# Growth and Gonad Changes in Stony Sea Urchin, *Paracentrotus Lividus* (Lamark, 1816) Fed Artificially Formulated Feed and Benthic Macrophyte Diet

# *Utjecaj hranidbe prirodnom i umjetnom hranom na rast i morfometrijske promjene u gonadama hridinskog ježinca, Paracentrotus lividus (Lamark, 1816)*

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**Summary**

**This study reported the efficiency of artificially formulated feed and benthic macrophyte diet on growth and gonad development of cultured stony sea urchin, *Paracentrotus lividus* (Lamark, 1816). An initial sample of 720 individual urchins was gathered in coastal area of SE Adriatic, near Dubrovnik, Croatia and for the purposes of the experiment, was held in a flow-through system. Sea urchin were fed four test diets A, B, C and D. Diet A consisted of seaweeds collected in the natural habitat of sampled sea urchin, artificial diets B, C, and D contained a different ratio of ingredients. The feed mixtures differed in respect to corn meal content; B (22%), C (30%) and D (35%) as well as the distribution of fish meal content; B (15%), C (5%) and D (0%). Prior to the commencement of feeding, sea urchins in all groups were starved for 15 days. During the period of starvation, recorded total urchin weight, gonadosomatic index (GSI) and gonad weight values for all experimental groups, showed a declining trend except in the control group, where they stayed the same. After 30 days, the declining trend of morphometric values was recorded for treatment A and the control group. During the two months of the intensive feeding conditions in the regimes with artificially prepared feed, B, C and D a growing trend was recorded for all morphometric values, and it was particularly evident in the treatment C. In the last month of the experiment, a significant decline in the GSI and gonad weight values were observed for the groups, A and the control. The differences among the artificially formulated feeds expressed through increase of GSI and gonad mass values revealed that the best among them was artificial feed C whose variance in consistence of essential nutritional components fits between prepared feeds B and D. Based on the results of our study we conclude that the artificially formulated feed is adequate food for sea urchins in a controlled environment, especially when it contains a smaller animal protein component (5-10%) and a larger share of plant components (over 90%).**

**KEY WORDS**
**stony sea urchin**
***Paracentrotus lividus***
**artificially formulated feed**
**echinoculture**

***Sažetak***

***U radu je opisana učinkovitost hranidbe umjetnom hranom i bentoskim makroalgama na rast i razvoj gonada hridinskog ježinca, Paracentrotus lividus (Lamark, 1816) u kontroliranim uvjetima. Uzorci su prikupljeni u jugoistočnom dijelu Jadrana, u blizini otoka Lokruma, pokraj Dubrovnika te su za potrebe pokusa preneseni u protočni uzgojni sustav.. Nakon prilagodbe, ježinci su stavljeni na četiri hranidbena režima A, B, C i D. Režim A sastojao se od bentoskih makroalga prikupljenih u prirodnom staništu uzorkovanih ježinaca. Umjetno proizvedena hrana B, C, i D razlikovala se u strukturi smjese i kemijskom sastavu. Hranjive smjese su se razlikovale u udjelu kukuruznog; B (22%), C (30%) i D (35%) i ribljeg brašna; B (15%), C (5%) i D (0%). Na samom početku pokusa, nakon razdoblja izgladnjivanja zabilježeno je smanjenje vrijednosti ukupne mase, mase gonada i gonadosomatskog indeksa (GSI) u svim eksperimentalnim skupinama osim***

***KLJUČNE RIJEČI***
***hridinski ježinac***
***Paracentrotus lividus***
***umjetna hrana***
***ehinokultura***

***u kontrolnoj. Uzorkovanje nakon 30 dana ukazalo je na smanjenje izmjerenih morfometrijskih vrijednosti kod ježinaca na hranidbenom režimu A i u kontrolnoj skupini. Tijekom idućih 60 dana zabilježen je trend rasta morfometrijskih vrijednosti za ježince na hranidbenom režimu umjetnim hranama B, C i D, a posebno je bio izražen za hranu C. U posljednjem mjesecu pokusa zabilježen je značajan pad vrijednosti GSI-a i mase gonada u ježincima na hranidbenom režimu A i u kontrolnoj skupini. Između pojedinih hranjiva po rastu GSI-a i mase gonada najbolja se pokazala hrana C, koja se po bitnim hranidbenim komponentama nalazi između hranjiva B i D. Temeljem dobivenih rezultata možemo zaključiti da je umjetno pripravljena hrana odgovarajuća za hranidbu ježinaca u kontroliranim uvjetima, a posebno kad sadržava mali udio bjelančevina životinjskog podrijetla (5-10%) i veliki udio biljnih komponenti (> 90 %).***

## INTRODUCTION / *Uvod*

The stony sea urchin*, Paracentrotus lividus* (Lamark,1816) is one of the most prevalent echinoid species in the photophilic algal assemblages in the Mediterranean Sea (Tortonese & Vadon, 1987). This species has been given particular attention for its economic value (Palacin et al., 1998) and, mainly for its key role in maintaining the equilibrium of sublittoral communities (Sala et al., 1998). Its role as a grazer, as for many other echinoid species (McClanahan et al., 1995), has been widely investigated, stressing the role of habitat heterogeneity and complexity (McCoy & Bell, 1991), such as the presence of shelters (crevices), in regulating its effects on the presence and recovery of macroalgae, mainly on early successional species, by affecting patterns of succession (Benedetti-Cecchi & Cinelli, 1998).

Sea urchins are intensively harvested in many parts of the world, with their gonads as a delicacy (Lawrence, 2001). Some time ago, the sea urchin fishery was conducted by artisan fishermen and the product was sold locally (Boudouresque, 1987). However, today certain markets in the world, mainly Japan, absorb large amounts of sea urchins and this has increased the fishing pressure on the inter-tidal population of such species (Yokota, 2002). Increasing demand for sea urchin roe has led to over-fishing of natural populations (Conand and Sloan, 1989; Le Gall, 1990). Thereafter, the echinoid stocks should be strictly managed in order to be protected (Lesser and Walker, 1998; Yokota et al., 2002). Several possible solutions have been tested: reseeding natural habitats with farmed juveniles (Agatsuma and Momma, 1988; Gomez et al., 1995); mariculture (Fernandez 1996; Spirlet et al., 2000); raising sea urchins in immerged cages, alone (Spirlet et al., 2000; Robinson and Colborne, 1998); with other animals polyculture with salmons notably, (Kelly et al., 1998) or IMTA; and finally land-based, closed-system echiniculture allowing control of each phase of the echinoid biological cycle (Le Gall and Bucaille, 1989; Le Gall, 1990; Grosjean et al., 1998). *P. lividus* is potentially important as a local fishery resource for the tourist Adriatic coast of Croatia. This species is also a promising candidate for the emerging aquaculture industry of Croatia especially today when diversification of products is obligatory.

Sea urchin roe is a first class food and it is worldwide commercialized. Yet, the taste of gonads is variable and directly related to the biochemical composition of adults' diet. Consequently, this experiment addresses the questions emerging from the increasing demand of sea urchin roe and the feasibility of sea urchin aquaculture. The use of natural marine macrophytes is problematic due to the variability in their biochemical composition (which varies according to season or site). As a result, the use of food with a known and stable biochemical composition is necessary to study the effect of food quality on nutrition and growth of sea urchins. In order to maximize the feasibility of sea urchin farming (availability, stocking, etc.), the utilization of artificial feed seems indispensable.

Artificial feeds are successfully used elsewhere to rear echinoids or maintain them in aquaria (Lawrence et al., 1997; Klinger et al., 1997). One of the most necessary nutrients required in a diet is protein. It is a vital macronutrient and required by all eukaryotic organisms, including sea urchins for maintaining a proper physiological functions (Pearce et al., 2002). It is necessary to evaluate both the quality and quantity of protein for a particular sea urchin species, as a candidate for aquaculture, to gain understanding of how artificial feeds are utilized and reflected on gonad and somatic growth. The aim of this study is to monitor morphometric changes in adult sized *Paracentrotus lividus* fed formulated feeds with varying protein source and quantity.

## MATERIAL AND METHODS / *Materijali i metode*

On March 2006, a sample of 720 individual sea urchins was caught in the coastal rocky habitat near the city of Dubrovnik, Croatia. The sample was equally distributed among 12 tanks (volume: 20 x 60 x 40 cm) that were arranged in 3 columns and four rows. The three tanks at the top of the column were connected to influx pipe with filtered (1µm) ambient sea water. A plastic pipe, covered with fine mesh (500 µm) and fixed in the middle of each tank, collected filtered water and gravitationally filled each following tank that was located directly underneath. Vertical distance between tanks was 35 cm which allowed for additional aeration. The three tanks at the bottom of each column were connected to effluent piping that after passing through a filtration system, returned to the sea (figure 1.).

Throughout the course of the experiment the urchins were kept at the regime of ambient temperature and salinity. Recorded temperature ranged from 15 °C in March, at the start of the experiment to 23 °C in June, at the end of the experiment. The recorded salinity values ranged from 32 to 37 psu. Photoperiod regime was constant (12 h light / 12 h dark) throughout the experiment. Following the 15 day starvation period the urchins were fed 4 experimental diets during a period of 60 days.

Each horizontal series encompassed one of the feeding regimes (A, B, C, D) and was performed in a triplicate. Feeding was based on food servings in 3 day intervals, 1 g/urchin/day for macrophytes and 0,5 g/urchin/day for extruded feeds. Regime

Figure 1 The system design for the feeding experiment of stony sea urchin, *Paracentrotus lividus*
*Slika 1. Dizajn sustava za hranidbu hridinskog ježinca, Paracentrotus lividus*

A consisted of seaweeds collected in the vicinity of sea urchin natural habitat, and represented were species of macrophytes belonging to three phylums; Chlorophyceae, Rhodophyceae and Phaeophyceae, although, most abundant were green algae of order, Ulvales (80%). Regimes B, C and D consisted of artificially prepared feed, and differed in respect to the mixture structure and chemical composition.

## Artificial feed composition

Artificial feeds B, C and D were manufactured in „Pliva-Kalinovica"- Zagreb factory, and composition of each is shown in table 1. The feed mixtures differed in respect to corn meal content, where the smallest allotment is presented in feed B (22%), median in C (30%) and highest in feed D (35%). The other important difference was in respect to the content of fish meal, where the highest allotment is presented in feed B (15%), median in C (5%) and was absent in feed D. All other components are equally represented in each experimental feed mixture B, C, D.

Table 1 The structure of each experimental feed - B, C and D
*Tablica 1. Struktura svake pojedine pokusne hrane - B, C i D*

| FEED | B | C | D |
|---|---|---|---|
| Components (%) | | | |
| | | | |
| CORN MEAL | **22** | **30** | **35** |
| WHEAT MEAL | **20** | **22** | **22** |
| SOY MEAL | **15** | **15** | **15** |
| FISH MEAL | **15** | **5** | **0** |
| CORN STARCH | **15** | **15** | **15** |
| SOYA LECITHIN | **3** | **3** | **3** |
| SUNFLOWER OIL | **3** | **3** | **3** |
| GELATIN | **5** | **5** | **5** |
| VAM | **2** | **2** | **2** |
| C- VIT (150 mg/kg) | | | |

In table 2 the essential chemical composition of feeds B, C and D is expressed in % in dry matter. The basic difference in experimental mixtures which is related to the content of particular fodder, more precisely it is in direct correlation to the content of fish meal (15, 5, 0 %) substituted by relevant content of corn meal. As expected, the difference is expressed in the varying nutritional value among the mentioned feeds (CP, NET and ash).

Table 2 Chemical composition of feeds; CP-crude protein, CF-crude fibre, NFE- Nitrogen-free extract
*Tablica 2. Kemijski sastav hranjiva*

| FEED | MOISTURE | CP | CFat | CF | NFE | ASH |
|---|---|---|---|---|---|---|
| | % | % | % | % | % | % |
| **B** | **12.0** | **22.4** | **7.6** | **1.8** | **51.5** | **4.7** |
| | | | | | | |
| C | 11.0 | 15.8 | 7.8 | 2.1 | 60.2 | 3.1 |
| | | | | | | |
| D | 10.5 | 12.6 | 7.6 | 2.4 | 65.3 | 1.8 |

The same vitamin-mineral content is added to all artificially prepared feeds (B, C i D), as shown in table 3.

Table 3 Vitamin-mineral content
*Tablica 3. Vitaminsko-mineralni sastav*

| Component | Amount – measurement unit |
|---|---|
| Vitamin A - Retinol | 15000 ij |
| Vitamin D3 - Cholecalcipherol | 2500 ij |
| Vitamin E - Tocopherol | 200 ij |
| Vitamin B1 - Thiamine | 11 mg |
| Vitamin B2 - Riboflavin | 13 mg |
| Niacin | 19 mg |
| Pyridokxine - B6 | 11 mg |
| Pantothenic acid | 35 mg |
| Vitamin B12 - Cyanocobalamin | 0,1mg |
| Folate | 2 mg |
| Choline | 500 mg |
| Inositol | 80 mg |
| Mn | 35 mg |
| Zn | 30 mg |
| Fe | 20 mg |
| Cu | 1 mg |
| J | 0,5 mg |
| Co | 0,06 mg |

Every three days uneaten food was removed and the tanks were cleaned. Throughout the period of 75 days four events of measurements were performed (morphometric and histological), for that purpose, 20 urchins were sacrificed from each of the 4 feeding regimes. Following the morphometric analysis the gonads were conserved in a Bouins' solution for further histological analysis.

## RESULTS / *Rezultati*

At the beginning of experiment the recorded average gonad weight value was 4,2 g, while the average recorded GSI was 6%. After the starvation period, 15 days following the start of the experiment the recorded gonad weight and GSI values were on a decline. Average recorded value of gonad mass was 3,72 g and GSI, 5,5%. As the project progressed, in the artificial feed regimes (B, C, D) the recorded gonad weight and GSI values revealed an evident growing trend, however, with no apparent statistical differences among the three groups. The, average recorded gonad weight values in the three groups, at the beginning of the experiment was 4,4 g, and 6,4 g at the end of the experiment. The average recorded GSI values at the beginning and the end of the experiment for the three groups were 5,5% and 9% respectively. The average recorded values of gonad mass and GSI in the group that was on macrophyte feeding regime revealed a declining trend with the progression of time, the same was true for the control group, sea urchins from the natural population.

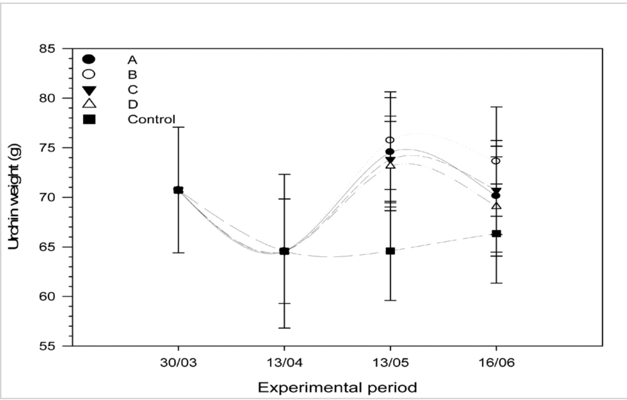


Figure 2 Recorded total mass values of stony sea urchin, *Paracentrotus lividus*, during the feeding experiment; A macrophyte diet, B, C and D – artificially formulated feed and control group (sea urchins from the natural population)

*Slika 2. Kolebanje vrijednosti ukupne mase hridinskog ježinca, Paracentrotus lividus, tijekom hranidbenog pokusa; A, B, C i D – umjetna hrana i kontrolna skupina ježinci iz prirodnog staništa*

After two months of intensive feeding, the values of total urchin mass in all regimes showed a declining trend. The recorded total mass values of urchins in the natural population were significantly lower than in other groups. Among the three groups with artificially prepared feed, significant statistical differences were recorded for the treatment B, while statistical differences among groups A, C, and D were not recorded (figure 2.).

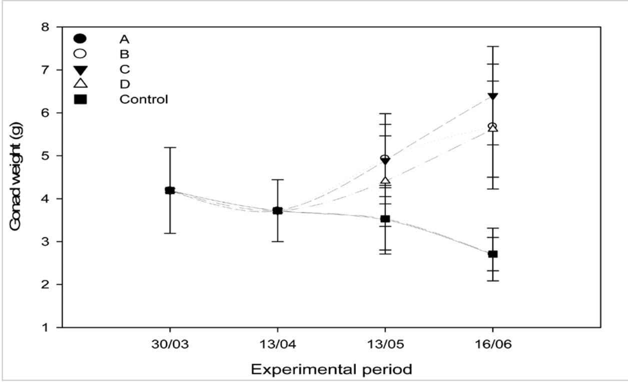


Figure 3 Recorded gonad mass values of stony sea urchin, *Paracentrotus lividus*, during the feeding experiment; A macrophyte diet, B, C and D – artificially formulated feed and control group (sea urchins from the natural population)

*Slika 3. Kolebanje vrijednosti mase gonada hridinskog ježinca, Paracentrotus lividus, tijekom hranidbenog pokusa; A -bentoske makroalge, B, C i D – umjetna hrana i kontrolna skupina (ježinci iz prirodnog staništa)*

During the period of starvation, recorded gonad mass values for all experimental groups, including the control showed a declining trend. Immediately following the onset of feeding, the gonad mass in the experimental groups with artificially prepared feed - B, C, D begun to increase in value, meanwhile in treatment A (macrophyte) it was still on the decline. The same was recorded in the natural population. The growing trend of gonad mass in the groups with the artificially prepared feed continued all throughout the duration of the experiment, this was particularly evident in treatment C. Similarly, the descending trend of the values of gonad mass was continuous in treatment A as well as in the control (figure 3.). Analysis of variance (ANOVA) showed that the differences among the arithmetic means are accidental and not significant.

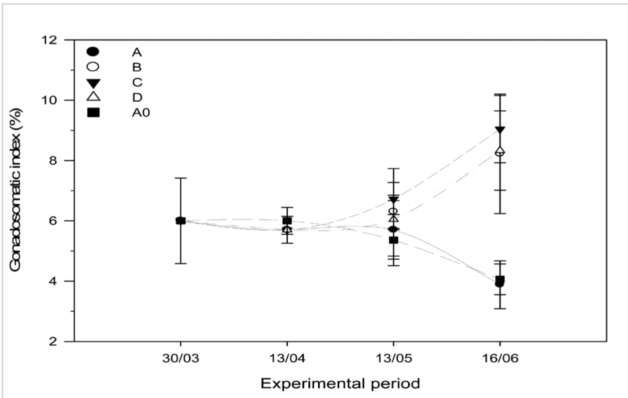


Figure 4 Recorded gonadosomatic index (GSI) values of stony sea urchin, *Paracentrotus lividus*, during the feeding experiment; A macrophyte, B, C and D – artificially formulated feed and A0 – control group (sea urchins from the natural population)

*Slika 4. Kolebanje vrijednosti gonadosomatskog indeksa (GSI) hridinskog ježinca, Paracentrotus lividus, tijekom hranidbenog pokusa; A – bentoske makroalge, B, C i D – umjetna hrana i kontrolna skupina ( ježinci iz prirodnog staništa)*

During the period of starvation, recorded gonadosomatic index (GSI) values for all experimental groups, showed a declining trend except in the control group, where they stayed the same. After 30 days, the declining trend of gonadosomatic index (GSI) values was recorded for treatment A and the control group. During the two months of the intensive feeding conditions in the regimes with artificially prepared feed, B, C and D a growing trend was recorded for GSI values, and it was particularly evident in the treatment C. In the last month of the experiment, a significant decline in the GSI values was observed for the groups, A and the control (figure 4.). Analysis of variance showed that the differences between all artificial and natural food groups are significant. The highest values of GSI were recorded for the group fed formulated feed C (figure 4.).

The physical properties of artificially formulated feed were not appropriate, the pellets were completely dissolved few hours after immersion, considerable amount of dissolved food remained unused.

## DISCUSSION / *Rasprava*

During the starvation period, gonad mass, GSI and total mass of the urchins indicated a declining trend. Similar effects of starvation on *P. lividus* were published by Sprilet (2001). Leoni et al. (2003) have concluded that sea urchins, during adverse conditions focus more on reproduction and nutrient reserves are mobilized for the needs of gametogenesis and maintenance of vital functions (not growth) which results in a decrease of gonad mass and GSI. With the evolution of the feeding experiment, gonad mass and GSI values were increasing in the experimental groups fed artificially prepared feed. However, the experimental group that was fed a macrophyte diet, as well as the control, urchins in the natural habitat, demonstrated a decline in the recorded values of gonad mass and GSI. Two months after the intensive feeding conditions, recorded values of gonad mass in the control group were significantly lower than in all other groups in the experiment. The sea urchins fed a macrophyte diet demonstrated an increase in gonad mass and GSI values, however, the recorded values were significantly lower than those measured in samples fed the artificially prepared feed. The less pronounced effect of intensive macrophyte diet in respect to artificially prepared feed was similarly described by Olave (2001) and Lawrence (1997) for the Chilean sea urchin, *Loxechinus albus*. Pearce (2004) however, described statistically significant differences in recorded GSI values for the species, *Strongylocentrotus droebachiensis* between groups, one being artificially prepared feed and the other, algal diet, as well as the natural population. Accomplished growth was recorded for all the experimental groups, including the natural population. In our research we found a significant decrease in the values of GSI for the group fed algal diet and the control. The disparity of results can be interpreted through revelation of histological analysis. In fact, at the beginning of the experiment histological analysis reveals that the population of *P.lividus* near the island of Lokrum is in the stage of mature gonads and ready to spawn. In the course of first and second sampling, through histological analysis, the event of spawning was determined for the control and partial spawning for the experimental group on macrophyte diet, which explains the decrease in recorded gonad mass values. At the beginning of the experiment described by Pearce (2004) the urchins have spawned, therefore all groups showed gonad growth in the course of the experiment.

The differences among the artificially prepared feeds expressed through growth of GSI and gonad mass values revealed that the best among them was artificial feed C whose variance in consistence of essential nutritional components (fish and corn meal, the percentage of protein, total nitrogen and ash content) fits between prepared feeds B and D. The main features of the artificial feed C as combination of plant and animal components, is the consistent of dominant plant component in the amount of 88%, with the addition of fish meal (5%) and gelatine (5%) which are both of animal origin. The similar results of intensive feeding of sea urchin, *Strongylocentrotus droebachiensis* were published by Pearce et al. (2002) where the best GSI percentages were obtained with the use of feeds containing 10 % and 5 % as opposed to 0 and 15 % of fish meal.

In scope of world echinoculture research, various articles have been published on the topic of artificially prepared feeds for sea urchins, with the aim of finding inexpensive and efficient fodder composed mostly of plant origin sources with a lesser amount of expensive fish meal, which casts a positive influence on growth and organoleptic quality of gonads. In the published work of Spirlet et al., (2001) the best results of gonad growth and GSI percentages were obtained with feeds containing higher contribution of soy meal and a slightly less significant results with a mixed diet containing about 30 % of fish meal, while algal diets were significantly weaker. Fernandez and Pergent, (1998) obtained best GSI percentages using mixed diet that contained up to 45 % of fish meal. In the above mentioned and the consecutive experiments, the same author published favourable results using mixed diet while the single origin feeds (plant or animal) obtained somewhat similar, however, less favourable results (Fernandez and Pergent 1998; Fernandez and Boudouresque 2000).

Based on the results of our study we can conclude that the artificially formulated feed is adequate food for sea urchins in a controlled environment, especially when it contains a smaller animal protein component (5-10%) and a larger share of plant components (over 90%). Percent gonad yield was not affected by protein concentration, but was significantly affected by protein source ratio.

The physical properties of formulated feed used in the experiment were not optimal due to the rapid degradation in water. Since urchins eat slowly, the availability of feed became limited in time. The feed must have a sufficient stability in sea water (Mortensen and Siikavuopio 2003); pellets that break down quickly may leach important dietary components more rapidly giving such feeds an inferior nutritional quality as compared to more stable feeds. Improving the stability of feed materials in seawater can expect much better results in cultivation.

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