19.39
Spring			
Dry matter (%)	12.0	12.0	90.0
Total protein (mg)	340	294	234
Ash (mg)	269	329	94
Lipid (mg)	20	14	70
Total energy (kJ)	13.39	12.82	19.39

were dried to a constant weight at 60 °C. Feces from the same aquaria were combined over the sampling period to provide sufficient fecal matter for analysis.

Apparent digestibility coefficients (ADC) for protein and energy were calculated using acid insoluble ash (AIA) as a marker where:

$$ADC\ (\%) = 100 - [100 \times (AIA_{food}/AIA_{feces}) \times (energy\ or\ protein_{feces}/energy\ or\ protein_{food})]$$

Digestible protein (DP) and digestible energy (DE) in the diets were calculated with digestibility values and diet compositions:

Digestible protein in diet (DP) :
(mg g^{-1} total protein in diet) × (ADC$_{protein}$%)

Digestible energy in diet(DE) :
(kJ g^{-1} gross energy in diet) × (ADC$_{energy}$%)

Digestible protein and energy intake were calculated as DP or DE × dry feed consumed animal^{-1} day^{-1}.

Protein and energy efficiencies (%) were calculated as [(final dry weight protein or energy per animal-initial protein or energy per animal)/(DP or DE intake)] × 100.

Identical analyses were applied to the diets, fecal matter and sea urchin body compartments. Dry matter was calculated by weight loss after 24 h drying at 105 °C. Protein was measured using the Kjeldahl method and multiplying N by 6.25. Lipid was measured after chloroform–methanol extraction (Folch, Lees & Sloane 1957). Samples were homogenized with a high-speed homogenizer for 5 min and lipid was extracted gravimetrically after separation and vacuum drying. Ash was calculated from the weight loss after

incineration for 24 h at 550 °C in a muffle furnace and AIA content according to Atkinson, Hilton and Slinger (1984). Gross energy content was measured by combustion in a bomb calorimeter (Parr Instrument Company, Moline, IL, USA) using benzoic acid as a standard. Protein and energy of gonads and whole animal are compared using dry weights.

Statistical procedures

Two-way analysis of variance (ANOVA) using season and diet as main factors were carried out using aquarium means ($n = 3$ per treatment) for the ADC values, dry feed consumption, sea urchin dry weight, dry body compartment indices and protein and energy (mg or kJ animal^{-1}) for *P. lividus* gonads and whole animal. ANOVA were conducted using aquarium means and a $P < 0.05$. For all ANOVA, the degrees of freedom are 1 (season), 2 (diet), 2 (interaction) and 17 (error). Pair-wise differences were compared using Tukey's test. The data showed homogeneity of variance (Cochran's *C*-test) and were normally distributed (Kolmogorov-Smirnoff test with Lilliefors modification). Reproductive stage, DP and energy intake and protein and energy efficiencies are compared qualitatively.

Results

Diet composition and digestibility

Composition of the three feeds for fall and spring are given in Tables 1 and 2. Seasonal variation was observed for the algae, especially for *G. conferta*, which had lower protein and energy values in fall compared with spring. The prepared diet did not vary seasonally. Apparent digestibility coefficients values for protein were significantly lower for *G. conferta* in fall than in spring and there were no seasonally significant differences for *U. lactuca* or the prepared diet (Table 3). In the fall, ADC for protein was significantly greater for *P. lividus* fed *U. lactuca* compared with those fed *G. conferta*. There were no significant differences for ADC for energy between seasons. Apparent digestibility coefficients for energy was significantly lower for *G. conferta*, while higher and similar for *U. lactuca* and the prepared diet in both seasons.

Feed intake

A decrease in feed consumption during the third week in the spring for *P. lividus* fed the prepared diet

Table 2 Diet content of prepared diet fed to *Paracentrotus lividus* in the fall and spring experiments as percent of total dry matter

| Diet ingredient	% of total dry matter
Corn	26.00
Wheat middens	26.00
Defatted soy flour	12.33
Fish meal	13.45
Kelp	15.00
Sodium phosphate	1.46
Soy lecithin	1.12
Ethoxyquin	0.23
Ascorbic acid	0.11
Vitamin/mineral premix	0.23
Potassium sorbate	0.34
Phosphoric acid	0.60
Fish oil	2.00
Carotene	0.80
Cholesterol	0.33

Table 3 Apparent digestibility coefficients (ADC%, mean ± SD) of protein and energy, digestible protein (DP) and digestible energy (DE) content of diets fed to *Paracentrotus lividus* in the fall and spring experiments

	Ulva lactuca	*Gracilaria conferta*	Prepared diet
Fall			
ADC (%)			
Protein	89.7 ± 1.9	75.2 ± 5.4	83.8 ± 1.6
Energy	89.8 ± 2.0	50.5 ± 4.6	82.4 ± 1.1
DP (mg g^{-1})	335	114	196
DE (kJ g^{-1})	14.1	5.8	15.9
DP/DE ratio (mg kJ^{-1})	23.8	19.6	12.3
Spring			
ADC (%)			
Protein	87.3 ± 1.4	88.5 ± 6.1	83.8 ± 1.4
Energy	85.4 ± 0.7	62.2 ± 7.9	82.4 ± 1.9
DP (mg g^{-1})	297	260	196
DE (kJ g^{-1})	11.4	7.9	15.9
DP/DE ratio (mg kJ^{-1})	26.0	32.6	12.3

Values for the prepared diet did not differ between seasons and an average value was used for all calculations. Seasonal diet composition values were used for algal diets.

treatment corresponded with spawning (Fig. 1). Sea urchins fed *U. lactuca* and *G. conferta* spawned during weeks 4 and 6 (Fig. 1). After spawning, *P. lividus* fed prepared diet treatment had a greater dry feed intake for the duration of the experiment. Sea urchins fed algal diets showed a slight increase in food consumption during the last 2–3 weeks.

Feed consumption on a dry matter basis was significantly different between season, diet and their
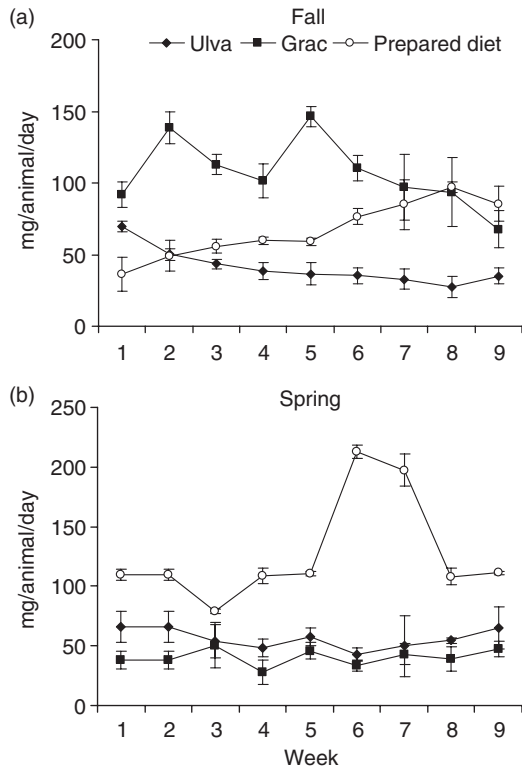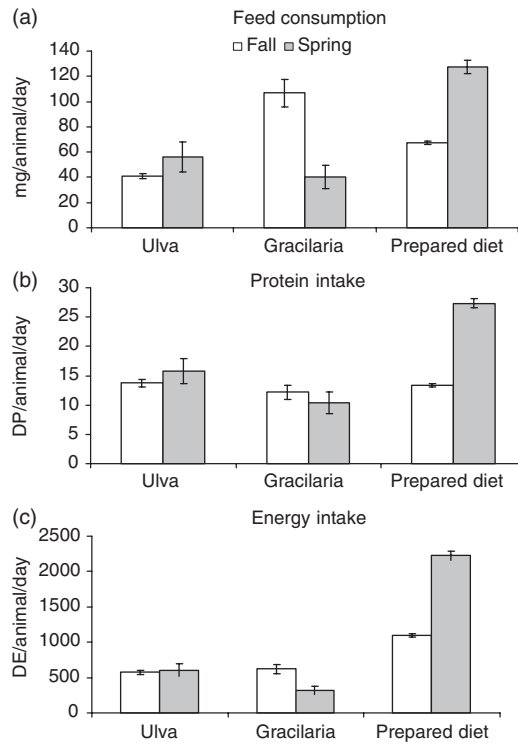
Figure 1 Daily dry feed consumed (mg animal^{-1} day^{-1}) for *Paracentrotus lividus* fed *Ulva lactuca*, *Gracilaria conferta* and a prepared diet on a weekly basis for 60 days. All values are mean ± SD, $n = 3$.

Figure 2 (a) Mean daily dry feed consumed per season, (b) daily digestible protein (mg) and (c) digestible energy (kJ) consumed per animal for *Paracentrotus lividus*.

interaction (Fig. 2). In spring, *P. lividus* consumed significantly more prepared diet than either algal species (Fig. 2). In fall, *P. lividus* consumed significantly more *G. conferta* compared with the prepared diet or *U. lactuca*. *Paracentrotus lividus* consumed significantly more prepared diet treatment in spring than fall, significantly more *G. conferta* in the fall than spring and equal amounts of *U. lactuca* in spring and fall (Fig. 2).

Whole animal dry weight

Significant differences between season and diet showed spring dry weights were greater than fall, primarily because of the large gonads. *Paracentrotus lividus* fed the prepared diet were significantly heavier than those fed algal diets. There were no significant differences between *P. lividus* dry weights in the algal treatments. The sea urchins were also slightly larger in spring than fall because of somatic growth of the laboratory cohort (Table 4).

Dry body compartment indices

The main factors, season and diet, significantly affected gonad index (Table 4). Spring gonad index was greater than fall (Table 4). *P. lividus* fed the prepared diet had significantly greater gonad indices than those fed *U. lactuca* or *G. conferta* in both seasons. There were no significant differences between gonad indices of *P. lividus* in algal treatments within seasons. Gut index was significantly affected by both season and diet. Fall gut indices were greater than spring indices. Gut indices for *P. lividus* fed the prepared diet were greater than both algal diets. Lantern indices were significantly affected by the diet/season interaction. Lantern indices for sea urchins from the algal treatments were significantly greater than those for *P. lividus* fed the prepared diet, but only in the fall. Test indices were significantly affected by season and diet, but not their interaction. Test indices were greater in the fall and greater for *P. lividus* in algal treatments compared with the prepared diet.

Gonad and whole animal protein and energy

Significant differences between season, diet and their interaction indicated that whole animal protein

Table 4 Growth and efficiency parameters of *Paracentrotus lividus* from fall and spring experiments

	Fall			Spring		
	Ulva lactuca	**Gracilaria conferta**	**Prepared diet**	**U. lactuca**	**G. conferta**	**Prepared diet**
Initial live weight (dry) (g urchin^{-1})	12.5 (3.9) ± 1.6 (1.2)	12.5 (3.9) ± 1.6 (1.2)	12.5 (3.9) ± 1.6 (1.2)	18.4 (6.0) ± 1.3 (0.1)	18.4 (6.0) ± 1.3 (0.1)	18.4 (6.0) ± 1.3 (0.1)
Final live weight (dry) (g urchin^{-1})	13.9 (3.6) ± 1.3 (0.21)	12.3 (3.7) ± 1.5 (0.3)	13.7 (4.6) ± 2.4 (0.1)	17.6 (5.7) ± 1.8 (0.1)	17.5 (5.4) ± 2.5 (0.3)	18.1 (6.6) ± 1.6 (0.2)
Wet gonad index* (dry) (%)	3.9 (4.3) ± 1.5 (0.1)	4.1 (3.8) ± 1.5 (0.1)	5.1 (7.3) ± 1.9 (0.1)	12.7 (9.1) ± 2.1 (0.1)	11.2 (7.9) ± 3.1 (0.1)	16.8 (13.7) ± 2.3 (0.1)
Dry Gut Index† (%)	1.3 ± 0.1	1.5 ± 0.1	1.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.5 ± 0.1
Dry Lantern Index‡ (%)	8.8 ± 1.1	8.4 ± 1.0	6.5 ± 1.0	7.4 ± 1.0	7.8 ± 2.3	6.6 ± 1.0
Dry Test Index§ (%)	86.3 ± 2.0	86.1 ± 2.7	84.2 ± 3.1	82.0 ± 3.7	83.2 ± 2.5	78.6 ± 3.5
Protein¶ efficiency %	8.7 ± 2.9	11.8 ± 2.6	24.0 ± 0.9	–	–	–
Energy¶ efficiency %	7.3 ± 2.6	7.6 ± 1.8	16.4 ± 0.7	–	–	3.0 ± 1.8

Initial wet weight (g) is whole animal live weight. Initial dry weight is the sum of all dry body compartments and does not include interstitial water and coelmic fluid. Gonad index is shown for wet and dry weights. Dry weights or indices follow wet weights or indices and are in parentheses. Dry gut, lantern and test indices are shown. All values are mean ± SD (*n* = 3 for all except initial sample where *n* = 20).

*Gonad initial: Fall, 3.9 (3.68) ± 1.2 (0.04); Spring, 16.1 (13.32) ± 2.8(0.04).

†Gut initial: Fall, 1.3 ± 0.1; Spring, 1.4 ± 0.1.

‡Lantern initial: Fall, 8.2 ± 1.2; Spring, 7.0 ± 1.0.

§Test initial: Fall, 86.9 ± 2.8; Spring, 78.2 ± 3.3.

¶Based on DP and DE.

(mg animal^{-1}) was greater in spring than fall for all diets (Tables 3 and 5). *Paracentrotus lividus* fed the prepared diet had significantly greater protein than sea urchins from the algal diets. There were no significant differences in whole animal protein between *P. lividus* fed the algal diets.

Spawning in all diet treatments during the spring experiment resulted in a decrease of gonadal protein (mg animal^{-1}) compared with the initial sample. Gonadal protein was significantly different between season and diet with spring values greater than fall (Table 5). Gonadal protein of *P. lividus* fed the prepared diet was significantly greater in sea urchins than gonads from algal treatments in both seasons. Whole animal and gonadal protein increased during the fall study and decreased during the spring.

Whole animal energy (kJ animal^{-1}) and gonad energy were significantly affected by season, diet and their interaction. Energy content of whole animal and gonads was greater in spring than fall for all treatments and sea urchins fed the prepared diet had significantly greater energy content than animals from the algal treatments (Table 5). There were no significant differences in energy of gonads or whole animal between the algal treatments. Whole animal energy content increased in the prepared diet treatment in both experiments and decreased for algal treatments in the spring. Gonadal energy content increased in the fall for all treatments. In the spring, *P. lividus* fed the prepared diet showed an increased gonadal energy content while sea urchin gonads from algal treatments decreased in energy content.

DP and energy intake and efficiencies

Daily DP intake in the fall was similar for all dietary treatments and amounted to 13.7 ± 0.7, 12.2 ± 1.3 and 13.4 ± 0.3 mg for *U. lactuca*, *G. conferta* and the prepared diet respectively (Fig. 2). In the spring, DP intake was higher in the prepared diet treatment because of higher food consumption (Fig. 2). During the spring experiment, total protein intake with prepared feed was twice as great compared with algal treatments (Fig. 2). Protein efficiency was two to three times greater in the prepared diet compared with algal treatments in the fall (Table 4). Protein efficiency was negative for all treatments in the spring.

High dietary energy in the prepared diet treatment resulted in high DE intake in fall and spring (Fig. 2). Digestible energy intake was two to three times greater in the prepared diet compared with algal treatments in the fall and spring respectively. Energy efficiency in the prepared diet was double that of algal diets in the fall and was positive in the prepared diet treatment in the spring.
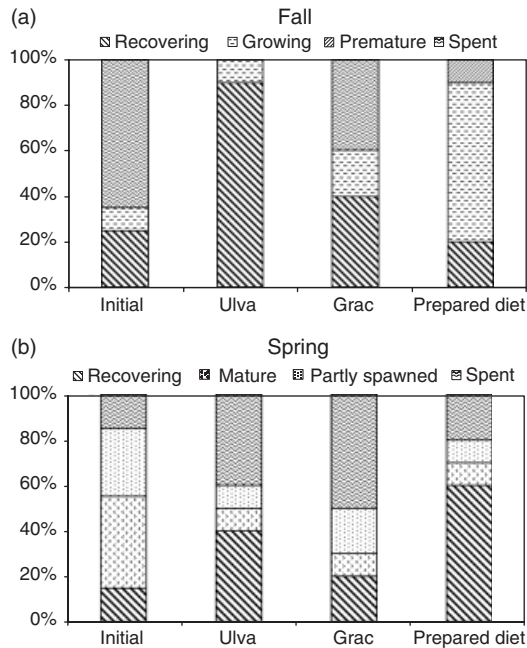
Reproductive stage

Initially in fall, *P. lividus* were in spent, recovering and growing stages (Fig. 3). At the end of the

Table 5 Protein and energy content of *Paracentrotus lividus* fed three experimental diets

| Factor | Treatment | Gonad | Body compartment | | | Whole animal |
			Gut	Lantern	Test	
Protein Fall	Initial sample	84.0 ± 6.8	29.0 ± 2.2	19.0 ± 0.7	156.0 ± 10.3	287.2 ± 12.3
	Ulva lactuca	99.4 ± 11.4	28.5 ± 2.8	23.8 ± 1.2	187.0 ± 10.5	342.3 ± 24.1
	Gracilaria conferta	102.4 ± 7.1	36.6 ± 10.5	25.6 ± 2.2	191.4 ± 16.3	356.0 ± 28.2
	Prepared diet	155.0 ± 21.9	39.0 ± 2.7	28.4 ± 1.0	239.8 ± 8.8	462.1 ± 11.7
Spring	Initial sample	517.1 ± 63.3	51.0 ± 1.4	35.2 ± 1	272.0 ± 8.4	876.0 ± 61.1
	Ulva lactuca	312.6 ± 74.3	37.2 ± 5.1	27.3 ± 1.1	246.0 ± 11.0	623.1 ± 67.0
	Gracilaria conferta	265.7 ± 54.1	35.3 ± 1.1	21.6 ± 3.1	238.1 ± 7.1	560.7 ± 60.4
	Prepared diet	450.5 ± 57.9	43.5 ± 2.1	28.7 ± 1.2	322.5 ± 11.4	845.1 ± 55.3
Energy Fall	Initial sample	3.5 ± 0.3	1.1 ± 0.2	0.5 ± 0.1	4.6 ± 0.3	9.7 ± 0.7
	Ulva lactuca	4.2 ± 0.5	1.0 ± 0.1	0.6 ± 0.1	5.8 ± 0.3	11.65 ± 0.9
	Gracilaria conferta	4.1 ± 0.5	1.4 ± 0.4	0.7 ± 0.1	5.8 ± 0.5	12.0 ± 0.9
	Prepared diet	8.1 ± 1.1	2.4 ± 0.2	0.8 ± 0.1	8.7 ± 0.3	19.9 ± 0.7
Spring	Initial sample	17.9 ± 3.1	1.8 ± 0.2	1.0 ± 0.1	9.4 ± 0.4	30.4 ± 2.8
	Ulva lactuca	12.3 ± 0.5	1.4 ± 0.2	0.8 ± 0.1	7.5 ± 0.3	22.0 ± 2.7
	Gracilaria conferta	9.2 ± 1.8	1.2 ± 0.1	0.7 ± 0.1	7.4 ± 0.2	18.4 ± 2.1
	Prepared diet	20.8 ± 2.7	2.4 ± 0.1	0.9 ± 0.1	10.2 ± 0.4	34.4 ± 2.6

Protein content is mg body^{-1} compartment or animal and energy is kJ body^{-1} compartment or animal. For the initial sample $n = 20$ and for all diet treatments, $n = 3$, mean ± SD.

Figure 3 Reproductive condition of *Paracentrotus lividus* for an initial sample and at the end of the fall and spring experiments. For each bar, $n = 10$, five male and five female sea urchins.

experiment, sea urchins fed *U. lactuca* were in the recovering stage while individuals fed *G. conferta* resembled the initial sample. Greatest changes in reproductive stage were found in gonads of *P. lividus* fed the prepared diet where gonads at the end of the experiment were recovering, growing and premature.

Initially in spring, *P. lividus* were in mature, partly spawned, spent and recovering stages. Spawning occurred during the spring. Most sea urchins fed *U. lactuca* and *G. conferta* were in the recovering and spent stage at the end of the study. In the prepared diet treatment, most *P. lividus* were in the recovering stage.

Discussion

Results from both seasons suggest that dietary energy content was the main limiting factor for *P. lividus* under conditions of this experiment. In the fall, DP intake was similar in all treatments, but gonad production was greatest in the animals fed the prepared diet. The dietary DP/DE ratios show that for each 24 mg of *U. lactuca* consumed in the fall, only 1 kJ of energy was available. The DP/DE ratio of the prepared diet was 12 mg kJ^{-1}, meaning, that twice as much energy in relation to protein was available

from the prepared diet. As dietary protein may function as an energy source apart from its essential role in growth, low energy intake in urchins fed the algal diets may have resulted in use of protein to meet absolute energy requirements, resulting in lower gonadal growth. This holds true for a number of aquatic species such as finfish (Lupatsch, Kissil, Skalan & Pfeffer 2001).

In the spring, because of spawning, protein efficiencies were negative in all treatments. Positive energy efficiency was found only in the prepared diet treatment where gonad index remained around 13%. Energy intake in urchins fed the prepared diet was double that of algal treatments. Although not measured, it is possible that greater gonad production for many species of sea urchin species fed prepared diets compared with algal diets may result from higher energy intake in the prepared diet treatments (Lawrence *et al.* 1997; Barker *et al.* 1998; Cook *et al.* 1998).

In the fall trial, *P. lividus* apparently consumed feed according to energy requirement. High feed intake in the *G. conferta* treatment plus low ADC for energy may have resulted in physiological limitations associated with the absorption of energy. Three times as much mass of algae was consumed as of prepared diet. Echinoids have a poor gut musculature and regulate gut water content to a constant volume (de Ridder & Jangoux 1982). In the fall, when gonads were small, *P. lividus* could consume large masses of algae but in spring when gonads were large at the start of the experiment, intake of both algal diets was lower than of the prepared diet. Lower feed intake together with lower protein and energy availability resulted in loss of gonadal production, protein and energy in the algal treatments.

The gut of sea urchins allows immediate storage of nutrient reserves (Lawrence & Klinger 2001). The increases in gut index seen in both seasons for *P. lividus* fed the prepared diet allow more digestion and absorption (Lawrence, Lawrence & Holland 1965; Bishop & Watts 1992). An abundant and high-quality food supply results in increases to the digestive system capacity which contributes to production (McBride, Lawrence, Lawrence & Mulligan 1999). Low gut index in the algal treatments suggests these diets did not contain high quality or quantity of nutrients and that storage of nutrients did not occur in these treatments. The prepared diet is an extruded product where carbohydrates are heated, possibly making them more digestible compared with algal carbohydrates (Whistler & Bemiller 1999; Akiyama *et al.* 2001). The prepared diet also contains vitamin

and mineral supplements and was consistent in quality. Seasonal differences in algal composition may affect sea urchin digestion and production (Fuji 1967; Agatsuma, Matsuyama & Nakata 1996; McBride *et al.* 1998).

In the prepared diet treatment in the spring, protein intake by *P. lividus* was low and protein efficiency was negative as a result of spawning. The change in reproductive stage within the gonad despite no change in gonad index shows the importance of histological analysis. Size of the gonad alone does not reveal how the food consumed was utilized by *P. lividus*. The slight increase in energy content for *P. lividus* in the spring is also indicated by the changes of the reproductive stage from mature to growing reproductive stage, in effect, the beginning of the next gametogenic cycle. Mature gonads released gametes and 70% of the individuals fed the prepared diet were in the growing stage in the prepared diet treatment in the spring. At this stage gonads contain less gametes, hence lower protein content and grow rapidly (Walker, Unuma, McGinn, Harrington & Lesser 2001).

Gonadal growth following spawning resulted from increase in nutritive cells. The histological changes in the gonads confirm the loss of gametes as the gonads progressed from mature to recovering and growing gonads. The availability of nutrients in the prepared diet positively affected gonadal growth during both seasons. The mature gonads of *P. lividus* at the beginning of spring were most likely near their maximum protein content. Protein level would be expected to decrease as gametes were lost during spawning (Pearse & Cameron 1991) although the protein loss was lowest in the prepared diet treatment. In an elegant dietary protein study with *Lytechinus variegatus*, dietary protein content affected gonad protein content by increasing the size of nutritive phagocytes and therefore protein storage capacity (Hammer, Hammer, Watts, Desmond, Lawrence & Lawrence 2004).

Food consumption rates also indicate spawning by their decrease and subsequent rise, particularly evident in the prepared diet. Lack of change in gonad index and reduced feed consumption in mature echinoids with high gonad index is widely reported (Fuji 1967; Ebert 1968; Meidel & Scheibling 1998). Minimum feed intake at spawning and maximum feed intake following spawning is known for *P. lividus* (Fernandez & Boudouresque 2000).

Mean temperature differences between fall and spring did not appear to affect ADC for protein and energy. Digestibility coefficients found with the diets tested here are similar to those found in other studies with *P. lividus* and other sea urchins (Frantzis & Grémare 1992; Klinger *et al.* 1998; Lawrence & Klinger 2001). Changes in digestibility coefficients may require greater seasonal changes in temperature, such as those reported by Fuji (1967).

Gonadal growth and development found in *P. lividus* fed the prepared diet indicate that besides protein, the energy content is one of the limiting factors for growth during the growing and mature stages of the reproductive cycle. This may partially explain why other studies show greater gonadal production with prepared diets compared with algal diets. Although low protein absorption has been shown to reduce gonad production, the significant effect of increased energy availability was seen in the high gonadal index and progression in the reproductive cycle of *P. lividus* fed the prepared diet. In the prepared diet treatment, available energy was sufficient and accounted for significantly greater gonadal production. Further studies examining a range of dietary energy content would be useful. Successful sea urchin aquaculture requires understanding energy needs for production, economic efficiency and understanding the interaction of energy with other dietary components.

References

Agatsuma Y., Matsuyama K. & Nakata A. (1996) Seasonal changes in feeding activity of the sea urchin *Strongylocentrotus nudus* in Oshoro Bay, southwestern Hokkaido. *Nippon Suisan Gakkaishi* **62**, 592–597.

Akiyama T., Unuma T. & Yamamoto T. (2001) Optimum protein level in a purified diet for young red sea urchin *Pseudocentrotus depressus*. *Fisheries Science* **67**, 361–363.

Atkinson J.L., Hilton J.W. & Slinger S.J. (1984) Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout. *Canadian Journal of Fisheries and Aquatic Science* **41**, 1384–1386.

Barker M.E., Keogh J.A., Lawrence J.M. & Lawrence A.L. (1998) Feeding rate, absorption efficiencies, growth, and enhancement of gonad production in the New Zealand sea urchin *Evechinus chloroticus* Valenciennes (Echinoidea: Echinometridae) fed prepared and natural diets. *Journal of Shellfish Research* **17**, 1583–1590.

Bishop C.D. & Watts S.A. (1992) Biochemical and morphometric study of growth in the stomach of the echinoid *Lytechinus variegatus* (Echinodermata). *Marine Biology* **114**, 459–467.

Boudouresque C.-F. & Verlaque M. (2001) Ecology of *Paracentrotus lividus*. In: *Edible Sea Urchins: Biology and Ecology* (ed. by J.M. Lawrence), pp. 177–216. Elsevier Science, B.V., Amsterdam, the Netherlands.

Byrne M. (1990) Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. *Marine Biology* **104**, 275–289.

Cohen I. & Neori A. (1991) *Ulva lactuca* biofilters for marine fishpond effluent. I. Ammonia uptake kinetics and nitrogen content. *Botanica Marina* **34**, 475–482.

Cook E.J., Kelly M.S. & McKenzie J.D. (1998) Somatic and gonadal growth of the sea urchin *Psammechinus miliaris* (Gmelin) fed artificial salmon feed compared with a macroalgal diet. *Journal of Shellfish Research* **17**, 1549–1556.

de Ridder C. & Jangoux M. (1982) Digestive systems: echinoidea. In: *Echinoderm Nutrition* (eds. by M. Jangoux & J. Lawrence), pp. 213–234. Balkema, Rotterdam, the Netherlands.

Ebert T.A. (1968) Growth rates of the sea urchin *Strongylocentrotus purpuratus* (Stimpson) related to food availability and spine abrasion. *Ecology* **49**, 1075–1091.

Edwards P.B. & Ebert T.A. (1991) Plastic responses to limited food availability and spine damage in the sea urchin, *Strongylocentrotus purpuratus*. *Journal of Experimental Marine Biology and Ecology* **145**, 205–220.

Fernandez C. (1997) Effect of diet on the biochemical composition of *Paracentrotus lividus* under natural and rearing conditions. *Comparative Biochemistry and Physiology* **118A**, 1377–1384.

Fernandez C. & Boudouresque C.-F. (1997) Phenotypic plasticity of *Paracentrotus lividus* (Echinoidea: Echinodermata) in a lagoon environment. *Marine Ecology Progress Series* **152**, 145–154.

Fernandez C. & Boudouresque C.-F. (2000) Nutrition of the sea urchin *Paracentrotus lividus* (Echinoidea: Echinodermata) fed different artificial food. *Marine Ecology Progress Series* **204**, 131–141.

Fernandez C.M. & Pergent G. (1998) Effect of different formulated diets and rearing conditions on growth parameters of the sea urchin *Paracentrotus lividus*. *Journal of Shellfish Research* **17**, 1571–1582.

Folch J., Lees M. & Sloane G.H. (1957) Simple method for isolation and purification of total lipid from animal tissues. *Journal of Biological Chemistry* **226**, 497–507.

Frantzis A. & Grémare A. (1992) Ingestion, absorption, and growth rates of *Paracentrotus lividus* (Echinodermata: Echinoidea) fed different macrophytes. *Marine Ecology Progress Series* **95**, 169–183.

Fuji A. (1967) Ecological studies on the growth and food consumption of a Japanese common littoral sea urchin, *Strongylocentrotus intermedius* (A. Agassiz). *Memoirs of the Faculty of Fisheries Hokkaido University* **15**, 83–160.

Hammer B.W., Hammer H.S., Watts S.A., Desmond R.A., Lawrence J.M. & Lawrence A.L. (2004) The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegates* (Echinodermata:Echinoidea). *Marine Biology* **145**, 1143–1157.

Klinger T.S., Lawrence J.M. & Lawrence A.L. (1998) Digestion, absorption and assimilation of prepared feeds by echinoids. In: *Echinoderms: San Francisco* (ed. by R. Mooi & M. Telford), pp. 713–722. A.A. Balkema, Rotterdam, the Netherlands.

Lawrence J., Fenaux L., Corre M.C. & Lawrence A. (1991) The effect of quantity and quality of prepared diets on production in *Paracentrotus lividus* (Echinodermata: Echinoidea). *Echinoderm Research 1991* 107–110, ISBN 90 5410 0494.

Lawrence J.M. & Klinger T.S. (2001) Digestion in sea urchins. In: *Edible Sea Urchins: Biology and Ecology* (ed. by J.M. Lawrence), pp. 103–113. Elsevier Scientific B.V., Amsterdam, the Netherlands.

Lawrence J.M., Lawrence A.L. & Holland N.D. (1965) Annual cycle in the size of the gut of the purple sea urchin *Strongylocentrotus purpuratus*. *Nature* **205**, 1238–1239.

Lawrence J.M., Olave S., Otaiza R., Lawrence A.L. & Bustos E. (1997) Enhancement of gonad production in the sea urchin *Loxenchinus albus* in Chile fed extruded feeds. *Journal of the World Aquaculture Society* **28**, 91–96.

Lozano J., Galera J., López S., Turon X., Palacín C. & Morera G. (1995) Biological cycles and recruitment of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. *Marine Ecology Progress Series* **122**, 179–191.

Lupatsch I., Kissil G.Wm., Skalan D. & Pfeffer E. (2001) Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream (*Sparus aurata* L.). *Aquaculture Nutrition* **7**, 71–80.

McBride S.C., Lawrence J.M., Lawrence A.L. & Mulligan T.M. (1998) The effect of protein concentration in prepared feeds on growth, feeding rate, total organic absorption, and gross assimilation efficiency of the sea urchin *Strongylocentrotus franciscanus*. *Journal of Shellfish Research* **17**, 1563–1570.

McBride S., Lawrence J.M., Lawrence A.L. & Mulligan T.M. (1999) Ingestion, absorption, and gonad production of adult *Strongylocentrotus franciscanus* fed different rations of a prepared diet. *Journal of the World Aquaculture Society* **30**, 364–370.

McClintock J.B. (1986) On estimating energetic values of prey: implications in optimal diet models. *Oecologia* **70**, 161–162.

Meidel S.K. & Scheibling R.E. (1998) Annual reproductive cycle of the green sea urchin *Strongylocentrotus droebachiensis*, in differing habitats in Nova Scotia. *Marine Biology* **131**, 461–478.

Pearse J.S. & Cameron R.A. (1991) Echinodermata: echinoidea. In: *Echinoderms and Lophophorates*, Vol. VI (ed. by A.C. Geise), pp. 513–662. Boxwood Press, Pacific Grove, CA, USA.

Shpigel M., Ragg NL., Lupatsch I. & Neori A. (1999) Protein content determines the nutritional value of the seaweed *Ulva Lactuca* L for the abalone *Haliotis tuberculata* L. and *H. discus hannai* Ino. *Journal of Shellfish Research* **18**, 227–233.

Spirlet C., Grosjean P. & Jangoux M. (2001) Cultivation of *Paracentrotus lividus* (Echinodermata: Echinoidea) on extruded feeds: digestive efficiency, somatic and gonadal growth. *Aquaculture Nutrition* **7**, 91–99.

Unuma T., Kooichi K., Furuita H., Yamamoto T. & Akiyama T. (1996) Seasonal changes in gonads of cultured and wild red sea urchin *Pseudocentrotus depressus*. *Suisanzoshoku* **44**, 169–175.

Walker C.W., Unuma T., McGinn N., Harrington L.M. & Lesser M.P. (2001) Reproduction of sea urchins. In: *Edible Sea Urchins: Biology and Ecology* (ed. by J.M. Lawrence), pp. 5–26. Elsevier, Amsterdam, the Netherlands.

Whistler R.L. & BeMiller J.N. (1999) *Carbohydrate Chemistry for Food Scientists*. Eagan Press, St Paul, MN, USA, 241 pp.