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INGESTION, ABSORPTION AND ASSIMILATION EFFICIENCIES, AND PRODUCTION IN THE SEA URCHIN *ARBACIA DUFRESNII* FED A FORMULATED FEED

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ABSTRACT Sea urchins have been used as a source of food from prehistoric times and as a research animal model since the 19th century. They presently are harvested in many parts of the world. In Argentina, sea urchins have been studied only from biological perspectives. Of the 14 species of sea urchins found off the coast of Argentina, *Arbacia dufresnii* is the most abundant. It is an omnivorous species that exists in dense populations in Nuevo Gulf. Biomass production in sea urchins, especially gonad yield, is related to food quantity and quality. In the field, *A. dufresnii* has a small size and low gonad biomass and gamete production. Therefore to test the hypothesis that a high-quality formulated food would produce more biomass and gonad yield than that found in the sea urchins from a natural population, sea urchins were reared in a laboratory aquaculture system for 8 wk in autumn when gametogenesis occurs. In April, 30 sea urchins were collected and dissected to establish the initial condition (Baseline). Another 32 sea urchins were collected in April and maintained until June in aquaria at constant temperature and salinity and fed a formulated feed (Fed). At the conclusion of the experiment, 30 sea urchins were collected from the field population in June (Field) to establish the population condition in the Field and for comparison with the Fed sea urchins. Fed sea urchins had a 20% greater gain in weight resulting from an increase in both somatic and gonadal tissue beyond that of the field population. All organs increased in weight in females and all organs except the lantern in males. The absorption efficiency in Fed sea urchins was over 80%. Fed sea urchin had organic biomass production higher than Field sea urchins. Differences were found in the gonad cellular composition: Fed females had a unimodal oocyte size–frequency distribution, in contrast to a multimodal distribution in Field females. Fed males had fewer mature gametes than Field males. Both testes and ovaries had more nutritive phagocytes in Fed sea urchins than in Field sea urchins. Proximate composition of gonads, however, was similar in Fed and Field sea urchins. Fed individuals showed a remarkable increase in biomass production. The biochemical and cellular composition of the gonads reflected this. This indicates that *A. dufresnii* fed a highly nutritional food is able to assimilate nutrients with high efficiency and produce an increased gonad yield.

KEY WORDS: echinoidea, production, artificial fed, *Arbacia dufresnii*

INTRODUCTION

Sea urchins have been consumed by humans since prehistoric times (Lawrence 2007). They presently are harvested in many parts of the world (Brown & Eddy 2015). In addition, since the 19th century, sea urchins have been used as a model in research. Since the description of the genome of *Strongylocentrotus purpuratus* (Stimpson, 1857) (Burke et al. 2006), interest in sea urchins has increased. In Argentina, sea urchins have been studied only from biological perspectives (review in Brogger et al. 2013, Epherra et al. 2015a, 2015b, Parra et al. 2015, Zárate et al. 2016). Of the 14 species found off the coast of Argentina, *Arbacia dufresnii* (Blainville, 1825) is the most abundant; however, there are spatial and temporal differences in its reproductive cycle and population parameters between populations off the coast of Patagonia. In Nuevo Gulf (NG), the sea urchin has a high-density population with small individuals. In San Jorge Gulf (SJOG), it has a low-density population with large individuals (Epherra et al. 2015a). Maximum size has been used as an indication of size constraints based on food availability (reviewed in Lawrence & Lane 1982, Levitan 1988). Sea urchins from SJOG are not only larger but also have a greater gonad yield and mature gametes during the entire year. In NG, the sea urchins have mature gametes for a short period of the year and less synchrony of gonadal stages (Epherra et al. 2015b). Parra et al. (2015) suggested the differences between the two populations may be related to food availability since reproductive output was higher in the population from SJOG owing to the larger size of gonads and gametes. Sea urchins with good nutritional state often invest not only in gonad production but also in somatic growth (Guillou et al. 2000, Tavares 2004, Walker et al. 2007, Dodge & Edwards 2012). Feeding habit in sea urchins influences biomass production because food composition has a pronounced effect, especially on gonad yield (Marsh et al. 2013). Sea urchins are often described as herbivorous, but omnivory seems to be the most common feeding habit. The type of food ingested by a sea urchin is crucial. Ingestion of food does not imply absorption because not all nutrients are digested and absorbed. Ingestion,

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digestion, and absorption are important factors that determine production in sea urchins (Lawrence et al. 2013). In the field, these factors are very difficult to determine. Therefore, the nutritional state of sea urchins often is established indirectly through the repletion index (Fernandez 1998), estimation of biomass production of gonads (Laegdsgaard et al. 1991, Tavares 2004, Walker et al. 2007), and size of the Aristotle's lantern (Black et al. 1984, Ebert 1996, Minor & Scheibling 1997), or comparison between populations (Epherra et al. 2015b, Parra et al. 2015). In contrast, it is possible in carefully controlled culture conditions to determine these factors and analyze the effect of food quality on production.

Often, prepared diets support sea urchin growth better than natural diets in the field because feeds can be prepared that are more nutritional than algae (Lawrence et al. 1997, Olave et al. 2001, Pearce et al. 2004, Chang et al. 2005). The components of these diets are relevant since minimum values of protein and adequate ratios of protein:carbohydrate (P:C) are necessary for a maximum gonad production (Hammer et al. 2004, 2006). Therefore, the aim of this integrative study is to test the hypothesis that a high-quality formulated food would produce more biomass and gonad yield than that found in the sea urchins from natural population in an omnivorous sea urchin as *Arbacia dufresnii*.

MATERIALS AND METHODS

Collection of Sea Urchins

Sea urchins ($\bar{x}$ = 33.64 ± 1.99 SD mm diameter) were collected (n = 62) on April 10, 2014 from Bahía Nueva, NG (42.70° S, 65.60° W) and transported to the Experimental Aquarium at CENPAT at Puerto Madryn. A second collection from the field population (Field) ($\bar{x}$ = 32.35 ± 2.25 SD mm diameter, n = 30) was made on June 18, 2014. Specimens were measured and dissected (see below).

Experimental Design

A week after collection, on April 17, 32 of the 62 sea urchins (henceforth Fed) were placed into individual containers (15 × 15 × 15 cm) and distributed into four aquaria (n = 8 per aquarium) to ensure an empty gut before feeding began. The containers were made of plastic screen (1-mm mesh) that allowed water circulation but retained feces and food. The aquaria were 90 l in size with water circulation, a biofilter and an air pump to ensure good oxygenation of the water. Twenty-five percent of the water volume was changed weekly. The sea urchins were maintained on a 12 h light:12 h dark photoperiod with a salinity of 32‰ and temperature between 14°C and 16°C (similar values to field conditions). Water quality was maintained within optimal parameters. Sea urchins were fed every 3 days with a weighed amount of formulated feed. Feeding parameters (ingestion, defecation, and absorption) were recorded weekly beginning 3 days after the first feeding. They were fed for 50 days, until June 5, 2014, and then measured and dissected (see below).

Dissection and Measurement of Organic Matter and Proximate Constituents

Thirty sea urchins from the initial collection (henceforth Baseline), all fed sea urchins (henceforth Fed), and 30 sea urchins from the second collection from the field population (henceforth Field) were measured at two perpendicular points across the ambitus (test diameter) by using calipers, weighed by immersion (mass determined in water) to the nearest milligram, and dissected. Sea urchins were cut outside the peristomial membrane. The test with spines, Aristotle's lantern, gut, and gonads were separated. The gut (esophagus, stomach, and intestine) was rinsed in sea water in a finger bowl to remove food. Each organ was weighed to the nearest milligram (wet weight). A portion of the gonads was placed into a fixative (Davidson' solution) for histological analysis. All organs were placed in a 60°C oven, dried for 48 h to constant weight, and reweighed to the nearest milligram [dry weight (DW)]. The dry tissues were ground to a powder with a mortar and pestle. Because organ size changes allometrically with body size, results are presented as adjusted DW obtained by ANCOVA using diameter as covariable (see Statistics section).

TABLE 1.

Composition of formulated feed.*

Ingredient	Percentage weight (as is or as fed basis)
Wheatstarch	18.04
Soy protein	7.70
Casein	4.00
Cellulose	3.00
Beta carotene	1.00
Vitamin premix	0.60
Mineral premix	17.73
Other marine ingredients	31.00
Other nonmarine ingredients	16.90

* Feed provided by Texas A&M AgriLife Research.

The amount of organic matter (OM) was determined by ashing. A known weight of powder of each organ (weighed to the nearest milligram) was placed into a porcelain crucible and incinerated in a muffle furnace at 500°C for 4 h. After incineration, the crucibles were cooled to room temperature in a desiccator and the ash was weighed to the nearest 0.1 mg. The percent organic material was calculated as (sample DW – ash weight/sample DW).

The powder of the gonads was analyzed for proximate composition. Trichloroacetic acid (TCA)-soluble carbohydrates were measured according to Dubois et al. (1956), with glycogen as the standard. Soluble proteins were measured by the method of Lowry et al. (1951), with bovine serum albumin as the standard. Total lipids were measured according to Zöllner and Kirsch (1962), with cholesterol as the standard. Unmeasured organic material (UOM) (refractive proteins and nonprotein nitrogen, mainly, nucleic acids in testes) was calculated by subtraction (Parra et al. 2015). Energy content was calculated by multiplying the content of each biochemical component by conversion coefficients in Brody (1945) and expressed in kJ/ind. The energy content was not calculated for males because DNA in the testes was not measured. Therefore, the reproductive effort was calculated for females as the difference between the energy content of soluble proteins, lipids, and TCA-soluble carbohydrates in the Baseline sea urchins and Field and Fed sea urchins in June (Pérez et al. 2010).

TABLE 2.

Proximate composition of formulated feed.

Component	Concentration (%)
Lipids	7.38
Soluble protein	17.02
Insoluble protein	2.3
Carbohydrates	37.75
Ash	32.22
UOM	3.33

Growth

The test diameter of each fed sea urchin (Fed) was measured every 10 days for 8 wk. The diameter (in millimeters) was measured across two perpendicular points along the ambitus using calipers.

Ingestion, Defecation, and Absorption

Sea urchins in each container were fed a weighed amount (400 mg) of formulated feed (Tables 1 and 2) every 3 days. Feed was placed into a 60°C oven, dried for 48 h to constant weight, and weighed to the nearest milligram (DW). The percent moisture was used to calculate the amount of feed fed in terms of DW. The remaining uneaten food and the feces produced during these 3 days were removed and dried as above.

Feeding parameters were calculated gravimetrically in terms of DW and OM weight (Lawrence et al. 2013) weekly. *Ingestion* was calculated as proffered food – uneaten food. *Defecation* was calculated as the amount of feces (mg) of each individual produced every week. *Absorption* was calculated as *Ingestion – Defecation*. The *absorption efficiency* (AE) or *apparent digestibility* (endogenous material is not considered) was calculated directly in DW as: Ingested food – Defecation)/(Ingested food) × 100 and indirectly by using the concentration of OM in the food and feces as suggested by Chang et al. (2005) by using the Conover formula as follows: [(Proffered food – Defecation)/(1 – Defecation) (Proffered food)] × 100.

Production, Food Conversion Ratio, and Assimilation Efficiency for Sea Urchins Fed a Formulated Feed

The amount of OM for each body component was calculated as the difference in between the DW and the ash weight. The amount of OM in each body component was summed to calculate the total amount of OM/ind.

Organic matter production was calculated as: OM of Fed sea urchin or Field sea urchin – OM of Baseline sea urchin from the initial sample. The *food conversion ratio* (FCR) for the Fed sea urchins was calculated for the entire experiment as: (Total amount of ingested OM/(Total amount of OM in Fed sea urchins – Total amount of OM in Baseline sea urchins from the initial sample). *Gross* assimilation efficiency for the Fed sea urchins was calculated as: [(Total amount of OM in Fed sea urchins – Total amount of OM in Baseline sea urchins)/amount or OM ingested] × 100. *Net assimilation efficiency* per individual for the Fed sea urchins was calculated as: [(Total amount of OM in Fed Sea urchins – Total amount of OM in Baseline sea urchins)/amount of OM absorbed] × 100)]. All calculations were made following Watts et al. (2013).

Cellular Composition of Gonads

Gonadal tissue was fixed in Davidson solution for 24 h and then preserved in 70% ethanol. Gonads were dehydrated in increasing ethanol concentrations and embedded in paraffin wax, cut into sections with a microtome at 7 mm, and stained with hematoxylin and eosin Y. Gonadal stages were categorized according to Epherra et al. (2015b). Oocyte diameter frequency distribution in all females was determined by image analysis of the ovary sections by using the software Fiji (Schindelin et al. 2012). Only oocytes sectioned through the nucleus were measured. In all males, thickness of the spermatogenic cell layer (spermatogonia, spermatocytes, and spermatids), spermatozoa, and nutritive layers was measured at the center of the lumen by using transverse sections of at least six acini per male. The size of each acinus was also recorded by measuring the radius.

Statistics

Growth was analyzed by fitting different mixed-effects models, the growth models were fitted by using maximum

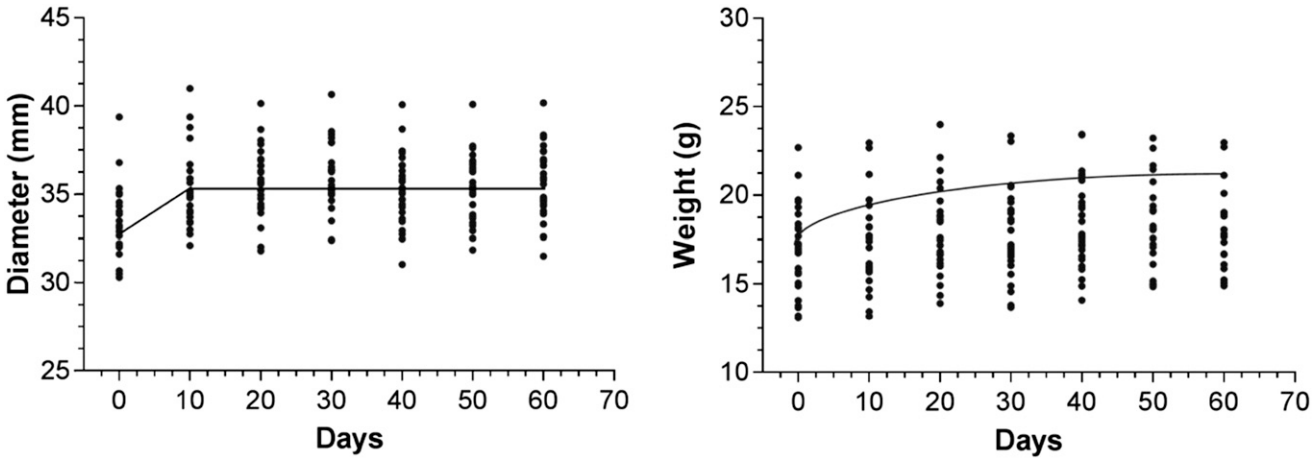

Figure 1. ***Arbacia dufresnii*: test diameter (mm) and individual weight (g) over time during the experiment. The line corresponds to adjusted logistic function and quadratic function, respectively. Days 0–42, $n = 32$; days 43–60, $n = 30$.**

likelihood. The performance of each model was assessed by information-theoretical procedures. To quantify the plausibility of each model given the data and the set of models, the Akaike information criteria (AIC), differences in AIC (Δi) and AIC weights (wi) of all possible models were obtained (Burnham et al. 2011, Symonds & Moussalli 2011). Only the best fit is reported. A logistic function was fitted for sea urchin oocyte diameter.

Differences between sexes of the DW of the gonads, gut, test, and lantern were evaluated by using separate one-way ANCOVA analysis with test diameter as covariate and Baseline, Field, and Fed sea urchins were used as factors. Differences between organic biomass production of females and males were also evaluated by using separate one-way ANCOVA analysis with test diameter as covariate and Field and Fed sea urchins as factors.

Proximal composition of gonads in females and males of Baseline, Field, and Fed were tested by using one-way ANOVA. Variations in oocyte diameter frequency distribution between Field and Fed sea urchins were assessed through a Kolmogorov–Smirnov two-sample test by using Bonferroni correction procedures (Siegel & Castellan 1988). Variations of spermatogenic columns, spermatozoa, and nutritive phagocytes layers between Field and Fed sea urchins were evaluated by using separate one-way ANCOVA analysis with the radius of the acini as a covariate. Repeated-measures ANOVA analyses were used to test differences in the amount of ingested DW and OM, feces DW and OM, absorption DW and OM, and AE (apparent digestibility) DW and OM over time. Because of the lack of sphericity (Mauchly's criterion), univariate analyses were used (Crowderm & Hans 1990). The assumptions of normality (Shapiro–Wilk test) and homogeneity of variances (Cochran's C test) were verified before all ANCOVA and ANOVA analysis. All statistical analyses were performed with STATISTICA 7.0 (StatSoft, Inc., Tulsa, OK). A significance level of 5% was assumed throughout the study.

RESULTS

Growth

Test diameter was fitted to a logistic function (Fig. 1) according to the following:

$$D\,(\mathrm{mm}) = \frac{35.54}{1 + e(-0.017 \times (t + 16.5))}$$

where D is diameter, 35.54 mm is the maximum value, 0.017 mm/day is the growth rate, and 16.5 mm the initial size.

The individual weight was fitted to a quadratic function (Fig. 1) according to the following:

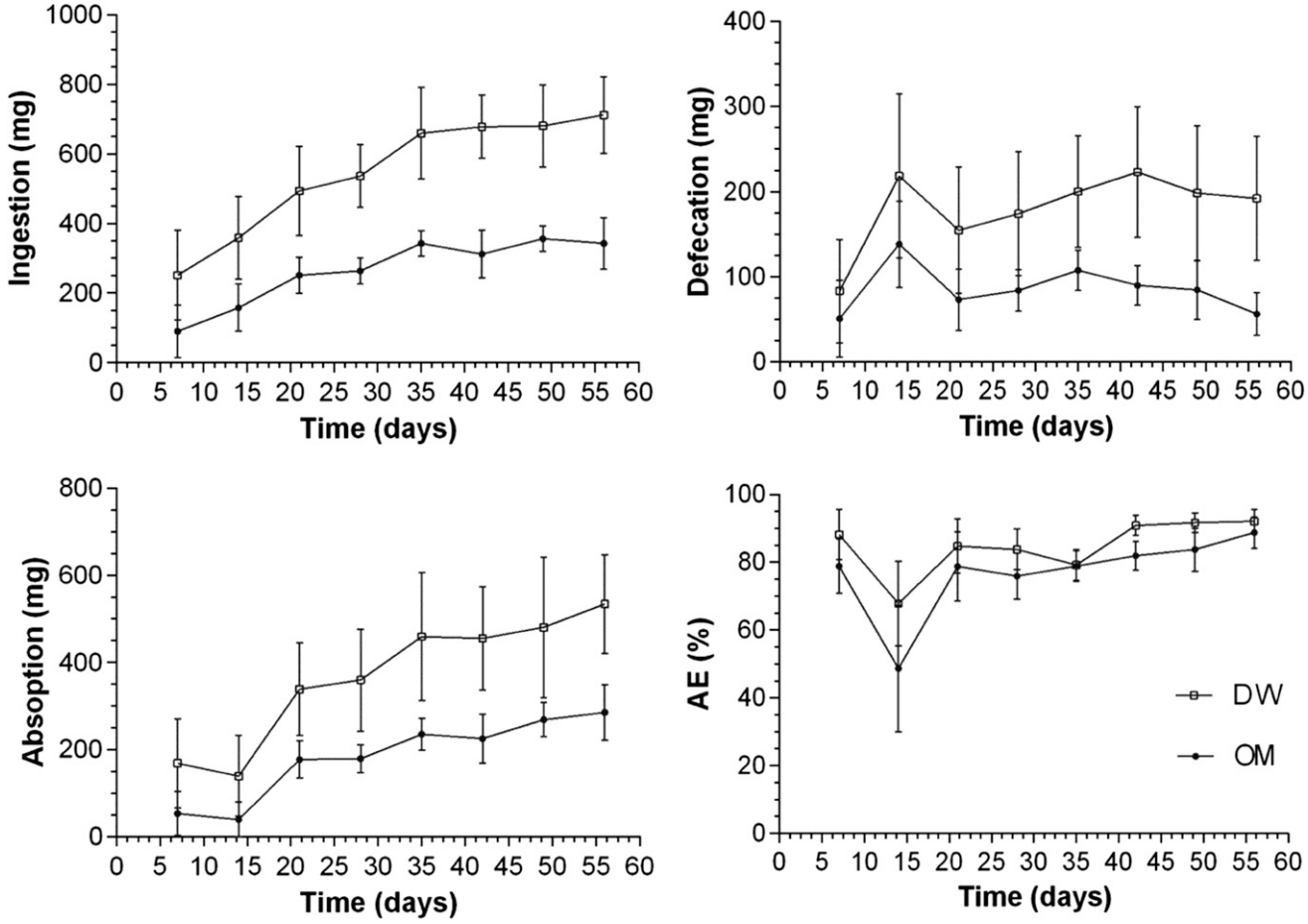

Figure 2. *Arbacia dufresnii*: ingestion, defecation, absorption, and AE % are reported in terms of mg DW and OM. All calculations were made on Fed sea urchins. Mean ± SE, $n = 30$.

$$W(t) = -0.05t + 5.73t^2 + 1674$$

where W is weight, –0.05 is the growth decrease in growth rate in mg, 5.73 mg/day is the growth rate, and 1,674 the initial weight in mg.

Ingestion, Defecation, and Absorption

Feeding parameters over time are shown in Figure 2. There were no differences in these parameters between sexes both for DW and OM ($F = 1.54$; $F = 1.64$; $P > 0.05$). The amount of ingestion changed significantly during the experiment ($F = 115.87$; $P < 0.05$). At the beginning, a steep increase occurred and continued to increase to reach a nearly constant value of ~300 mg OM/ind./wk (~42 mg/ind./day) after about 30 days of feeding. Ingestion measured as DW showed the same pattern. The values, however, were twice the OM ingestion. Defecation also changed significantly with the time ($F = 63.14$; $F = 65.12$; $P < 0.05$). The maximum value occurred at the beginning of the experiment with 140 mg OM/ind./wk (~20 mg/ind./day) and then decreased with time. By the end of the experiment, defecation was ~50 mg OM/ind./wk (~7 mg/ind./day) until the end. Defecation measured as DW of feces showed a similar pattern; however, the values were nearly twice the OM defecation. Absorption changed significantly during the experiment ($F = 130.09$; $F = 143.9$; $P < 0.05$). It decreased slightly at the beginning of the experiment and increased sharply in the 3rd wk to ~180 mg OM/ind./wk (~26 mg/ind./day), and then continued to increase slowly, reaching ~288 mg OM/ind./wk (~41 mg/ind./day) at the end of the experiment. Again, absorption measured as DW showed a similar pattern; however, the values were nearly twice the OM absorption. The AE changed significantly during the experiment ($F = 44.72$; $F = 45.32$; $P < 0.05$) due to a sharp decrease during the 2nd wk coincident with a higher defecation rate. After the 3rd wk, the AE of OM had nearly steady values of around 80%, whereas AE in DW had a value around 90%.

Organ Weights

The changes in the adjusted DW of Field and Fed sea urchins are shown in Figure 3. The adjusted gonad weight of Field sea urchins was not significantly greater than that of Baseline sea urchins collected at the beginning of the experiment. The increase in adjusted gonad weight of the Fed sea urchins was significantly greater than that of the Field sea urchins ($F = 39.76$; $P < 0.05$). No differences were found between sexes in the adjusted gonad weights in both Fed and Field sea urchins ($F = 0.54$; $P > 0.05$). The adjusted gut weight of Field sea urchins decreased from the Baseline sea urchins collected at the beginning of the experiment. On the other hand, the Fed sea urchins had a significantly higher value than Baseline and Field sea urchins ($F = 7.95$; $P < 0.05$). No differences in the adjusted gut weight were found between sexes in both Field and Fed sea urchins ($F = 0.06$; $P > 0.05$). The

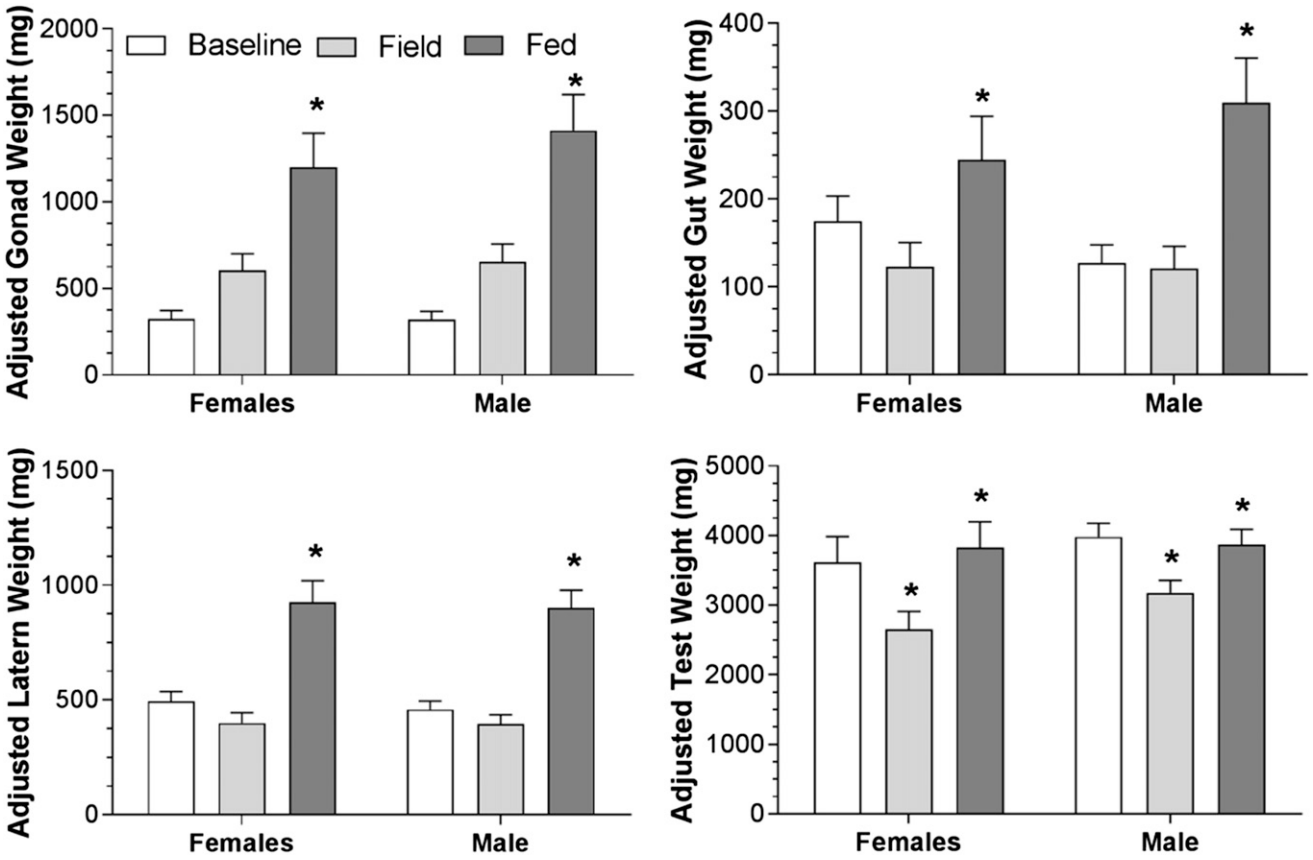

Figure 3. ***Arbacia dufresnii*: adjusted organs DW in females and males. Baseline: values of sea urchins from the population in April. Field: values of sea urchins from the population in June. Fed: values of fed sea urchins in June. Mean ± SE, $n = 30$. * Denotes significant differences.**

TABLE 3.

***Arbacia dufresnii*: change in of OM (mg) of Field and Fed females and males.**

	Female		Male	
Organ	**Field**	**Fed**	**Field**	**Fed**
Gonads	3.8	112.5	29.3	178.9
Gut	2.5	138.5	33.2	123.7
Lantern	24.3	34.5	–7.6	–18.4
Test	–546.9	89.2	–557.4	28.7

Marginal means from the ANCOVA, n = 30.

adjusted lantern weight in Field sea urchins also decreased from the Baseline sea urchins. Again, the adjusted lantern weight of Fed sea urchins was significantly higher than the Baseline and Field sea urchins ($F = 29.9$; $P < 0.05$). No differences in the adjusted lantern weight were found between sexes in both Field and Fed sea urchins ($F = 0.29$; $P > 0.05$). The adjusted test weight of Field sea urchins was not significantly greater than that of Baseline sea urchins. The increase in the adjusted test weight in Fed sea urchins was significantly higher than that of Field sea urchins ($F = 36.6$; $P < 0.05$). No differences were found between sexes in adjusted test weight of both Field and Fed sea urchins ($F = 0.07$; $P > 0.05$).

Production, Food Conversion, and Assimilation Efficiency

In females and males, change in biomass of OM was significantly higher in Fed sea urchins than in Field sea urchins for gut, gonad, and lantern (Table 3). The total amount of OM produced per individual was significantly higher in Fed sea urchins regardless of sex ($F = 5.54$; $P < 0.05$). In Fed males, the test had almost 20 times more OM than Field males, whereas Fed females had only ~6 times. In contrast, in Fed females, the most significant difference was in the biomass production of the gonads, which produced over 29 times more OM than Field females, whereas in Fed males the increase was ~6 more times than Field males. These differences in OM production in the gonads are related to their proximal composition. In females, UOM and carbohydrate concentrations and amounts were significantly higher in Fed sea urchins ($F = 3.7$; $F = 22.23$, respectively; $P < 0.05$); nearly a 10-fold increase in UOM amount was observed (Fig. 4, Table 4). In males, the carbohydrate concentration and amount in testes was significantly higher in Fed sea urchins ($F = 30.77$; $P < 0.05$), a ~5 times increase in carbohydrate amount was observed (Fig. 4, Table 4). Soluble proteins were the main source of energy in both Field and Fed ovaries, although lipids were also important in Fed females (Table 5). The reproductive output was less in Field females (0.59 kJ) than in Fed females (1.41 kJ); however, the FCR and assimilation efficiency were very similar between sexes in Fed sea urchins (Table 6).

Cellular Gonad Composition

Gonad stages of Field and Fed males and females in June are shown in Figure 5. Qualitatively, ovaries of field females were in growth and premature stages. There were few developing oocytes in the acinal wall and near the lumen. Nutritive phagocytes were depleted and secondary oocytes and ova were present in the lumen. Ovaries of Fed females were in the growth stage. Developing oocytes were present along the acinal wall and near the lumen. Nutritive phagocytes were numerous, filling the acinus and surrounding the oocytes. The oocyte diameter frequency distribution of Field and Fed sea urchins is significantly different (Fig. 6). The oocyte diameter frequency distribution of Field sea urchins was multimodal, whereas that of Fed sea urchins was unimodal (KS = 0.02; $P < 0.05$). Qualitatively, testes of both Field and Fed males were in the growth stage, however, nutritive phagocytes were more abundant in Fed sea urchins, filling the acinus and surrounding the spermatocytes. Fed sea urchins also had larger spermatocyte columns, whereas Field sea urchins had a higher amount of spermatozoa (Fig. 7).

DISCUSSION

Under the conditions of this experiment, *Arbacia dufresnii* fed a formulated feed did not increase in diameter. By the end of the experiment, however, Fed sea urchins were 20% heavier, probably due to increase of all the organs. Weight gain seems to be a better indicator of growth than test diameter, as found in the sea urchin *Pseudocentrotus depressus* (A. Agassiz, 1864)

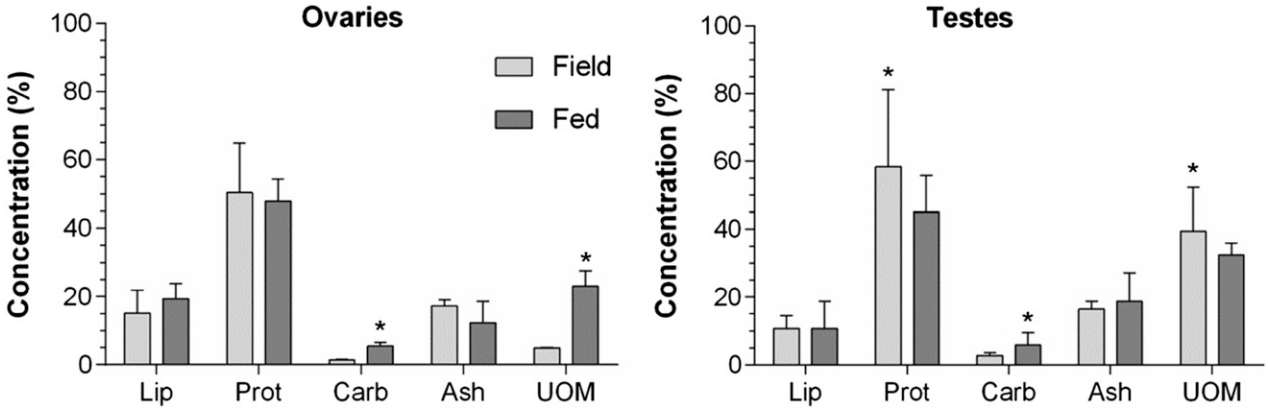

Figure 4. ***Arbacia dufresnii*: concentration of proximate constituents (% DW) in ovaries and testes of Field and Fed sea urchins in June. Lip, lipids; Prot, soluble protein; Carb, soluble carbohydrates; Ash, ash; UOM, undetermined OM. Mean ± SE, $n = 30$. * Denotes significant differences.**

TABLE 4.

***Arbacia dufresnii*: total amount of proximate constituents (mg) in gonads of Field and Fed sea urchins in June (mean ± SE).**

	Female		Male	
Component	Field	Fed	Field	Fed
Lipids	15.1 ± 6.88	19.68 ± 4.69	10.73 ± 1.58	10.77 ± 1.91
Soluble protein	50.36 ± 6.33	58.21 ± 14.51	69.12 ± 10.29	39.4 ± 6.68
Soluble carbohydrates	1.46 ± 0.48	5.47 ± 3.66	2.73 ± 1.67	5.9 ± 1.69
Ash	15.86 ± 3.10	12.01 ± 5.17	13.58 ± 5.69	13.35 ± 6.76
UOM	5.37 ± 1.89	16.4 ± 5.43	51.55 ± 3.71	32.73 ± 6.99

by Akiyama et al. (2001). Ingestion of food and absorption increased and defecation decreased over time to reach an almost constant value. The consumption rate was high at the beginning of the experiment, probably due to the previous starvation period. During the experiment, the food supply was maintained *ad libitum* and the rate of ingestion decreased and then remained fairly constant. These results are similar to those reported for herbivorous species of sea urchins such as *Echinus esculentus* (Linnaeus, 1758) (Bonsdorff 1983) and *Lytechinus variegatus* (Leske, 1778) (Lawrence et al. 2003).

The AE (apparent digestibility) was 80% when calculated using OM and 90% when calculated directly using DW. Similar values were found in the herbivorous *Lytechinus variegatus* fed a formulated feed with a high percentage of protein (Hammer et al. 2004). The FCR in Fed *Arbacia dufresnii* was similar to that of *L. variegatus* fed a feed with 23% protein (Hammer et al. 2004) even though *A. dufresnii* was fed a feed with a lower protein percentage (19%). Usually, elevated protein levels in food result in decreased feed intake and increased somatic and gonad growth (Watts et al. 2013). Ingestion, absorption, and AE, however, increased in sea urchins in this experiment. This resulted in an increase of somatic and gonadal growth of all individuals greater than that of the field population. The fact that Fed sea urchins doubled their gonad weight in comparison with the Field sea urchins and also increased the somatic tissues suggests an ability to efficiently use the extra nutrients found in the artificial feed. Organic biomass production was remarkably high in Fed sea urchins. All organs increased in weight in females and all organs except the lantern in males. The gut and gonads had the highest biomass production in Fed sea urchins, whereas Field sea urchins had only a slight increase in gonad and gut production. A decrease in food availability often causes preferential nutrient allocation to tissues with high energy demand such as gonads (Marsh et al. 2013). Even though, Field sea urchins may be subject to a number of environmental factors that may affect their production beside food availability, the population in the site of the sampling inhabits an area with low currents and seawater temperature remains quasi—constant during autumn (Rivas & Ripa 1989, Rivas et al. 2016). The proximate composition of gonads of Fed and Field sea urchins was very similar. Carbohydrates in gonads of Fed females, however, were higher than in Field females and carbohydrates also increased in Fed males. To understand the proximal composition of the gonads, it is necessary to analyze the histological composition (Marsh et al. 2013). In the female gonads of Field sea urchins, there was a lack of synchrony of oocyte development; oocytes of different diameter and maturation were found. This suggests heterogeneous conditions in the field. In contrast, oocyte development was more orderly in Fed sea urchins with a unimodal size–frequency distribution. In fact, oocyte development was somewhat delayed, because almost no mature ova were found. This suggests preferential accumulation of nutrients in the gonads prior to maturation of gametes. The increase in gonadal growth over the somatic growth (precocious gonads) often results from a surplus of nutritional energy that cannot be effectively used for somatic growth (Lawrence 2000). In *L. variegatus,* unimodal oocyte size-frequency distribution was achieved only with a feed with 33% protein, whereas a bimodal distribution was observed with a feed with 21% protein and the same phenomenon of precocious gonads was found (Hammer et al. 2006). In Fed males, there was an evident increase in nutritive phagocytes and spermatogenic columns and a decrease in spermatozoa. Again, gonad development in Fed sea urchins was a little delayed from Field sea urchins. The same scenario of precocious gonads in the premature stage was found in the sea urchin *Mesocentrotus* (as *Strongylocentrotus*) *franciscanus* (Linnaeus, 1758) fed feed with protein concentration varying from 15% to 25% (McBride et al. 1998). Unmeasured organic material in gonads of Fed females was also higher than in Field females. High values of UOM found in the gonads are generally related to a high number of cells (Parra et al. 2015). Oocytes and nutritive phagocytes in Fed sea urchins were more abundant than in Field sea urchins. In contrast, the lower values of UOM in males may be related to the lower number of spermatozoa. The other biochemical component that showed differences was carbohydrates. Gonads of both Fed females and males had higher values than Field sea urchins, probably because the feed has a higher percentage of digestible carbohydrates

TABLE 5.

***Arbacia dufresnii*: biochemical energy content of ovaries (kJ/ind.) for sea urchins from the initial sample, Field and Fed sea urchins (mean ± SE, $n = 12$).**

Component	Initial Sample	Field	Fed
Lipids	0.10 ± 0.01	0.16 ± 0.04	0.42 ± 0.11
Soluble protein	0.20 ± 0.05	0.72 ± 0.19	1.22 ± 0.23
Soluble carbohydrates	0.01 ± 0.00	0.02 ± 0.01	0.09 ± 0.06

TABLE 6.

***Arbacia dufresnii*: food conversion efficiency, gross, and net assimilation efficiencies for Fed sea urchins.**

Sex	Female	Male
Food conversion efficiency	3.16	2.85
Gross assimilation efficiency	6.81	7.53
Net assimilation efficiency	11.10	10.40

Marginal means from the ANCOVA, $n = 30$.

than natural food in the natural environment. Carbohydrate levels in gonad tissue appear to be directly related to dietary carbohydrate, *L. variegatus* fed with varying carbohydrate levels showed that sea urchins fed with higher concentrations in the feed had higher carbohydrate in gonads (Hammer et al. 2006). In the sea urchin *Paracentrotus lividus* (Lamarck, 1816), gonads had higher carbohydrate concentrations in sea urchins fed a vegetable-based diet than an animal-based diet (Fernandez 1997). The proximal composition of the diet has a crucial effect on growth and survival in sea urchins. Often carbohydrate and protein concentrations in sea urchin organs depend on the dietary composition of their food (Hammer et al. 2006). Proteins are one of the important nutrients for sea urchins due to their multiple roles in many biological processes (Watts et al. 2013). It appears that there is a minimum requirement of protein for survival in several herbivorous species, below which mortality increases (Akiyama et al. 2001, Pearce et al. 2002, Hammer et al. 2004, 2006).There also is an upper limit beyond which increased proteins are not beneficial and indeed result in less growth (Eddy et al. 2012). On the other hand, carbohydrates provide energy in sea urchins. Even though the direct effect of dietary carbohydrates on sea urchin is poorly understood, it appears that sea urchins adjust feed intake to satisfy energy

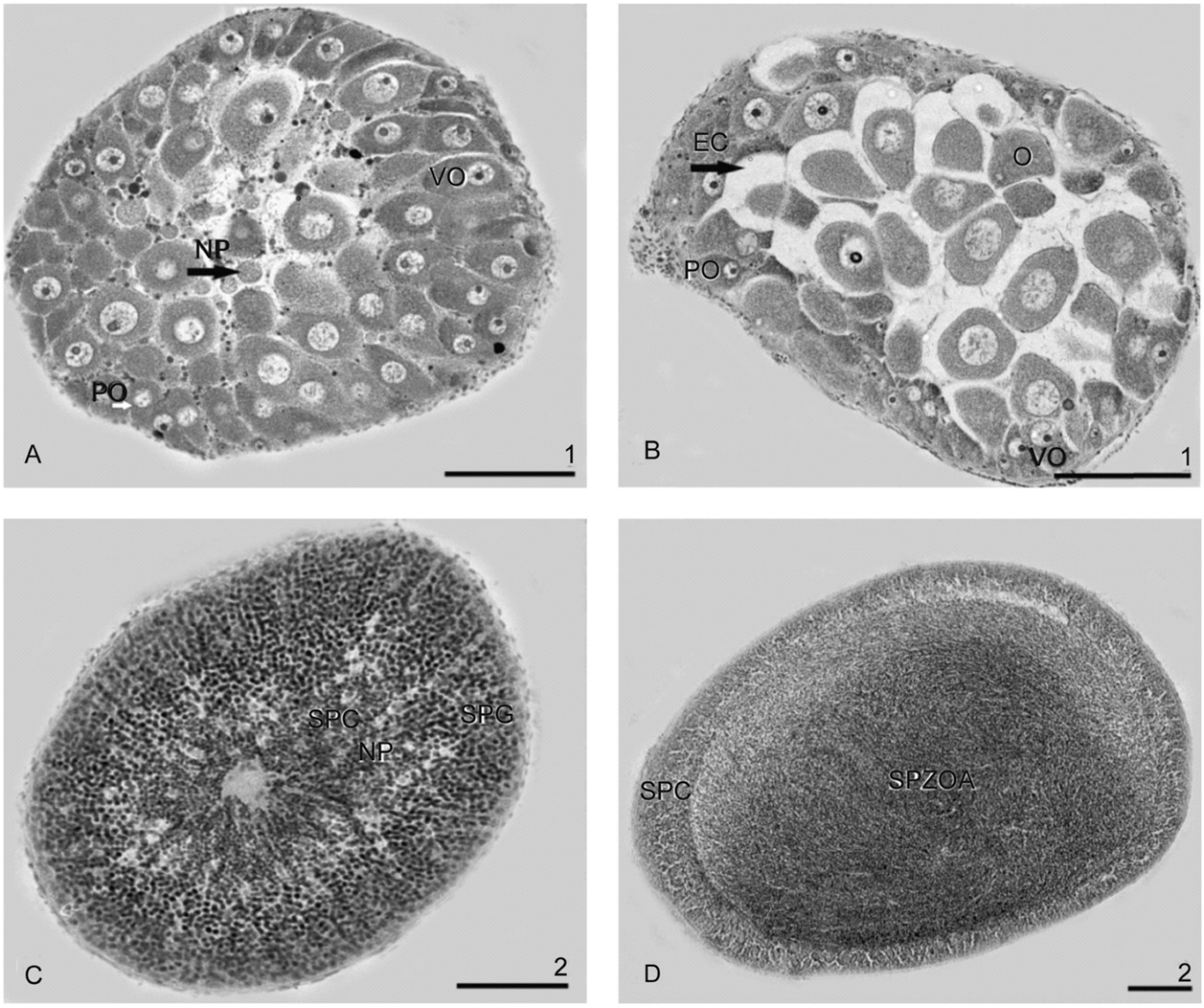

Figure 5. ***Arbacia dufresnii*: histological sections of ovaries and testes. (A) Fed female, (B) Field female, (C) Fed male, and (D) Field male. NP, nutritive phagocytes; SPC, spermatogenic column; SPG, spermatogonia; SPZOA, spermatozoa, O, ova; PO, previtellogenic oocyte; VO, vitellogenic oocyte. Scale bars: 1, 100 μm; 2, 50 μm.**

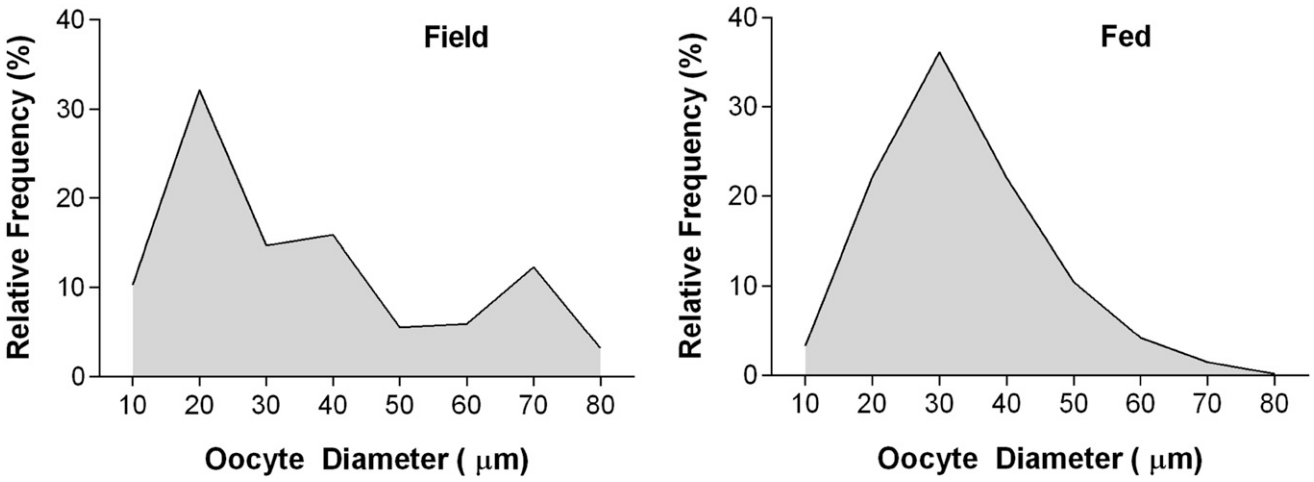

Figure 6. ***Arbacia dufresnii*: oocyte diameter relative frequency distribution of sea urchins. Field: values of sea urchins from the population in June. Fed: values of fed sea urchins in June. $n = 30$.**

requirements when availability of carbohydrates is low (Watts et al. 2013). The P:C ratio in the diet may alter biochemical and cellular composition of gonads (Hammer et al. 2006). The differences found in the proximate and cellular composition of the gonads between Field and Fed sea urchins were probably due to difference in the P:C ratio in the food. In the Field, *A. dufresnii* had very low carbohydrate levels (~2%) in the gonads that were significantly lower than those of Fed sea urchins (~5%). Although, carbohydrate concentrations were higher in Fed sea urchins, values were still very low in comparison with herbivorous species (Montero-Torreiro & García-Martínez 2003, Hammer et al. 2006, Mol et al. 2008, Arafa et al. 2012, Verachia et al. 2012). The low values found in *A. dufresnii* fed a feed with a high percentage of carbohydrates suggest a low assimilation of carbohydrates in this omnivorous species. The formulated feed fed to the sea urchins had an intermediate P:C ratio (0.5), which may have promoted gamete growth and development in *A. dufresnii*. In contrast, Hammer et al. (2006) found a high P:C ratio (2.5) in the diet was necessary to promote gamete growth and development in *L. variegatus*. When carbohydrate levels are limiting, dietary protein is often used as an additional energy source in herbivorous species (Schlosser et al. 2005, Hammer et al. 2006). It seems that the same happens in omnivorous species. The energy values of soluble proteins of Fed females were more than ten times the energy values of carbohydrates. In the field, food with high levels of carbohydrate ingested by *A. dufresnii* seems to be scarce, judging by the very low percentage in the gonads. In Fed sea urchins, gonads did not have an increase in carbohydrates even though the feed had a high percentage of this nutrient. The sea urchins belonging to the genus *Arbacia* are thought to be not only omnivorous but with tendency to carnivory (Vásquez et al. 1984, Fernandez & Boudouresque 1997, Penchaszadeh & Lawrence 1999, Hill & Lawrence 2003, Cobb & Lawrence 2005, Wangensteen et al. 2011, Gianguzza & Bonaviri 2013). In fact, *Arbacia lixula* (Linnaeus, 1758) has a great digestive enzyme pool with similar amylase and lipase activity but much higher total protease activity than herbivorous sea urchin species such as *Sphaerechinus granularis* and *P. lividus*, indicating that *Arbacia* has an effective use of a great variety of food, especially of animal origin (Trenzado et al. 2012). Therefore, it is likely algae are not a major food item in this species. Instead lipids are better assimilated than carbohydrates. Lipid content of gonads was not different in Field and Fed sea urchin; however, lipids provided more energy in Fed sea urchins than in Field sea urchins. The fact that lipids from Fed sea urchins provided more energy than in Field sea urchins indicates an increased accumulation of lipids. This study shows that a high-quality formulated food produces a remarkable increase in biomass production and gonad yield, probably due to high assimilation efficiency of protein and lipids in the culture conditions.

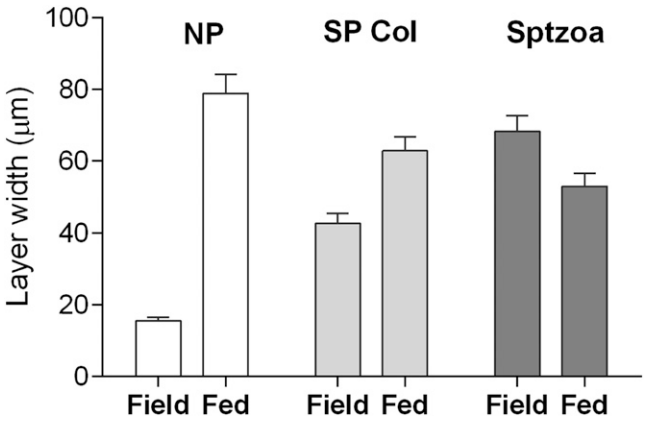

Figure 7. ***Arbacia dufresnii*: width of nutritive phagocytes (NP), spermatocyte columns (SP Col), and spermatozoa (Stpzoa) layers of sea urchins. Field: values of sea urchins from the population in June. Fed: values of fed sea urchins in June. Mean ± SE, $n = 30$.**

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