Raking: an innovative and sustainable echinoculture method to produce “caviar” without sacrificing sea urchins

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Article

Keywords:

Posted Date: June 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2963167/v1

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Abstract

Sea urchin aquaculture represents a promising tool to achieve sustainable aquaculture, promote sector diversification and obtain high-priced products using low-trophic species. However, although echinoculture has been practised for several decades, this sector has not yet achieved economic sustainability and large-scale development mainly due to problems linked to long-term sea urchin rearing cycles. In this paper we present a groundbreaking production method, called "Raking", for sea urchin caviar production that represents a technological advancement both in terms of production approach and in the final product. Raking, in fact, is a no-kill method for the harvesting of eggs as a final product (sea urchin caviar) from only-female batches of sea urchins, meaning that the same sea urchins are employed through several production cycles. This method, therefore, helps overcome important biological and economic constraints of echinoculture, such as high mortality in the early development stages and the slow speed of growth to reach viable market size. This new production method was compared with a traditional gonad enhancement method known as Bulking. Our results showed that multi-cycle production using the Raking method proved more profitable and sustainable than the Bulking method, and in fact allowed us to obtain a cyclic ovulation with a total regenerative capacity of the ovary of about 3/4 months, employing the same sea urchin batch in each productive cycle. In addition, the sea urchin caviar harvested in this way was appreciated by assessors in terms of its sensory qualities and was actually preferred to the traditional gonad products, when assessors were informed of the sustainability and ethical criteria of the Raking production method.

1. Introduction

The Blue Economy refers to ocean-based activities which adopt a sustainable approach in order to encourage economic development without affecting ocean health\(^1\). Aquaculture is widely recognized as the most promising food sector for the promotion of blue growth, satisfying global protein demand (Garlock et al., 2020). However, to achieve the blue economy goals, a reconfiguration of animal protein production in aquaculture and the shift toward low-trophic species production, characterized by high nutritional profiles and poor diet requirements, is of overriding importance.\(^2\)–\(^4\)

Sea urchin aquaculture falls fully within the framework of the blue economy\(^5\). In fact, commercial sea urchin species are low trophic organisms (generalist herbivores) that do not require highly enriched feed with animal supplements to grow\(^6\),\(^7\).

Moreover, sea urchins have a high economic value, since their gonads are considered a culinary delicacy and are one of the highest-priced seafoods worldwide, with the demand often exceeding the fishery supply\(^8\) (McBride, 2005). This high market demand has led to the over-exploitation of local wild stocks, causing the rapid depletion of many natural populations\(^9\). In this context, aquaculture is one of the most promising tools to achieve a sustainable supply alternative to wild exploitation\(^10\),\(^11\). Although echinoculture has been practised for several decades, it still has not achieved economic sustainability\(^8\).
In recent years, many companies worldwide have tried to meet the strong demand for sea urchins by experimenting with various farming techniques. However, despite the strong research effort, economic sustainability is still to be attained on a productive scale. Two types of echinoculture have been set up: the full cycle aquaculture where the whole rearing is completed in captivity (Fig. 1), and the gonad enhancement aquaculture (known also as the Bulking method), where the gonads of adults collected in the wild are enhanced using an intensive feeding method before the extraction (Fig. 1). Clearly, bulking aquaculture remains directly reliant on harvesting and wild stock exploitation.

Meanwhile, since sea urchins are generally characterized by slow somatic growth, full life-cycle aquaculture is hindered by the lengthy rearing period to reach viable market size (around 3/4 years), which represents the main challenge of echinoculture to achieve economical profitability. Therefore, overcoming these constraints through new productive solutions and cutting-edge technologies remains the major priority in this sector.

In this paper, we present a groundbreaking method, called "Raking", for sea urchin caviar production. The design of the Raking method represents a technological advancement both in the production approach and in the final product itself. Unlike traditional methods, which envisage the entire gonad as the final consumer product, thus imposing the sacrifice of adult sea urchins at the end of the breeding cycle, the Raking method provides eggs as a final product in the form of "sea urchin caviar", without requiring the sacrifice of the sea urchins, which can thus go through several production cycles. Caviar production through Raking is obtained by breeding female-only batches, which are induced to spawn with cyclical cadence through a combination of wet-dry and thermal stimulation. This echinoculture approach provides a solution to both the traditional constraints of the lengthy breeding time to reach market size and the exploitation of wild stocks. Importantly, this is a no-kill production method, for which the same sea urchin stock is employed in multiple spawnings. In the research presented here, the Raking method is trialed on the purple sea urchin Paracentrotus lividus, in order to assess both the feasibility of caviar production and the sustainability and quality of the end-product in line with the aims of the blue economy. Finally, a sensory quality analysis and a consumer appreciation were conducted to compare this new end-product with that coming from the traditional method, which instead were produced by rearing sea urchin batches through the Bulking method in parallel to the female-only batches reared through the Raking method.

2. Materials and Methods

2.1. Raking Method General Description

Fully mature females are selected after the induction of spawning through a combined dry and thermal stimulation (§ 2.2) in order to establish a female-only rearing batch to be employed in Raking rearing cycles. The batches are then reared in Recirculating Aquaculture Systems inside perforated floating baskets, with ad libitum feeding regime, facilitating at the same time the cleaning procedures during the rearing period. Finally, at the end of the rearing period, the Raking method is employed to obtain egg...
production through spawning induction: each perforated floating basket with the reared sea urchins is
removed from the RAS, washed with a sea water jet and left to dry for three hours. Subsequently,
spawning is induced in a cone bottom tank with warmed sea water for thermal stimulation. After a few
hours, the females spawn, producing a high quantity of eggs, which then drop from the perforated
floating baskets and accumulate at the bottom of the tank. The egg solution is collected through a drain
valve and centrifuged to remove the seawater, compacting the eggs into a thick paste which represents
the sea urchin caviar or the final product of this method. Finally, the caviar produced is pasteurized and
can be canned or directly consumed (Fig. 2)

2.2. Experimental Set Up

Adult specimens of Paracentrotus lividus (N, 600) were collected in the wild at 1–8 m depth in the central
Tyrrenian Sea, Italy (Santa Marinella: 42°03′00″Nord, 11°49′09″Est). The specimens were then
transported to the Laboratory of Experimental Ecology and Aquaculture (LESA, University of Rome “Tor
Vergata”) inside tanks filled with seawater and equipped with aerators. Once in the lab, the sea urchins
were transferred and maintained inside perforated floating baskets (at a density of 20 specimens per
basket) in 600 L closed circulation aquaria equipped with biological and mechanical filtration and water
temperature conditioner (20°C). The sea urchins were starved for a week in order to void their digestive
tract before inducing spawning. Spawning was then induced by combining wet-dry and thermal
stimulation. In this stage, the sea urchins were kept out of the water for three hours, with their tops
covered to prevent desiccation. The specimens were then placed in induction buckets filled with sea water
and maintained at 24 ° C, in order to stimulate gamete release.

2.3. Raking Rearing Experiment

At the end of the spawning induction, mature females, once completely voided their gonads, were
selected and sorted in order to establish a female-only batch to be employed in the Raking rearing
method. From this pool (TWWg: 58.4 ± 6.06 g, TD: 48.7 ± 0.63 mm; mean ± SD), 30 specimens were
randomly selected to assess the gonadal state at the start of the experiment (T0). The female batch (90
specimens) were randomly divided into 15 tanks in Recirculating Aquaculture System. Each sea urchin
was placed in perforated floating baskets (6 sea urchins per tank) and reared at 20 ± 0.5°C with 8:16
photoperiod. During the three-month rearing period, algae (Ulva and Laminaria;1:1 in dry weight) were
administrated daily ad libitum to the female batch with the addition of 10% krill meal as animal
supplement. The leftover food and feces of the sea urchins were removed on daily basis, and new food
was supplied in each tank after the cleaning procedures. After three months of rearing ad libitum, the sea
urchins were starved for three days in order to void the digestive tract. Finally, the Raking method was
performed on female batches in order to induce spawning.

Each perforated floating basket with reared sea urchins was removed from the RAS, washed with a water
jet and left out of the water (in a conditioned room 19°C) for three hours, with a cover over it to prevent
the desiccation of the sea urchins. Subsequently, spawning was induced by thermal stimulation by
placing the baskets after dry stimulation in a cone bottom tank filled with sea water at 24° C. After a few
hours, the females spawned a large quantity of eggs, which dropped from the perforated floating baskets and accumulated at the bottom of the tank. These eggs were then collected through a drain valve and filtered (with a 150 µm mesh sieve) to remove eventual coarse particles or aculea. Subsequently, the egg solution was centrifuged at 2500 rpm for 10 minutes to remove the seawater, compacting the eggs into a thick paste which represents the sea urchin caviar or the final product of the Raking method. The caviar produced was pasteurized at 70°C in order to be canned or directly consumed.

For each female basket, the volume and weight of eggs produced was assessed after centrifugation, and the Ovosomatic index (OI %) was calculated as follows:

\[
\text{OI} = \frac{\text{EWWg}}{\text{TWWg}} \times 100
\]

Where EWWg is the wet weight (g) of the eggs and TWWg is the total wet weight (g) of the sea urchins.

After the first (three-month) Raking production, the sea urchins were moved back into the experimental RAS for another month of rearing. Raking production was performed again to collect the remaining eggs from the female batches. The full productive cycle was therefore completed in four months. This full Raking cycle was performed in three cycles in one year, allowing us to assess a year-round production (4 + 4 + 4 months of Raking cycles).

### 2.4. Bulking Rearing Experiment

At the end of the spawning of the second sea urchin pool, 90 sea urchins of both sexes (TWWg: 47.0 ± 8.3 g, TD: 47.7 ± 2.7 mm; mean ± SD) in a spent stage/post spawning were randomly assigned to 15 tanks in a RAS system to undergo gonad enhancement rearing using the Bulking method. These sea urchins were reared in parallel, under the same conditions and at the same feeding regime as the female-only batches for caviar production (using the Raking method). After three months of rearing, the gonads of this mixed pool of male and female sea urchins were extracted, weighed, and the gonad index (%) was calculated.

### 2.5. Sensory Qualities of Final Products

The sensory quality of the sea urchin caviar produced by the Raking method was compared with sea urchin gonads produced by the traditional Bulking method. In particular gonads produced by the Bulking cycle were divided into a growing stage and mature stage pools. After this, each pool was half homogenized and the other half was maintained whole. In this way four end-products (gonads in two development stages and in two different forms) were compared to the sea urchin caviar in terms of sensory quality by an external panel of 40 assessors. The final products evaluated by the panel were: Sea urchin Caviar obtained by the Raking method (RC); Immature stage Gonads (IWG); Mature stage Gonads (MWG); Immature Homogenized Gonads (IHG); and Mature Homogenized Gonads (MHG) obtained by the traditional Bulking method (Table 1).

The participants were regular consumers of sea urchins, men and women of a wide age group (21 to 76 years) and from very different social and cultural backgrounds. Each assessor rated around 1 g of each of the five final products, classifying them on the basis of general liking, color and taste, on a 5-point
scale ranging from 1-“extremely unpleasant” to 5-“extremely liked” (Table 1) and were then asked to complete a consumer survey in blind. After this first evaluation, the panelists were fully informed about the production processes of each final product and were then asked to complete a second consumer survey questionnaire to evaluate the general appreciation for the Caviar and how respecting animal welfare and an environmental-friendly procedure such as Raking could influence them in a final purchasing decision. Hence, we assessed the consumers’ perception of these products on the basis of their compliance with sustainability criteria (product life-cycle phases and socio-ecological sustainability principles\(^30\)) and why consumers should or should not appreciate certain characteristics.

### 4.5. Data Analysis

Results were expressed as mean values ± standard deviation (SD). Prior to analysis, raw data were diagnosed for normality of distribution and homogeneity of variance by means of a Levene test and a Kolmogorov-Smirnov test respectively (Whitlock and Schluter, 2010). Samples were close to normal distribution (Kolmogorov-Smirnov p > 0.05) with similar variances (Levene’s p > 0.05). One-way analysis of variance ANOVA was carried out to analyze the differences in quantitative production between the Raking and Bulking methods and the sensory qualities between the five products (Sea urchin Caviar (C); Immature stage Gonads (IWG); Mature stage Gonads (MWG); Immature Homogenized Gonads (IHG); and Mature Homogenized Gonads (MHG). Where p values were generated in ANOVA, Tukey multiple comparison tests were used to evaluate differences among pair-wise means (p < 0.05).

### 3. Results

#### 3.1. Production Performance

The Raking method was performed in this experiment in three cycles, allowing us to obtain three Raking cycles in a year-round production. Each rearing cycle lasted three months, after which the sea urchins were induced to release the eggs before being returned to the experimental RAS for another month (for a total of 3 + 1 months). Subsequently, Raking production was performed again to collect the remaining eggs from the female batches. Hence, the full productive cycle was completed in four months.

The Raking method didn’t affect the survivorship of reared sea urchin female batches and no mortality occurred during the year-long experiment. In fact, although three sea urchins died in the early first cycle (during the first two weeks) and were promptly replaced, no further mortality was recorded over the course of the following production cycles.

After the first spawning induction carried out on wild-caught sea urchins, males and females were divided and the gonad were observed to have undergone a spent stage in both pools. Subsequently, at the beginning of the experimental rearing, both male and female (1:1) batches underwent the Bulking cycle while female-only batches were used to perform the Raking cycles. At the end of both the rearing cycles, the production results, in terms of wet gonads and spawned eggs, were obtained respectively through the Bulking and Raking method (Fig. 4). The amount of gonads obtained from the Bulking method (524.4 g)
was higher than the caviar produced with a single Raking cycle, (359.4 g, 384.4 g, 412.0 g respectively in the first, second and third cycle). However, focusing on the whole year-round production, the total amount of caviar (1313.1 g) obtained was more than double compared to gonad production, despite the same number of sea urchins being employed in the two methods. This result highlighted the benefits of cyclical production using the Raking harvesting method compared to the traditional method. The advantage is, in fact, even greater considering that caviar production can be performed with the same sea urchins for multiple years in several production-cycles.

Concerning the Gonado-somatic and Ovo-somatic indexes, the values obtained are reported in Fig. 4 and followed the same trend described above for the absolute production of gonads and caviar. The Gonado-somatic index of the sea urchins employed in the Bulking method was 15.6 ± 0.4% and resulted significantly higher than the GI baseline of 2.3 ± 0.9%. On the other hand, the Ovo-somatic index of females employed in the Raking method was similar among the three production cycles (10.2 ± 0.5%, 11.0 ± 0.6% and 11.8 ± 0.6%, respectively in the first, second and third cycle).

### 3.2. Product Assessment

The sensory assessment of the final products from both Raking and Bulking was performed by a panel of assessors, who compared both color and taste. The mean score value of the Raking Caviar color (RC) was the highest (3.7 ± 0.1), followed by Immature Whole Gonads (IWG) color (3.50 ± 0.2). Although no significant differences emerged between them, these values were both significantly higher than those obtained from Mature Whole Gonads (MWG), Immature Homogenized Gonads (IHG) and Mature Homogenized Gonads (MHG) (respectively of 2.5 ± 0.2, 1.5 ± 0.2, 1.3 ± 0.1). These results therefore showed a higher appreciation in terms of color for Caviar and Immature Whole Gonads than the other final products.

The sensory evaluation also highlighted significant differences among final products in terms of taste. IWG recorded the highest average value (3.3 ± 0.1), followed by IHG (3.1 ± 0.2). These results were not significantly different, but both were higher than values achieved in RC, MHG and MWG products (respectively 2.2 ± 0.1; 1.8 ± 0.2 and 1.6 ± 0.2) (Table 1).

Following the sensory evaluation, the panelists were then fully informed about the production processes of each final product and were given a second questionnaire to evaluate the general appreciation and purchasing decision of final products, considering also animal welfare and the environmental-friendly standards of production. Hence, the consumers’ perception of these products on the basis of their compliance with sustainability criteria were assessed (product life-cycle phases and socio-ecological sustainability principles). From the assessors’ questionnaire, the average score obtained from Caviar was 4.3 after the full information on the production process had been provided. It emerged that 77.5% of assessors preferred sea urchin caviar compared to the other final products, relying on the sustainability and ethical criteria. Out of the remaining assessors, 15% and 2.5% preferred respectively the Whole Gonads and the homogenized gonads, and 5% did not express preferences (Table 1).
Table 1


<table>
<thead>
<tr>
<th>Sensory qualities</th>
<th>Final Products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IWG</td>
</tr>
<tr>
<td>Color score blinded</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>Taste score blinded</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>General product score blinded</td>
<td>4.3±0.1</td>
</tr>
<tr>
<td>Product choice in blind</td>
<td>55%</td>
</tr>
<tr>
<td>General product score after method information</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>Product choice after method information</td>
<td>15%</td>
</tr>
</tbody>
</table>

4. Discussion

4.1. Raking Method and Caviar Production

This study presents a new productive approach in echinoculture, that we named the “Raking method”. This method allows the production of sea urchin caviar, an innovative end-product which is obtained through short, consecutive rearing cycles and, importantly, without the need to sacrifice sea urchins. The caviar pilot-scale production sustainability was compared with sea urchin end-products obtained with a traditional gonad enhancement method, known as Bulking.

During three Raking cycles (year-round rearing), a high production of sea urchin caviar, through a cyclic ovulation was achieved with the same number of sea urchins, showing a regenerative capacity of the ovaries of about 3 months. The Raking productiveness, in fact, highlighted a continuous vitellogenesis and oocyte maturation of reared female gonads which were in an “active growth stage”. The permanent potential reproductive ability, which allows for the continuous replacement of promptly released eggs from sea urchins, has already been described in well-fed sea urchins. From our results it emerged that when this sea urchin ability is combined with a not-invasive spawning induction method such as Raking, it enables sea urchins to be used for permanent egg production in controlled environments, independently of seasonal variations in gonadic development cycles in the field. The Raking method makes this possible, keeping sea urchins in productive systems for long period of time. In fact, the caviar produced does not require the sacrifice of the specimens, conversely to the traditional methodology which envisages the entire gonad as the final consumer product. The reproductive effort observed in *P. lividus*...
females during the present study, leads to the assumption that at least three annual caviar collections can be performed in this species. This result, considering that the eggs can represent 80% of the ovary volume in full-mature stage, suggested that this method allows for a multiplication of productivity and hence also economic profits compared to the traditional method of extracting the gonads.

4.2. Sensory Qualities

In the production of new products, sensory evaluations are important to measure and interpret how consumers will respond. In particular, sensory properties are crucial for seafood acceptance and choice of consumers. In this study, the results given by the sensory panelists, who were employed to perform the comparison between the sea urchin end-products, suggest that the quality of the "sea urchin caviar" as a final product was similar to mature gonads, though less intensely flavored than immature gonads. This result revealed that sea urchin caviar is a valid alternative to the "roe" for human consumption. Amongst the various visual properties comprised in appearance, color was shown to be the first quality attribute and key purchase determinant, impacting the consumers’ visual assessment and freshness perception of the products. More specifically, the color of the caviar was more appreciated by assessors than that of the Immature Whole Gonads (IWG), which are commonly indicated as the preferred products of consumers. On the contrary, the color of Mature Whole Gonads (MWG) and Homogenized Whole Gonads (MHG and IHG) was less appreciated.

Clearly, taste is another key aspect employed in determining the market suitability of a commercial product. This is crucial for sea urchin roe, since they are considered a delicacy with a very characteristic taste. In general, gonads containing large number of somatic cells (NPs) are preferred to fully mature gonads. However, a clear distinction emerges between the sexes. Once gametogenesis begins, the sensory qualities of sea urchin eggs start to differ between males and females and the preferred attributes, such as the sweetness, the fresh marine taste, as well as the attractive appearance and bright orange color are largely associated with female sea urchins. This is mainly related to the different levels of proteins and amino acids in the gonads, as these nutrients are the main determinants of taste for all edible sea urchin species. The ovaries are significantly higher in soluble protein concentration than the testis. This is a result of different metabolic pathways followed by the Major York Protein (MYP), which is the main nutrient resource accumulated in Nutritive phagocytes (NPs) of immature gonads to synthesize ova and sperm. In males, the majority of MYP is consumed by the end of spermatogenesis. In females, on the other hand, a significant part of the stored MYP is transported in the oocytes and forms yolk granules. As a result, the testes are filled with sperm without MYP, while the mature ovaries contain eggs with high amount of MYP. In line with this, in our study, the IWG and IHG were more appreciated than the other final products, including the caviar. However, the appreciation score of the sea urchin caviar taste was in any case higher than that of mature gonads (whole and homogenized), which are still commonly commercialized sea urchin products.

Finally, although the color and taste of sea urchin caviar were well-appreciated by assessors, the main benefit of this end-product is above all related to the high stability of its sensory qualities since it is...
completely independent from gender and gonadal maturation stage. The caviar is in fact obtained in a highly controlled environment and the final centrifugation process removes the remaining seawater, obtaining a stable end-product. Conversely, roe color and taste are strongly affected by many factors, such as intestine contamination during dissection, developmental stage, gender, age, etc\(^{17,43,44}\).

In addition to sensory qualities, consumer preferences are driven by several key factors, such as economic convenience, sustainability and ethical principles, health and innovation\(^9\). On this basis, new products are welcome when responding to changing lifestyles and consumer preferences since they are able to make the sector more cost-effective and valuable\(^8\). Sustainability and ethical principles, now more than ever, have become a guideline for industrial development\(^{45}\). The importance of this factor emerged clearly from our results, since the socio-economic sustainability of Raking was considered as a key factor in the final purchasing decision of the assessors. In fact, although the caviar sensory quality was appreciated slightly less than that of the Immature Whole Gonads when compared in the blind test (32.5 \% vs 55 \%), the appreciation score increased significantly (77.5 \%) when assessors were informed about the sustainable principles underlying the Raking production method.

### 4.3. General Considerations and Future Prospectives

Sea urchins are now considered as a novel, trendy and healthy product, and interest in their production has been increasing worldwide\(^9\). However, the labor cost and the problems linked to long term rearing could hinder the future development of sea urchin aquaculture. In this context, a no-kill caviar production such as the Raking method could provide a groundbreaking new method, allowing producers to overcome the main bottlenecks of the current echinoculture system. In fact, not needing to sacrifice specimens for gonad extraction means that the production is no longer hindered by having to replace the full rearing batch each time, making the production cycle much more profitable. Furthermore, adopting this approach, it is possible to take full advantage of the high fecundity and massive number of eggs easily spawned by sea urchins, a peculiar characteristic of these organisms for which they are indicated as a gamete production powerhouse\(^{46}\). Therefore, this no-kill caviar production method overcomes the need to complete the full-cycle rearing, which is economically disadvantageous due to the very slow somatic growth of sea urchins (3/4 years in captivity to reach viable market size) and high mortality rates during the early stages. In addition, when compared with the Bulking method, Raking enables producers to overcome both sensory quality variability, and reliance on gender and gonadal maturation, as well as addressing concerns about sustainability due to the strong dependence on wild sea urchin stocks to fully renew the rearing batches (every 3/4 months). This latter aspect, indeed, strongly hinders the economic effectiveness of echinoculture. In this context, Raking, since it is fishery independent, is a promising method to meet the twin challenges of food security and environmental sustainability which are two pillars of the Blue Transformation promoted by FAO. In fact, this method allows the production of a healthy luxury seafood from a low-trophic species, whose low ecological impact aquaculture is desirable to prompt culture diversification, especially in industrialized countries where the majority of production is based on high-trophic level species. Another important advantage of sea urchin culture is the reduction of water pollution from nitrogenous compounds, since the amount of animal protein supply required by this
form of aquaculture is very low\textsuperscript{7}. On this point, future research into new sustainable, well-performing and cost-effective diets remains crucial\textsuperscript{28,47}. Therefore, further investigations are needed to produce an excellent feed, in order to prompt the quantity and quality of the sea urchin caviar produced by the Raking method. Adopting the Raking method, where factors such as gonadal maturation and gender diversity are overcome, the direct effects of different diets on caviar production could be more easily evaluated compared to traditional gonad production.

Finally, taking into consideration sea urchin ecology and their compatibility in co-culture with other groups (i.e. sea cucumbers), the development of echinoculture can encourage the establishment/setting-up of an effective community-like IMTA aquaculture \textsuperscript{11}. Moreover, by adopting a more sustainable and profitable rearing method, such as Raking, it will be possible to make use of many land-based marine plants that have been abandoned since the relocation of some production (i.e. sea bass and sea bream) in sea-based floating cages. This could prompt progress in terms of economic income, new jobs, food security, feed reduction and wild stock conservation, which are desirable and necessary for the achievement of the Blue Growth challenges related to the food sustainability \textsuperscript{2,48}.

In conclusion, although Raking clearly presents groundbreaking advantages when compared with traditional production methods, further research is needed to investigate caviar production through long-time and multiple cycles. The number of reproductive cycles is fairly high in sea urchins, but the precise number remains unexplored. In fact, there would be biological constraints that could force a renewal of the female-only batches from which the “sea urchin caviar” is obtained after several spawning cycles. This aspect should be investigated in the future to gain a more comprehensive picture of the potential of this method.

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Figures
Comparison of sea urchin production methods. **A) Full-life cycle aquaculture:** a.1) artificial reproduction. a.2) larval rearing until metamorphosis. a.3) grow-out phase, juvenile rearing in wavy plates in a raceway system. a.4) sub-adult rearing. a.5) sea urchin gonad extraction. a.6) post-production processes to achieve the final product. **B) Bulking method:** b.1) wild sea urchin fishing. b.2) gonad enhancement. b.3) sea urchin gonad extraction. b.4) post-production processes to achieve final product. **C) Raking method:** c.1) rearing of female-only batches. c.2) spawning induction through a combination of dry and thermal shock and egg collection. c.3) post-production processes to achieve “sea urchin caviar”. c.4) beginning of new production cycle with the same female batches.
**Figure 2**

*Paracentrotus lividus* performance results. Ovo-somatic index (OI %) and Gonado-somatic index (GI %) of wild-caught sea urchins and of reared sea urchin batches after the first, second and third production cycles of Raking and Bulking methods. The different letters indicate significant differences ($p < 0.05$) among the female-only groups (one-way ANOVA followed by the Tukey comparison test).
Figure 3

Schematic representation of the Raking productive cycle. A) Rearing in a controlled environment of a female-only batch of sea urchins. B) Rearing tanks with sea urchins inside taken out of the water for 3 hours C) Conical tank, with heater, to induce the egg release through thermal stimulation; egg collection from the tank bottom through a drain valve. D) Centrifugation for sea water removal E) Caviar pasteurization. F) Canned sea urchin caviar, the end-product of the productive cycle.

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