Conditioning of the Mediterranean Scallop *Pecten Jacobaeus* (Linneaus, 1758) in Recirculation Systems with Different Types of Feed

Kondicioniranje jakobove kapice Pecten jacobaeus (Linneaus, 1758) u recirkulacijskim sustavima s različitim vrstama hrane

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Abstract

Mariculture bivalve production is limited to a small number of species, so there is a need for the introduction of new species. The Mediterranean scallop *Pecten jacobaeus* (Linnaeus, 1758) seems like a potential bivalve mariculture candidate, but there is a lack of spat from nature for farming purposes. Two experiments on conditioning of *P. jacobaeus* broodstock in recirculation systems were conducted, the first one in April 2023 and the second one in March 2024. Condition indices were calculated: condition (CI), muscle (MI), gonadosomatic (GSI) and gonad index (GI) in both experiments. For the second experiment, a histology examination of the gonads was also conducted. In the first experiment broodstock fed with live algae *Isochrysis galbana* for 21 days, showed a rise in GSI and GI values, with constant CI and a slight decline of MI. In the second experiment, broodstock was fed for 25 days with two different types of feed: Group 1 with live algae I. galbana, while Group 2 concentrated frozen algae mix of 3% of the dry body mass per day. Better results were achieved with the live feed, evidenced by higher CI and MI results in Group 1. The GSI and GI results, along with gonad histological examination, indicate that gonads developed with both types of feed, but the process appeared to be accelerated with live feed. We conclude that gonad development in Group 1 was at the expense of ingested food, while in Group 2, at the expense of storage of nutrient reserves from muscle and other tissues.

KEY WORDS

mariculture
Pecten jacobaeus
broodstock conditioning
condition index
gonadosomatic index
gonad histology

Sažetak

Proizvodnja školjkaša u marikulturi ograničena je na mali broj vrsta pa postoji potreba za uvođenjem novih vrsta. Jakobova kapica Pecten jacobaeus (Linnaeus, 1758) se čini kao potencijalni kandidat za uvođenje u marikulturu, ali nedostaje mlađi iz prirode za potrebe uzgoja. Provedena su dva pokusa kondicioniranja matičnog jata P. jacobaeus u recirkulacijskim sustavima, prvi u travnju 2023. i drugi u ožujku 2024. Izračunati su indeksi stanja: kondicijski (CI), mišićni (MI), gonadosomatski (GSI) i gonadni indeks (GI) u oba pokusa. Za drugi pokus također je provedeno histološko ispitivanje spolnih žlijezda. U prvom pokusu matično jato, hranjeno 21 dan živom algom Isochrysis galbana, pokazalo je porast vrijednosti GSI i GI, uz konstantan CI i lagani pad MI. U drugom pokusu matično jato hranjeno je 25 dana dvjema različitim vrstama hrane: Grupa 1 sa živim algama I. galbana, dok Grupa 2 koncentriranom mješavinom smrznutih algi u iznosu od 3% suhe mase tkiva dnevno. Bolji rezultati postignuti su živom hranom, što je dokazano boljim CI i MI rezultatima u grupi 1. Rezultati GSI i GI s histološkim pregledom gonada sugeriraju da su se gonade razvijale s oba tipa ishrane, ali čini se da je proces ubrzan sa živom hranom. Zaključujemo da je razvoj spolnih žlijezda u grupi 1 bio na trošak unesene hrane, dok je u grupi 2 bio na trošak skladišnih rezervi hranjivih tvari iz mišića i drugih tkiva.

KLJUČNE RIJEČI

marikultura
Pecten jacobaeus
kondicioniranje matičnog jata
indeks stanja
gonadosomatski indeks
histologija gonada

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1. INTRODUCTION / *Uvod*

The high demand for seafood is putting a lot of pressure on marine wild stocks, of which many are overexploited by commercial fishing. Aquaculture, as one of the fastest-growing sectors in food production in the world [1], can provide an answer to mitigate these adverse effects [2] and, with good management practices, could significantly contribute to global food production in the future [3]. Due to the need for ecologically responsible and sustainable development [1, 3], aquaculture is turning to the cultivation of low-trophic species, such as bivalves, which do not require additional feeding, but filtering particles from the water purifying the water and contributing to its quality [1, 2]. Today, mariculture is the main market supplier of bivalve products, but this supply is often limited to a small number of species [1], so there is a need to diversify its supply by the introduction of new species.

Scallops (Pectinidae Rafinesque, 1815) are interesting for introduction into aquaculture due to the high demand and high prices they achieve on the market. In 2021, scallop aquaculture production reached around 2 million tons [1, 4], but this production is limited to a small number of species. By far leading producer is China (>90%) with predominated production of species *Argopecten irradians* (Lamarck, 1819), followed by Japan with production of *Mizuhopecten yessoensis* (Jay, 1857) [4] and Peru for species *Argopecten purpuratus* (Lamarck, 1819) [4, 5]. In Europe, scallop production was mostly focused on king scallop *Pecten maximus* (Linnaeus, 1758) [6], but significant commercial production was never achieved due to the high costs of production and competition from commercial fishing, so aquaculture production declined to around 10 tons in 2021 [4].

The Mediterranean scallop *Pecten jacobaeus* (Linnaeus, 1758) is the largest scallop in the Mediterranean and Adriatic Sea [7] and is genetically very closely related to king scallop *P. maximus* [8, 9]. While scientific research on *P. maximus* was extensive, research on *P. jacobaeus* is rare and focused mainly on studying its biological characteristics, like growth and reproduction [10 - 16] and in small part their potential for aquaculture [11, 14, 17, 18, 19]. The biggest obstacle for farming of this species is the unavailability of a reliable and cheap spat source.

Understanding the reproductive cycle of bivalves, in relation to environmental conditions, is essential for the process of scallop seed production [20]. Regulation of gametogenesis is primarily under genetic influence, while the timing of gametogenesis is influenced by environmental factors [20, 21] among which the temperature has been considered as the most important [21], as it initiates the process of gametogenesis. As gametogenesis requires a lot of energy, food availabilty for most bivalves is an even equally important factor [22]. Scallops go through the process of energy accumulation and energy utilization related to gametogenesis, which has been related to seasonal food levels [20], but also to the utilization of stored energy reserves from adductor muscle and other organs [21, 23, 24].

During the bivalve broodstock conditioning in the hatcheries, the environmental conditions are manipulated to stimulate the development of gonads to their ripeness and to initiate spawning throughout the year [25, 26], but also to ensure egg quality and larvae viability [25, 27]. The nutrition of broodstock has an important role in gamete development, and quantity and quality of food are major factors affecting broodstock conditioning in hatcheries [27]. The effect of nutrition on broodstock conditioning is species-specific, so for the best results, an optimal diet should be established. Scallops primary food is phytoplankton, but they can also feed on detritus, bacteria and other organic matter that they filter from the water [28]. As the nutritional value of microalgae varies between species, it is necessary to find the right combination of microalgae species that will provide broodstock with all their nutritional demands [28, 29]. Therefore, better results are achieved with a diet that consists of more algae species, than with most single species diets [25, 27, 28]. As microalgae production represents the major bottleneck in bivalve hatcheries and is often prone to crashes, formulated algae diets are good replacement options [28].

The aim of this study was to provide information on *P. jacobaeus* broodstock conditioning in recirculation systems with different types of food, live and formulated algae diet. Obtained data can be useful for the potential future introduction of this species into commercial aquaculture.

2. MATERIAL AND METHODS / *Materijali i metode*

2.1. Animals, feed and experimental design / *Životinje, hrana i plan pokusa*

Two experiments on the conditioning of *P. jacobaeus* were conducted during the period of natural spawning at the collection site. Prior to each experiment *Pecten jacobaeus* specimens were collected by SCUBA diving from the Krka River estuary at the approximate geographical coordinates 43°45′ N 15°50′ E at the depths ranging from 8 to 20 m. Specimens were transported to the laboratory of the University of Zadar, cleaned from fouling, and placed in 120 dm^3 cylindrical recirculation tanks with artificial sea water made by a mixture of RO purified water and commercial salt (ASF Instant Ocean) at 35 salinity. Temperature was kept at constant levels at 15 –16 °C and aeration with aeration stones was provided in each tank. For the feeding. microalgae *Isochrysis galbana* was cultured in a 1250 dm^3 algae photobioreactor (Industrial plankton PBR 1250L). Seawater was made with a mixture of RO purified tap water and commercial salt (ASF Instant Ocean). Before inoculation of algae, the seawater was enriched with medium. Microalgae were grown under continuous light at an intensity of 1200 µEm-2s-1, at a temperature of 22°C, and were harvested daily in the late-exponential growth phase. Before feeding broodstock, the algae density was determined daily by standard algae cell counts (Bürker chamber).

The first experiment / *Prvi pokus*

In the first experiment, which started in March 2023 and ended in April 2023, a total of 30 specimens were placed in three tanks, 10 in each, and were acclimatized for five days at an ambient temperature of 16 °C and salinity at 35, during which they were not fed. Afterwards, six specimens were taken for the analysis of indices at the start of the experiment and the remaining 24 specimens were fed for 21 days with live algae *Isochrysis galbana* in the amount of 3% of their total soft tissue dry mass. At the end of the experiment six specimens were analyzed for condition indices calculation.

The second experiment / *Drugi pokus*

In the second experiment, which started in March 2024 and ended in April 2024, a total of 92 specimens were placed in six recirculation tanks and were acclimatized for 10 days at ambient temperature of 15 °C and salinity of 35, during which they were not fed. Afterwards, 21 specimens were taken for the analysis of indices and 9 for the analysis of gonad histology before the feeding commenced. The remaining 62 specimens were separated into two groups, 31 specimens in each, that were placed into two separate feeding lines, each consisting of three tanks, with 10–11 specimens, and were fed with two different types of feed for 25 days in the amount of 3% of their total dry soft tissue mass. The first group (Group 1: tanks A, B, C) was fed with live algae *Isochrysis galbana* and the second group (Group 2: tanks D, E, F) with concentrate of frozen algae consisted of species *Tetraselmis sp*., *Thalassiosira weissflogii* and *Thalassiosira pseudonana*. Feeding was carried out once a day in the evening with closing circulation overnight and opening it in the morning. For the duration of the experiment broodstock was exposed to natural light, temperature was on average 16.2 °C (± 1.3), salinity 35, pH ranged from 7.58-7.88, dissolved O2 was on average 6.0 (±0.3), O2 saturation was on average 80% (± 4%) and ammonium levels were on average 50 µmol/L. At the end of the experiment, 21 specimens from each group were analyzed for condition indices calculation, and an additional 9 from each group for analysis of gonad histology.

2.2. Condition indices / *Indeksi stanja*

For analysis of condition indices, the shells were separated from soft tissues, which were further dissected for gonads (male and female parts separated to calculate male-to-female ratio), adductor muscle and the remaining soft tissue. Shell dimensions height, length, and width (precision up to 1 mm) and wet weight of the shell and soft tissue parts were measured. Gonads were also visually inspected to estimate their ripeness. All soft tissue parts were then placed separately on the pre-weighted aluminum folium papers and together with the shell were oven-dried for 48 h at 60 °C and weighed afterwards (precision up to 0.001 g).

Moisture content (MC) percentage was derived from the formula:

$$\mathrm{MC} = \frac{\text{soft tissue weight} - \text{soft tissue dry weight}}{\text{soft tissue wet weight}} \times 100$$

Condition index (CI) was calculated according to [31]:

$$\mathrm{CI} = \frac{\text{total soft tissue dry weight(g)}}{\text{shell dry weight(g)}} \times 100$$

Muscle index (MI) was calculated according to [31]:

$$\mathrm{MI} = \frac{\text{muscle dry weight(g)}}{\text{total tissue dry weight(g)}} \times 100$$

The gonadosomatic index was calculated in two ways. For distinguishing these two indices, the one according to [20], which uses the relation of gonads to total soft tissue dry weight we will refer to as gonadosomatic index (GSI):

$$\mathrm{GSI} = \frac{\text{gonad dry weight(g)}}{\text{total soft tissue dry weight(g)}} \times 100$$

while the other, with the relation of gonads to shell weight as according to [32], we will refer to as gonad index (GI):

$$\mathrm{GI} = \frac{\text{gonad dry weight(g)}}{\text{shell weight(g)}} \times 100$$

2.3. Histology / *Histologija*

The analysis of the gonad histology in the second experiment was performed on a total of 27 specimens. Bivalves were opened and gonad tissue samples were extracted and fixed in 10 vol% formaldehyde. Samples were then dehydrated with ethanol treatments of increasing concentrations, after which the gonad tissue was embedded in paraffin, cut at 5 µm, put on glass slides and stained with hematoxylin and eosin. Sections were examined under a microscope (Olympus BX43F) to evaluate the gonad development stage by applying the gonad development scale of [16]: undifferentiated, early development, late development, ripe, partially spawned, spent, and reabsorption. When two stages occurred simultaneously in one single section, the staging criteria decision was based upon the condition of the major proportion of the preparation. Furthermore, one photograph of each female gonad slide was taken under 10x magnification (cellSens Entry 4.1 program), and the diameter of all oocytes with visible nucleus was measured using the Image J software [33].

2.4. Statistical analysis / *Statistička analiza*

All data are expressed as a mean ± standard deviation (SD). Graphs were generated using Microsoft Excel while statistical analyses were performed using SigmaPlot 14.0 (Systat Software, Inc.). Depending on the results of the Shapiro–Wilk normality test, either One-way ANOVA or Kruskal–Wallis one-way ANOVA on ranks was used to evaluate the effect of diets. Post hoc pairwise comparisons between indices values of different groups (feed types) were conducted using the Holm–Sidak method or Tukey's test. Statistical significance was set at $p < 0.05$.

3. RESULTS / *Rezultati*

3.1. The first experiment / *Prvi pokus*

Shell length of the analyzed specimens at the start of the experiment ranged from 10.8–13.7 cm (12.22±1.07), and at the end of the experiment from 11.7–13.2 cm (12.27±0.58). Total soft tissue dry weight at the beginning of the experiment was, on average, 11.19 g (±3.18), and at the end of the experiment, 10.24 g (±1.93). MC changed from the start to the end of the experiment for all soft tissue parts: for the total soft tissue from 83.55% to 82.59%, for the gonads from 83.48% to 79.49%, and for the muscle from 75.29% to 71.01%. All condition indices changed from the start to the end of the experiment: CI from 12.19±1.42 to12.12±1.42, MI from 53.87±3.44 to 53.29±2.75, GSI from 10.51±2.74 to 13.00±1.86, GI from 1.27±0.32 to 1.56±0.16. Recorded differences were not statistically significant (Fig 1.).

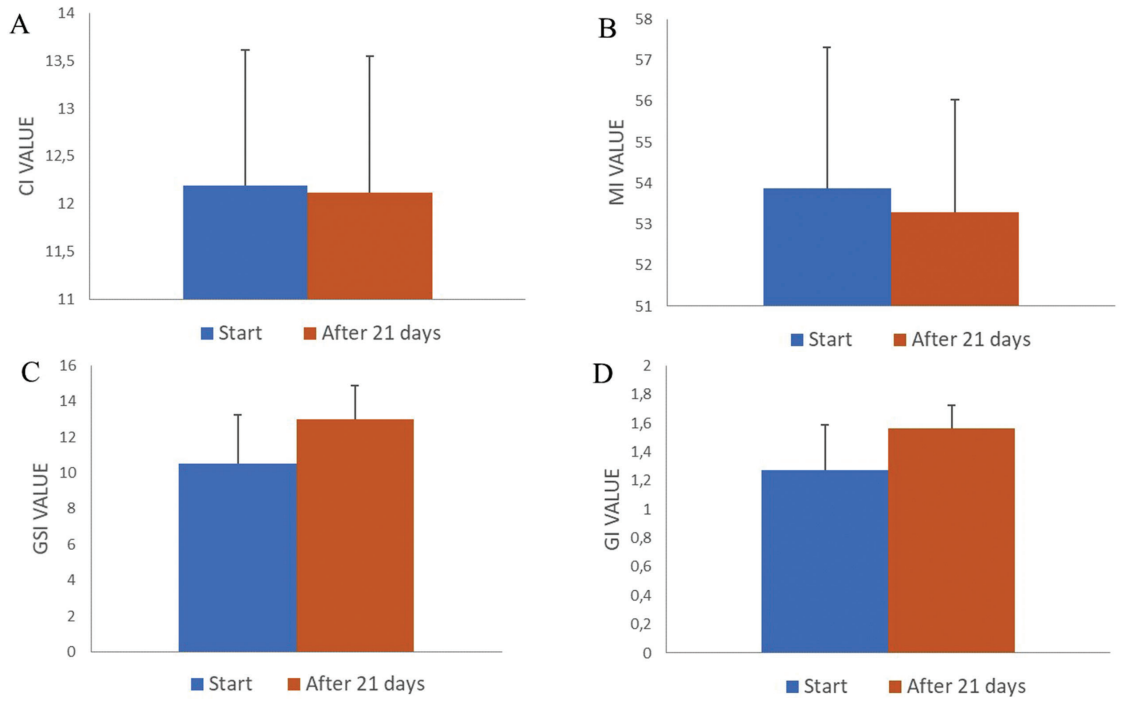

Figure 1 *Pecten jacobaeus* broodstock indices values (mean ± SD; n=6) at the start and 21 days after conditioning: A) condition index (CI), B) muscle index (MI), C) gonadosomatic index (GSI) and D) gonad index (GI).

Slika 1. Vrijednosti indeksa matičnog jata Pecten jacobaeus (srednja vrijednost ± SD; n = 6) na početku i 21 dan nakon kondicioniranja: A) indeks kondicije (CI), B) mišićni indeks (MI), C) gonadosomatski indeks (GSI) i D) gonadni indeks (GI).

3.2. The second experiment / *Drugi pokus*

Shell length of the analyzed specimens at the start of the experiment (Start) ranged from 8.4–12.4 cm (10.25±1.16), and total soft tissue dry weight was, on average, 5.34±1.62 g (Tab 1.). MC at the start was, on average, 83.36% for the total soft tissue, 82.95% for the gonads, and 76.96% for the muscle. At the Start, the condition index (CI) was 8.98±1.13, the muscle index (MI) 51.03±3.38, the gonadosomatic index (GSI) 10.65±2.53 and the gonad index (GI) 0.95±0.22, respectively (Fig. 2).

Shell length of the analyzed specimens at the end of the experiment fed with live *I. galbana* feed (Group 1) ranged from 7.3–11.9 cm (9.61±1.55) and total soft tissue dry weight was on average 5.80±2.38 g (Tab.1). For Group 1, MC was on average: 81.87% for the total soft tissue, 83.16% for the gonads and 73.93% for the muscle. Condition indices for Group 1 changed compared to Start and were on average: 10.20±1.54 for CI (12.76% rise, $p < 0.001$), 48.27±3.63 for MI, 9.97±4.40 for GSI and 1.01±0.47 for GI (Fig 2.). There was one mortality recorded for Group 1 (4.5%).

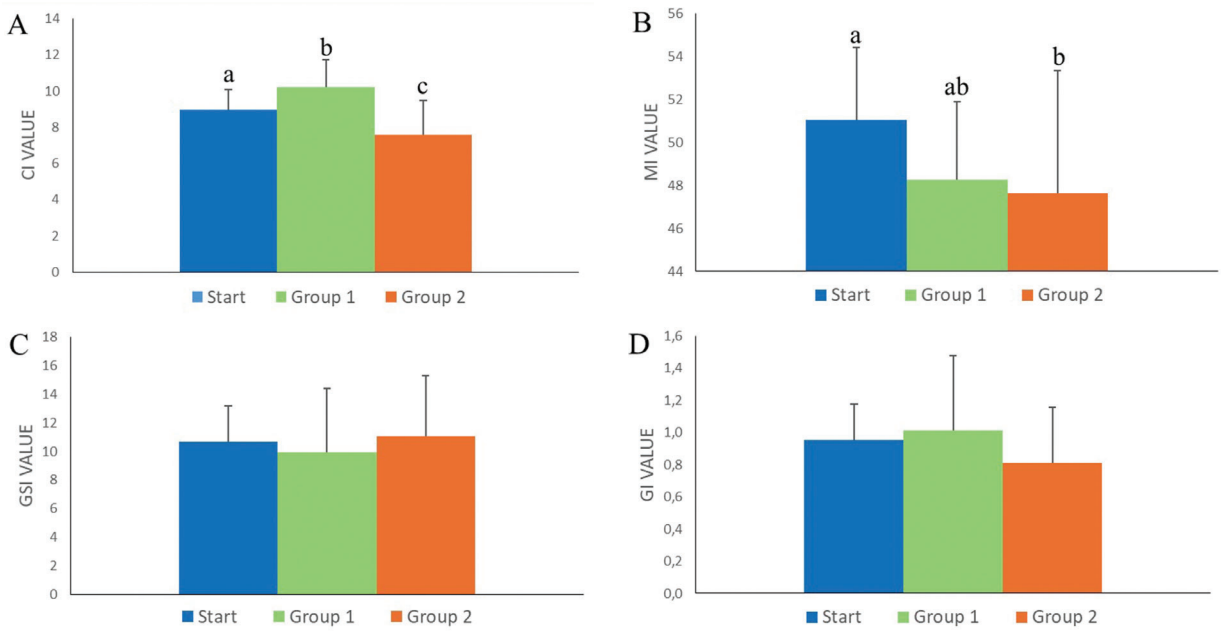

Figure 2 *Pecten jacobaeus* broodstock indices values (mean ± SD, n =21) at the start and 25 days after conditioning with two types of feed: live algae *I. galbana* (Group 1) and concentrated frozen algae mix (Group 2): A) condition index (CI), B) muscle index (MI), C) gonadosomatic index (GSI) and D) gonad index (GI). Letters on bars represent significant statistical differencies between group values

Slika 2 Vrijednosti indeksa matičnog jata Pecten jacobaeus (srednja vrijednost ± SD, n = 21) na početku i 25 dana nakon kondicioniranja dvjema vrstama hrane: žive alge I. galbana (Skupina 1) i koncentrirana mješavina smrznutih algi (Skupina 2): A) indeks kondicije (CI), B) mišićni indeks (MI), C) gonadosomatski indeks (GSI) i D) gonadni indeks (GI). Slova predstavljaju značajne statističke razlike između grupnih vrijednosti.

Shell length of the analyzed specimens at the end of the experiment fed with concentrated frozen mix algae feed (Group 2) ranged from 6.50–13.20 cm (10.00±1.83), and total soft tissue dry weight was, on average, 4.67±2.43 g (Tab 1.). For Group 2, MC was on average: 84.08% for the total soft tissue, 83.96% for the gonads and 78.05% for the muscle. Condition indices for Group 2 changed compared to Start and were on average: 7.59±1.88 for CI (16.76% decline, p< 0.001), 47.62±5.72 for MI (6.93% decline, p<0.05), 11.04±4.23 for GSI and 0.81±0.34 for GI (Fig. 2). For Group 2 there was one mortality recorded (4.5%).

3.3. Histology results / *Rezultati histologije*

Histological examination of gonads showed that the analyzed specimens after acclimatization were mostly in the ripe stage of development, with signs of the beginning of the reabsorption process in female gonads parts and late development and ripe development stages with signs of partially spawning in male gonads parts which suggests that broodstock was collected from nature at a time of ripening of gonads and spawning. After 25 days of the experiment, analysis showed that many gonad samples showed signs of spontaneous spawning, with a predominant development stage of partially spawned. Other specimens were mostly in the ripe stage of development, and few of the specimens showed signs of prespawning reabsorption in female gonads parts (Fig. 3., Supplementary Tab. 1). Photomicrographs examples of present gonad development stages are presented in Fig. 4. (female) and 5. (male), and all processed samples are shown in Supplementary Fig. 1. (female) and Fig. 2. (male). Measurement of oocyte diameter showed that mature oocytes (MO) were predominant, with the appearance of atresic oocytes (AO) in various ratios for different samples. Previtellogenic oocytes (PVO) were more abundant in Group 1 than in Group 2 (Supplementary Fig. 3).

Table 1 *P. jacobaeus* broodstock shell and dry soft tissue mass (g) (mean±SD, n=21) at the start and after 25 days of conditioning with two types of feed: live algae *I. galbana* (Group 1) and concentrated frozen algae mix (Group 2)

Tablica 1 Masa ljušture i suhog mekog tkiva (g) matičnog jata P. jacobaeus (srednja vrijednost ± SD, n = 21) na početku i nakon 25 dana kondicioniranja dvjema vrstama hrane: živim algama I. galbana (Grupa 1) i koncentriranom smrznutom mješavinom algi (Grupa 2)

	n	Shell (g)	Total soft tissue (g)	Gonads (g)	Muscle (g)	Remaining soft tissue (g)
Start	21	**58.40±**11.99	**5.34**±1.62	**0.57**±0.19	**2.74**±0.88	**2.03**±0.63
Group 1	21	**55.26**±16.97	**5.80**±2.38	**0.57**±0.34	**2.81**±1.18	**2.42**±1.06
Tank A	7	56.85±18.21	5.96±2.59	0.34±0.20	2.94±1.47	2.68±1.05
Tank B	7	51.70±15.30	5.22±1.68	0.57±0.23	2.56±0.89	2.10±0.79
Tank C	7	57.22±19.28	6.21±2.95	0.80±0.42	2.93±1.24	2.49±1.36
Group 2	21	**58.94**±19.29	**4.67**±2.43	**0.48**±0.28	**2.25**±1.32	**1.93**±1.07
Tank D	8	58.04±21.58	4.48±2.41	0.45±0.23	2.08±1.05	1.96±1.27
Tank E	7	57.09±18.55	4.31±1.90	0.51±0.23	1.99±0.87	1.81±0.86
Tank F	6	60.68±19.87	5.34±3.22	0.50±0.42	2.80±2.00	2.04±1.20

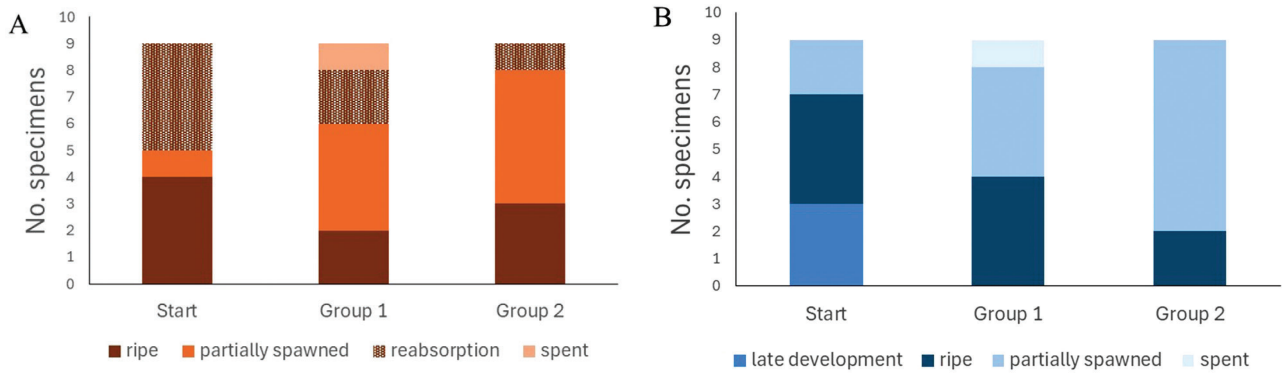

Figure 3 *Pecten jacobaeus* broodstock gonad development stages at the start and 25 days after conditioning with two types of feed: live algae *I. galbana* (Group 1) and concentrated frozen algae mix (Group 2): A) female gonads and B) male gonads.

Slika 3. Faze razvoja gonada matičnog jata Pecten jacobaeus na početku i 25 dana nakon kondicioniranja dvjema vrstama hrane: živim algama I. galbana (Grupa 1) i koncentrirana mješavina smrznutih algi (Grupa 2): A) ženske spolne žlijezde i B) muške spolne žlijezde.

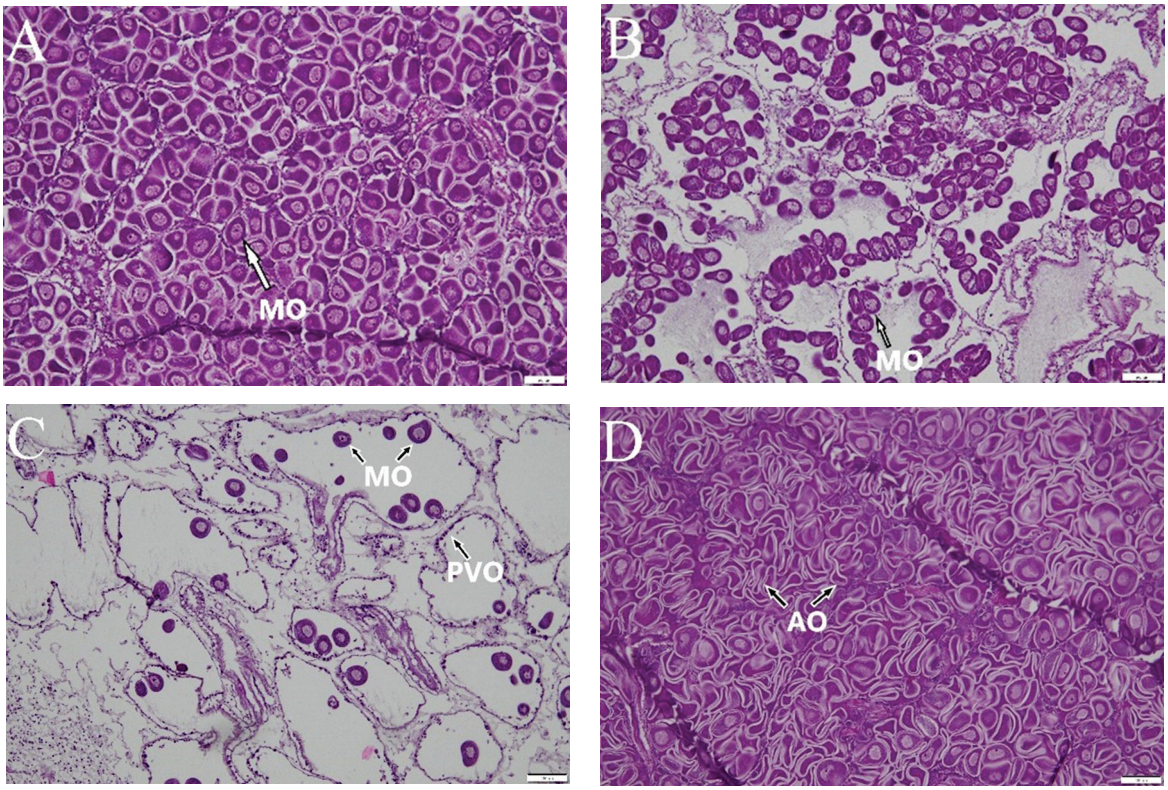

Figure 4 Examples of female *P. jacobaeus* gonads development stages: A) ripe, B) partially spawned, C) spent, D) reabsorption. PVO - previtellogenic oocyte, MO - mature oocyte, AO - atresic oocyte. Scale bar 100 µm.

Slika 4. Primjeri faza razvoja ženskih spolnih žlijezdi P. jacobaeus: A) zrele, B) djelomično u mrijestu, C) ispražnjene, D) reapsorpcija. PVO – previtelogena oocita, MO – zrela oocita, AO – atretična oocita. Mjerna traka 100 µm.

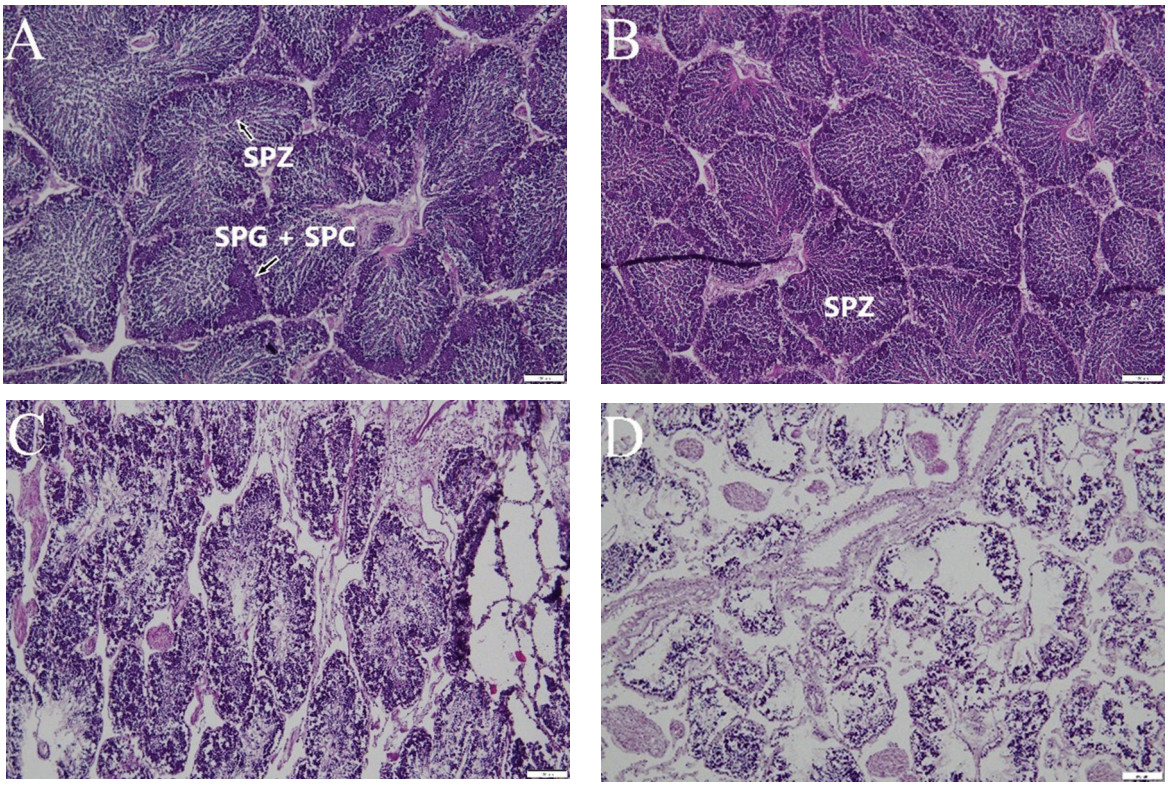

Figure 5 Examples of male *P. jacobaeus* gonads development stages: A) late development, B) ripe, C) partially spawned D) spent. SPG - spermatogonia, SPC - spermatocytes, SPZ – spermatozoa. Scale bar 100 µm.

Slika 5. Primjeri faza razvoja muških spolnih žlijezda P. jacobaeus: A) kasni razvoj, B) zrele, C) djelomično ispražnjene D) iskorištene. SPG – spermatogonija, SPC – spermatociti, SPZ – spermatozoidi. Mjerna traka 100 µm.

4. DISCUSSION / *Rasprava*

Gametogenic cycles may vary between different seasons, species and even within species at different locations [20], so the conditioning process can be rather long and often unreliable. While some scallop species can be brought into spawning throughout the year, for other species conditioning could not be achieved outside of spawning season [34, 35, 36] as for some species gametogenesis could not be initiated before post-spawning accumulation of reserves is completed [21]. Several scallop broodstock conditioning studies [e.g. 23, 27, 37, 38] have aimed at achieving the optimal algal composition for broodstock diet, but as best to our knowledge, there is no such information on *P. jacobaeus* broodstock. Studies of *P. maximus* have shown large differences in conditioning results, which have been attributed to the existence of separate local populations that respond differently to conditioning attempts [35, 36].

The first experiment results showed that broodstock conditioning fed with live algae *Isochrysis galbana* promotes gonads growth indicated by a rise in GSI and GI values, while CI of the scallops remains constant. A slight drop in MI might suggest that muscle energy reserves were also used to support gamete development. The experiment was carried out at a time when wild populations at collection sites in nature are spawning [14, 39], so gonad development could be due to the natural process of gametogenesis, as scallops already accumulated nutrient reserves.

In the second experiment, scallops from Group 1, fed with live algae *Isochrysis galbana*, showed a rise in CI, contrary to CI decline in Group 2, fed with a concentrated mix of frozen algae. This might suggest that live feed is better suited than frozen feed for promoting soft tissue growth, which is in accordance with results for sea scallops *Placopecten magellanicus* which shows that feeding scallops with a live diet under laboratory conditions promotes overall organ growth [34]. This can also be attributed to the fact that scallops preferentially feed on living algal cells, as was demonstrated for *P. maximus* [40]. As expected, since adult specimens were used and due to the short period of the experiment, there were no visible changes in the growth of the scallops.

The second experiment results suggest a decline in MI values in both Group 1 and Group 2, compared to Start. However, when we take into consideration that the formula for the calculation of MI considers total soft tissue mass, the results can be misleading. If we consider average dry muscle weight, then it is obvious that compared to the Start, there was a rise in muscle weight in Group 1 and a decrease in Group 2. Muscle moisture content decline in Group 1 and rise in Group 2 might suggest that when energy storage levels in the muscle decrease, the tissue moisture content rises, so lower MC Group 1 could indicate higher energy reserves in the muscle and vice versa [24]. This suggests that live feed was favourable for storing nutrient reserves in the muscle, but with frozen feed, the nutrient reserves from the muscle were used, which indicates that frozen feed alone was inadequate, and animals used their energy reserves for maintenance. For *P. maximus* it was suggested that partitioning of energy is divided between energy storage in adductor muscle and gonad development [41], so a decrease in muscle weight might also suggest that energy reserves were used for gonad development.

GSI results indicate better results are achieved in Group 2, fed with concentrated frozen feed than in Group 2, fed with live algae *I. galbana*. However, since the differences mentioned in GSI and GI formula calculations, these results are debatable. Because of this, GI results can be better suited for assessment of gonad development, as its formula does not consider other soft tissue parts but uses shell as a reference. GI results thus indicate opposite results, with a rise in values in Group 1, and a decline in Group 2. This is also supported when comparing the average dry gonad mass from the start, which shows a rather unchanged gonad mass in Group 1 and a decline in gonad mass in Group 2. Another factor that influenced GSI and GI results is spontaneous spawning that occurred to some extent in all tanks. The rise of the MC of gonads in Group 1 and Group 2 supports these findings as MC of gonads is lowest when gonads are ripe, and is on the rise after spawning [24, 42]. Histological examination of gonads confirmed that spontaneous spawning occurred in both feeding lines with predominant development gonad stage of partially spawned. As we detected that spontaneous spawning occurred only after the experiment was over, we cannot be sure if spawning resulted in the release of viable oocytes or in the release of aggregated eggs which do not result with successful spawning [36], so closer observation is necessary in future experiments. The water treshold temperature for initiation of spawning in *P. jacobaeus* is suggested at 15 °C [14], so lowering the temperature below this treshold should prevent occurrences of spontaneous spawning. For *Argopecten purpuratus* broodstock conditioning it was suggested that low temperatures, under adequate diet, are more appropriate for the normal development of gametes and for accumulation of energy reserves which are then used for gamete growth, while increasing temperature at the last stage of gamete development could accelerate gametogenesis [38].

Gonad histology also showed that specimens from the start of the experiment showed signs of the reabsorption process in female gonads. This could be explained because of food deprivation, as specimens were analyzed after the acclimatization process. After 25 days of feeding, signs of reabsorption were less obvious in both lines, which might suggest that when scallops were given food, the reabsorption process was deferred. Prespawning oocyte atresia can affect bivalve reproductive effort and fecundity [43] and can indicate that laboratory conditions were not adequate and thus could cause loss in the cases of hatchery production.

When GSI/GI results are considered with CI and MI results, it can be indicated that gonad development in Group 1 was at the expense of ingested food, while gonad development in Group 2, was at the expense of storage of nutrient reserves. The contribution of ingested food and energy reserves from muscle and other tissues is dependent on several factors, and no general pattern has been established [20, 41]. The utilization of energy reserves can vary with food levels, and if the food supply is adequate for both gonad development and accumulation of storage reserves, muscle reserves are not utilized [20].

For bivalve broodstock conditioning, a diet rich in lipids is of the utmost importance, as during gametogenesis, lipids are accumulated in gonads as the main reserve of oocytes [23, 24, 27, 38, 41, 42, 44]. Therefore, it is important to provide broodstock with a microalgae diet that is rich in lipids, especially in polyunsaturated fatty acids (PUFA), specifically eicosapentaenoic acid, 20:5(n-3) (EPA) and docosahexaenoic acid, 22:6(n-3) (DHA), as scallops, like other bivalves, cannot synthesise them [27]. For *P. maximus* broodstock conditioning, it

was suggested that better results were achieved with a diet with high levels of DHA or DHA/EPA ratio greater than 1, which was obtained with *I. galbana* diet [23, 44], which was then reflected in the broodstock gonads. While with an inadequate diet, the composition of fatty acids did not reflect those of diet as their constant levels in gonads were maintained on the part of energy reserves [23]. Our results suggest that the same could apply to *P. jacobaeus* broodstock conditioning, as better conditioning results were achieved with a diet containing live *I. galbana*, which has higher DHA than EPA [44], contrary to other used microalgae species formulated mix. Histology results could also indicate that a live diet with *I. galbana* was better suited for broodstock conditioning, because of more previtellogenic oocytes present in Group 1, which could suggest that the conditioning process was accelerated with live algae *I. galbana*, and rematuration of gonads after spontaneous spawning was already in progress. Some authors also suggested that the broodstock diet should be adjusted in accordance with the time when the broodstock was collected from nature and concluded that for the broodstock which is conditioned close to their natural spawning season in spring or summer, a diet rich in the *I. galbana* provides better larval yield, while a diet rich in diatoms gives better yields when the broodstock is conditioned outside of its natural spawning season [37]. Our results are in accordance with these findings, as for both experiments, *P. jacobaeus* broodstocks were collected at the time of spawning at the collection site, and better conditioning results were achieved with an exclusive *I. galbana* diet. Further research on *P. jacobaeus* broodstock conditioning should include conditioning of broodstock collected before spawning season in nature, to assess if conditioning is possible outside of spawning season for this species.

5. CONCLUSIONS / *Zaključak*

This study provides data on the *P. jacobaeus* broodstock conditioning in recirculation systems. Obtained results suggest that better conditioning results are achieved when broodstock is fed with a diet of live *I. galbana* microalgae, as gonad development was at the expense of ingested food, than with the mixture of concentrated frozen feed consisting of more microalgae species, where gonad development was at the expense of stored nutrient reserves. These results form the basis for further research aimed at inducing controlled spawning and production of larvae in recirculation systems, which will have implications for the possible introduction of this species into aquaculture.

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Conflict of interest: None

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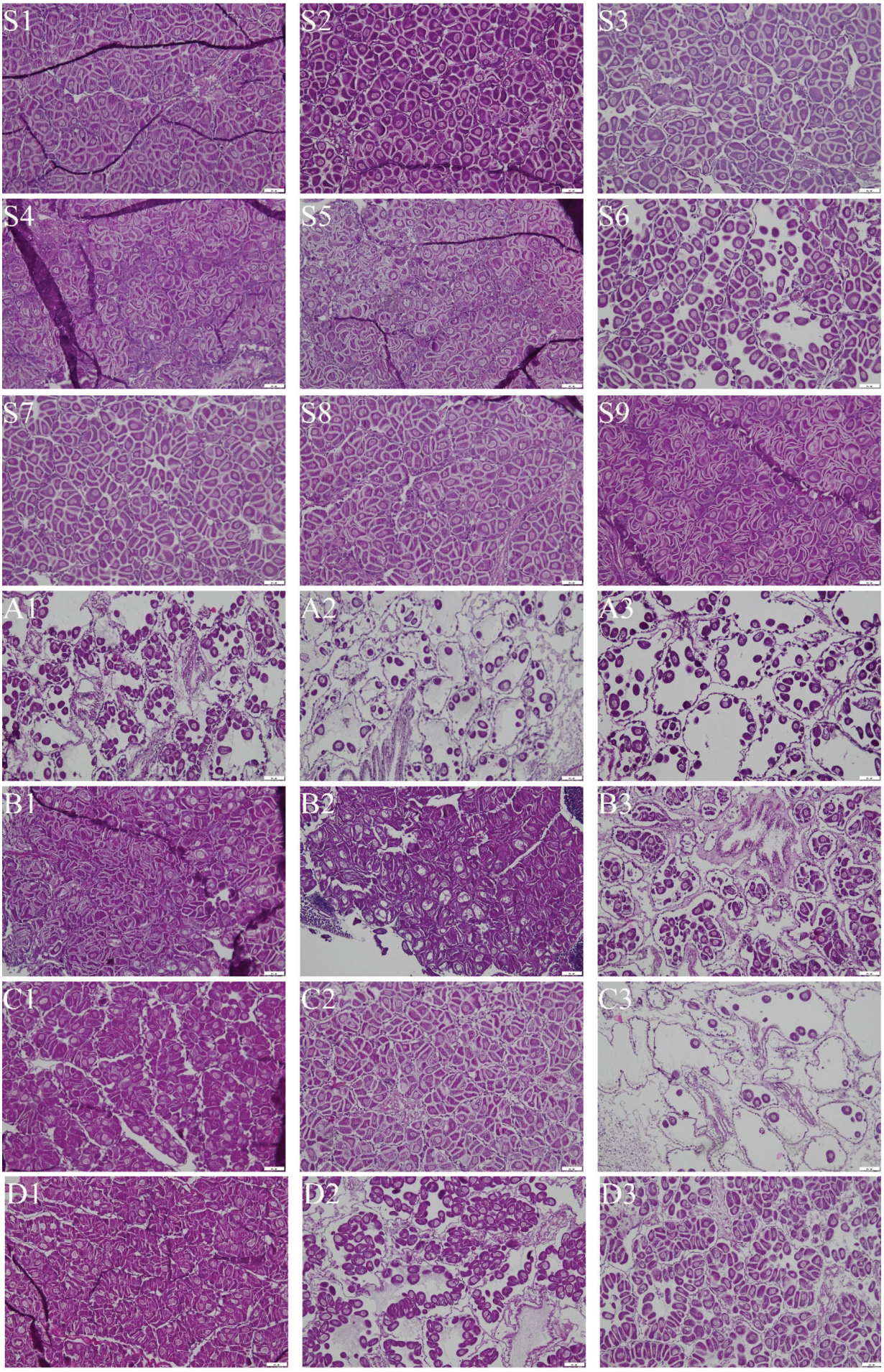
Supplementary Material / *Dodatni materijal*

Table 1 *P. jacobaeus* broodstock gonad development stages at start and after 25 days of conditioning with two types of feed: live algae *I. galbana* (Group 1) and concentrated frozen algae mix (Group 2)

Tablica 1. Faze razvoja gonada matičnog jata P. jacobaeus na početku i nakon 25 dana kondicioniranja s dvije vrste hrane: žive alge I. galbana (grupa 1) i koncentrirana mješavina smrznutih algi (grupa 2)

STAGE OF GONAD DEVELOPMENT								
Start	Female	Male	Line 1	Female	Male	Line 2	Female	Male
S1	ripe	ripe	A1	partially spawned	partially spawned	D1	ripe	ripe
S2	ripe	late development	A2	partially spawned	partially spawned	D2	partially spawned	partially spawned
S3	reabsorption	partially spawned	A3	partially spawned	ripe	D3	partially spawned	partially spawned
S4	reabsorption	ripe	B1	reabsorption	ripe	E1	ripe	ripe
S5	reabsorption	late development	B2	reabsorption	ripe	E2	partially spawned	partially spawned
S6	partially spawned	ripe	B3	partially spawned	partially spawned	E3	reabsorption	partially spawned
S7	ripe	partially spawned	C1	ripe	ripe	F1	ripe	partially spawned
S8	ripe	late development	C2	ripe	partially spawned	F2	partially spawned	partially spawned
S9	reabsorption	ripe	C3	spent	spent	F3	partially spawned	partially spawned


S1
S2
S3
S4
S5
S6
S7
S8
S9
A1
A2
A3
B1
B2
B3
C1
C2
C3
D1
D2
D3

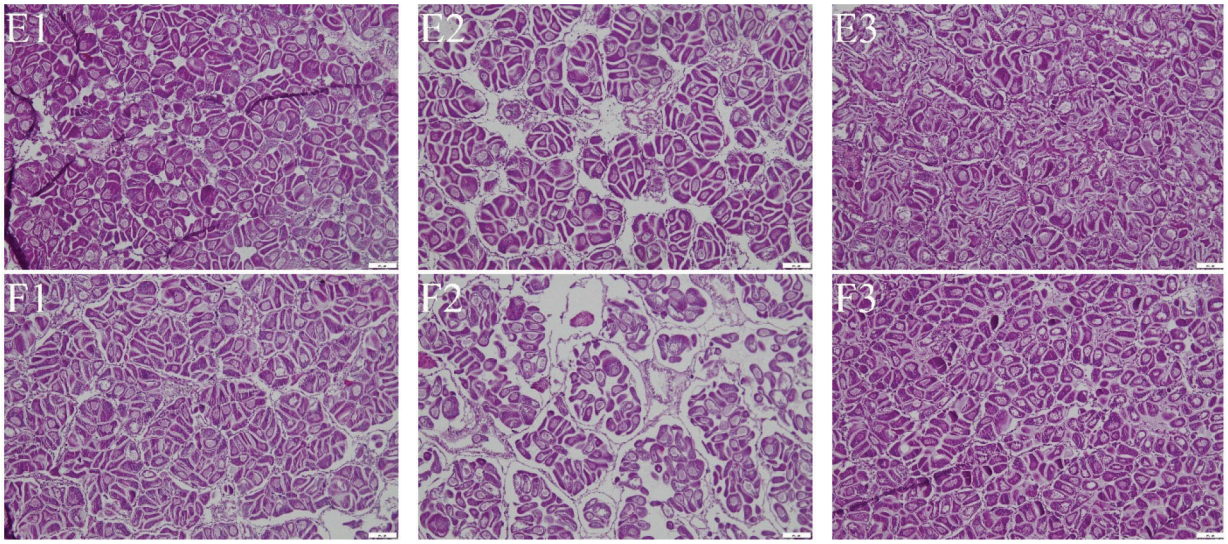
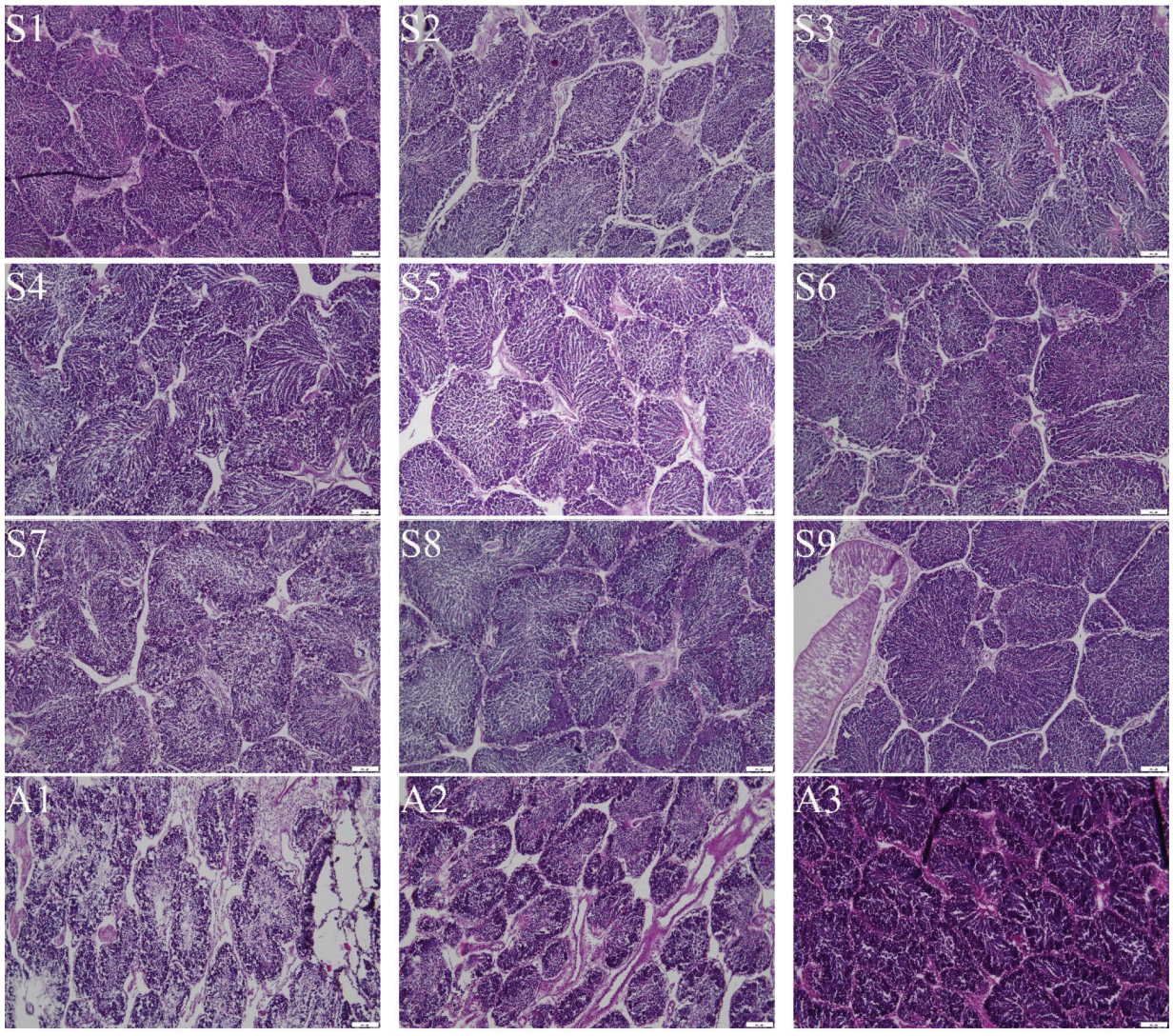


Figure 1 Photomicrographs of gonad development stages in female *Pecten jacobaeus* during broodstock conditioning: at start (S1-9), and after 25 days of conditioning with live *Isochrysis galbana* (A1-C3) and with formulated frozen mix of several algae species (D1-F3)

Slika 1. Fotomikrografije faza razvoja ženskih gonada Pecten jacobaeus tijekom kondicioniranja matičnog jata: na početku (S1-9) i nakon 25 dana kondicioniranja sa živom Isochrysis galbana (A1-C3) i s formuliranom smrznutom mješavinom nekoliko vrsta algi (D1-F3)



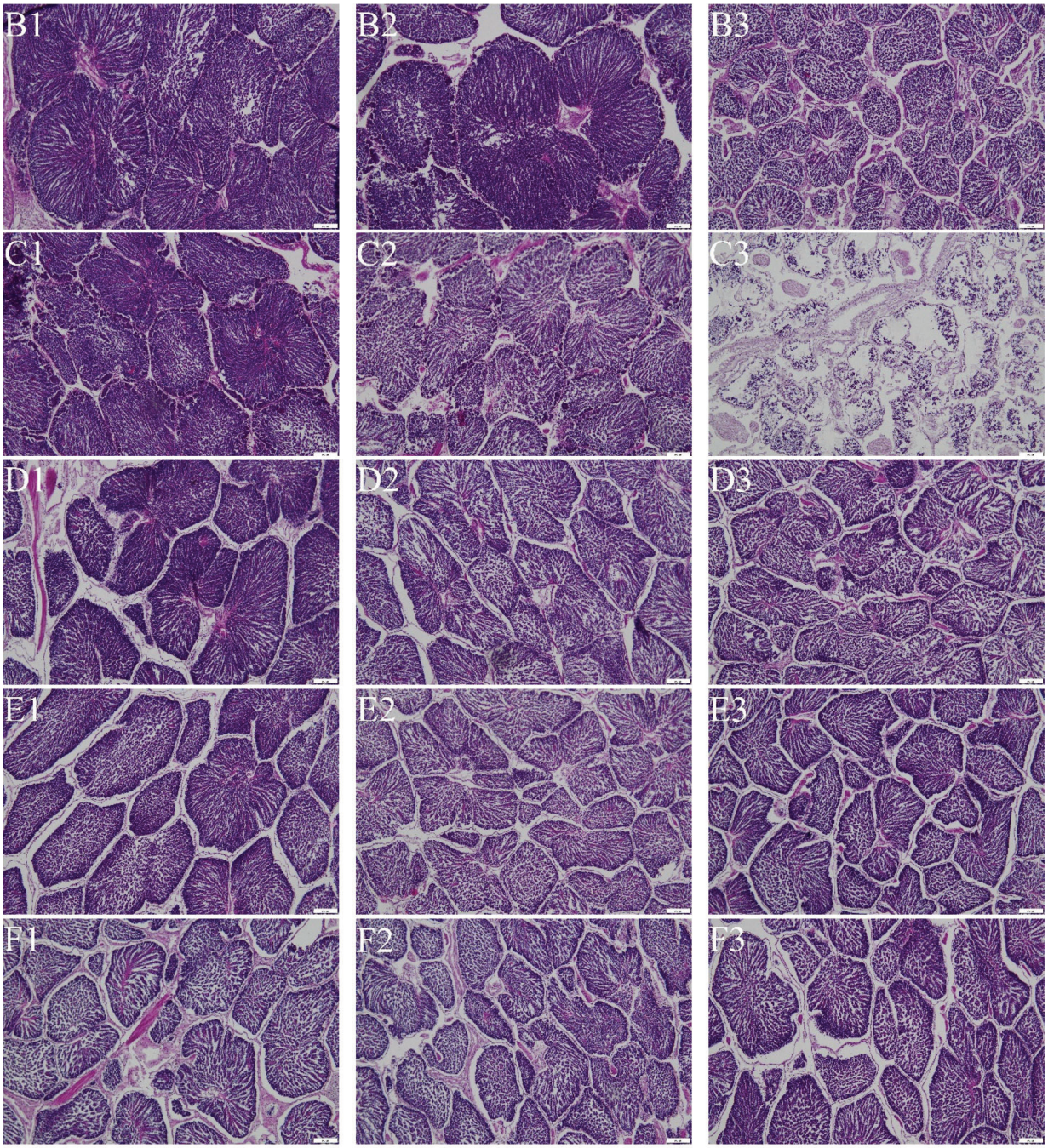
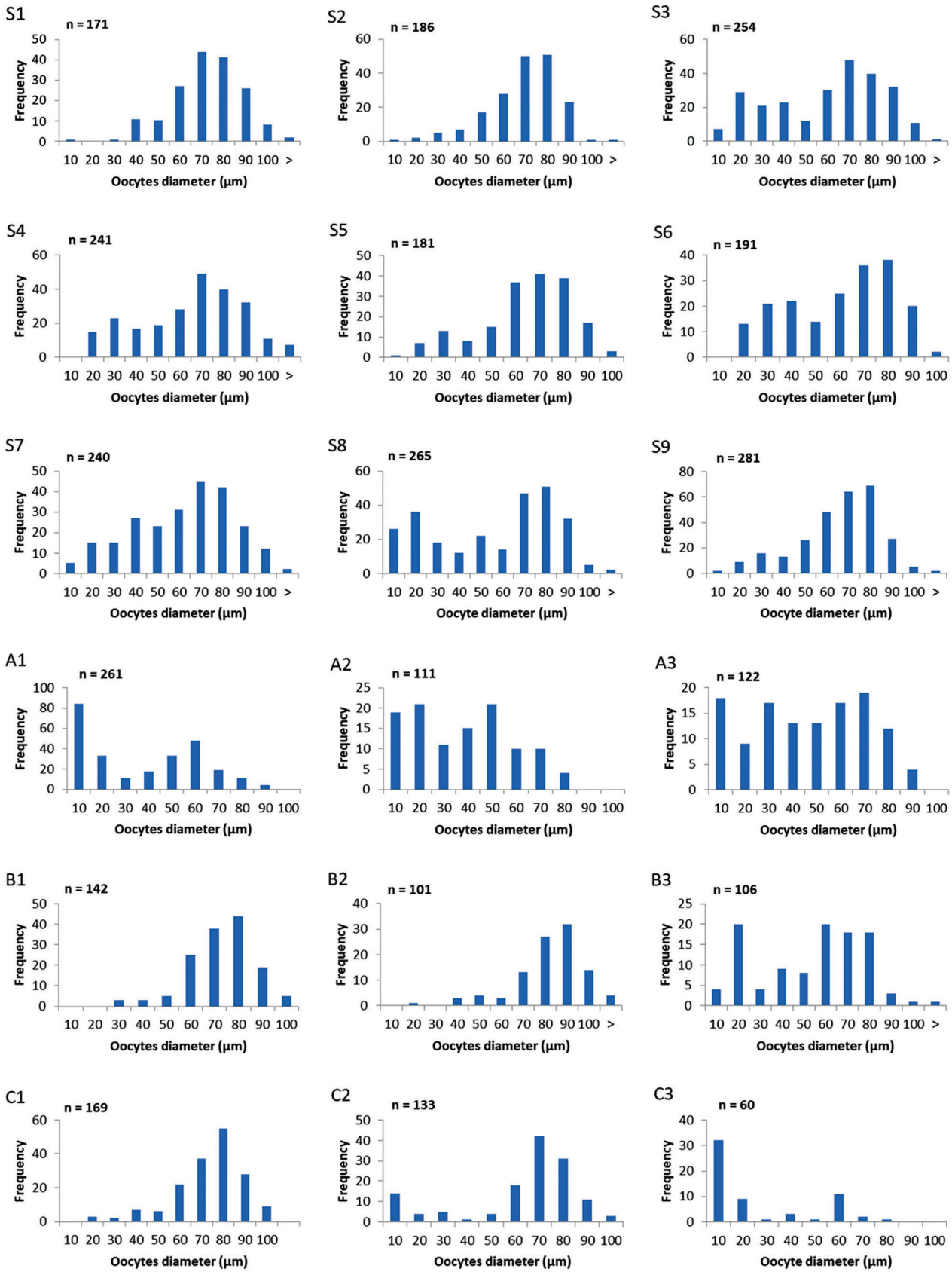


Figure 2 Photomicrographs of gonad development stages in male *Pecten jacobaeus* during broodstock conditioning: at start (S1-9), at start (S1-9), and after 25 days of conditioning with live *Isochrysis galbana* (A1-C3) and with formulated frozen mix of several algae species (D1-F3)

Slika 2. Fotomikrografije faza razvoja muških gonada Pecten jacobaeus tijekom kondicioniranja matičnog jata: na početku (S1-9) i nakon 25 dana kondicioniranja sa živom Isochrysis galbana (A1-C3) i s formuliranom smrznutom mješavinom nekoliko vrsta algi (D1-F3)


S1
n = 171
Frequency
Oocytes diameter (µm)
S2
n = 186
Frequency
Oocytes diameter (µm)
S3
n = 254
Frequency
Oocytes diameter (µm)
S4
n = 241
Frequency
Oocytes diameter (µm)
S5
n = 181
Frequency
Oocytes diameter (µm)
S6
n = 191
Frequency
Oocytes diameter (µm)
S7
n = 240
Frequency
Oocytes diameter (µm)
S8
n = 265
Frequency
Oocyte diameter (µm)
S9
n = 281
Frequency
Oocyte diameter (µm)
A1
n = 261
Frequency
Oocytes diameter (µm)
A2
n = 111
Frequency
Oocytes diameter (µm)
A3
n = 122
Frequency
Oocytes diameter (µm)
B1
n = 142
Frequency
Oocytes diameter (µm)
B2
n = 101
Frequency
Oocytes diameter (µm)
B3
n = 106
Frequency
Oocytes diameter (µm)
C1
n = 169
Frequency
Oocytes diameter (µm)
C2
n = 133
Frequency
Oocytes diameter (µm)
C3
n = 60
Frequency
Oocyte diameter (µm)

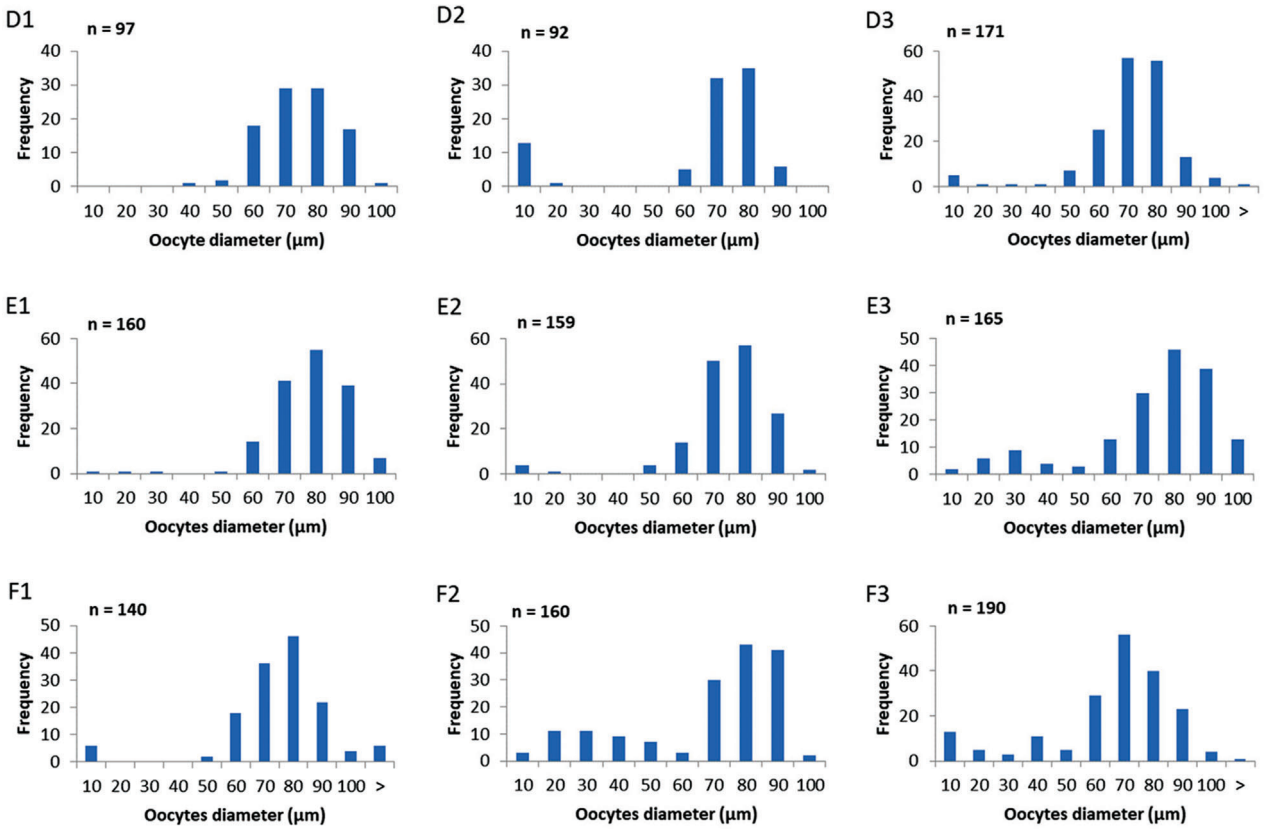


Figure 3. *Pecten jacobaeus* oocyte size distribution during conditioning: at start (S1-S9), at start (S1-9), and after 25 days of conditioning with live *Isochrysis galbana* (A1-C3) and with formulated frozen mix of several algae species (D1-F3)

Slika 3. Distribucija veličine oocita Pecten jacobaeus-a tijekom kondicioniranja: na početku (S1-9) i nakon 25 dana kondicioniranja sa živom Isochrysis galbana (A1-C3) i s formuliranom smrznutom mješavinom nekoliko vrsta algi (D1-F3)